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**RISK-ADAPTED THERAPY FOR YOUNG CHILDREN WITH EMBRYONAL BRAIN TUMORS,  
HIGH-GRADE GLIOMA, CHOROID PLEXUS CARCINOMA OR EPENDYMOMA (SJYC07)**

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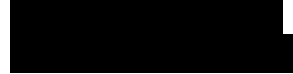
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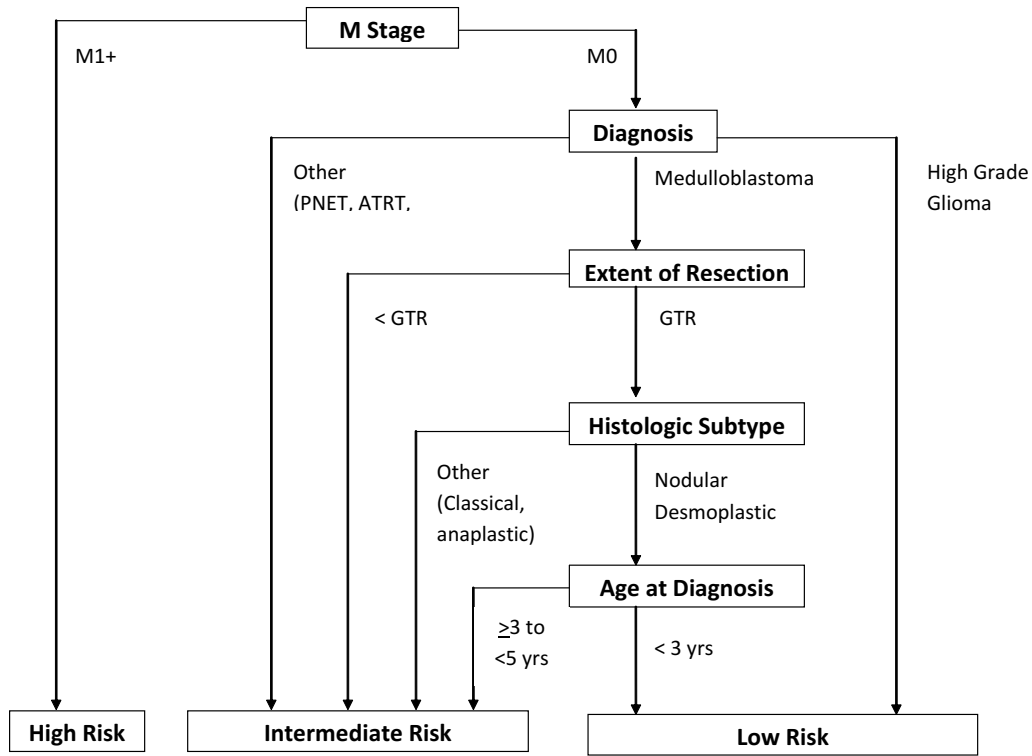
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**Protocol summary**

<p><b>SJYC07, Risk-Adapted Therapy for Young Children with Embryonal Brain Tumors, High-Grade Glioma, Choroid Plexus Carcinoma or Ependymoma</b></p>
<p><b>Principal Investigator:</b> Amar Gajjar, M.D.</p>
<p><b>IND holder:</b> Not applicable, non-IND study</p>
<p><b>Brief overview:</b> Children younger than 3 years of age with newly diagnosed medulloblastoma, supratentorial primitive neuroectodermal tumor (PNET), atypical teratoid/rhabdoid tumor (ATRT), high-grade glioma, choroid plexus carcinoma (CPC) or ependymoma. Children <math>\geq 3</math> and <math>&lt; 5</math> years of age with newly diagnosed non-metastatic medulloblastoma are also eligible</p>
<p><b>Intervention:</b> multi-modality, according to risk assignment                  Drugs: Methotrexate, vincristine, cisplatin, cyclophosphamide, vinblastine, carboplatin, etoposide, topotecan, erlotinib                  Other: Focal radiation therapy, craniospinal irradiation</p>
<p><b>Brief outline of treatment plan:</b>                  All participants will receive 4 identical cycles of induction chemotherapy including high-dose (<math>5 \text{ g/m}^2</math> or <math>2.5 \text{ g/m}^2</math> for patients less than or equal to 31 days of age at enrollment) intravenous methotrexate and standard dose vincristine, cisplatin, and cyclophosphamide. Patients enrolled on the high-risk arm will also receive low-dose vinblastine between induction cycles. Induction will be followed by risk-adapted consolidation therapy: low-risk patients will receive further conventional chemotherapy with carboplatin, cyclophosphamide, and etoposide; intermediate risk patients will receive focal radiotherapy (RT) to the tumor bed; high-risk patients will receive either chemotherapy with targeted intravenous topotecan and cyclophosphamide or optional craniospinal irradiation (CSI). CSI will be offered only to patients who reach 3 years of age by the end of induction only. After consolidation, all patients will receive 6 cycles of oral maintenance chemotherapy with cyclophosphamide, topotecan, and depending on the diagnosis, either erlotinib or etoposide (VP-16).</p>
<p>The diagram illustrates the treatment schedule in three phases: Induction (16 weeks), Consolidation (8 weeks), and Maintenance (24 weeks). Induction involves four cycles of MTX/VCR/CDDP/CPM (+VBL for High-risk). Consolidation involves two cycles: CPM/Carbo/VP16 (Low Risk), Focal RT (Intermediate Risk), and Topo/CPM or CSI (High Risk). Maintenance involves six cycles: PO CPM/Topo alternating with Erlotinib or VP16.</p>

**Study design:** Exploratory study utilizing risk-adapted multi-modality treatment.



**Sample size:** Target accrual is 90 medulloblastoma patients. The projected total accrual for the entire study including patients with other tumor types is 315.

**Data management:** Data management and statistical analysis will be provided locally by the Division of Neuro-Oncology and the Department of Biostatistics at St. Jude Children's Research Hospital.

**Human subjects:** The main risk to research participants will be the potential toxicities associated with the use of the multi-modality therapy. The research participants will be informed of the toxicities that have been associated with the study drugs and potential side effects of procedures recommended in this study. Adverse events will be monitored, treated, and reported following institutional and federal guidelines and regulations.

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## 1.0 OBJECTIVES

### 1.1 PRIMARY OBJECTIVES

1.1.1 Biology: To identify patterns of methylation profiling that are associated with progression-free survival among young patients with medulloblastoma treated with risk-adapted therapy.

*Responsible Investigators: Amar Gajjar, Giles. Robinson*

*Responsible Biostatistician: Arzu Onar-Thomas, Tong Lin*

1.1.2 Therapeutic: To estimate the event free survival distribution of young medulloblastoma patients treated with risk-adapted therapy.

*Responsible Investigator: Amar Gajjar*

*Responsible Biostatistician: Arzu Onar-Thomas*

### 1.2 SECONDARY OBJECTIVES

1.2.1 Biological aims

*Responsible Investigator: Amar Gajjar, Giles Robinson*

*Responsible Biostatistician: Arzu Onar-Thomas, Tong Lin*

- To perform high resolution genome-wide analyses of chromosomal abnormalities and gene expression patterns, and evaluate the relationship of these to other clinicopathological variables.
- To evaluate specific tumor types for molecular abnormalities with suspected prognostic or therapeutic significance.
- To evaluate the feasibility of collecting frozen and fixed tumor samples for analysis using high-resolution molecular biology tools.

1.2.2 Therapeutic aims

*Responsible Investigator: Amar Gajjar*

*Responsible Biostatistician: Arzu Onar-Thomas*

- To estimate the event free and overall survival of patients treated with the proposed risk-adapted therapy regimen, and to descriptively compare these survival rates to historical controls.
- To estimate the rates of local and distant disease progression in patients treated with focal radiotherapy (RT) to the post-operative tumor bed using a 5 mm clinical target volume margin.
- To estimate the objective response rate (sustained for 8 weeks) to induction chemotherapy including high-dose intravenous methotrexate for patients with residual or metastatic disease.



- To evaluate the feasibility and toxicity of administering low dose intravenous vinblastine in conjunction with induction chemotherapy to patients with metastatic disease.
- To evaluate the feasibility and toxicity of administering consolidation therapy including cyclophosphamide and pharmacokinetically targeted topotecan to patients with metastatic disease, and to estimate the sustained (for 8 weeks) objective response rate (complete response and partial response, as defined in section 9.1) to such therapy in patients with measurable residual disease after induction.
- To evaluate the feasibility and toxicity of administering oral maintenance therapy in young children.
- To use quantitative MR measures (volumetric, diffusion, and perfusion) of young brain tumor patients receiving chemotherapy including high-dose intravenous methotrexate to assess impact of treatment on developing brain.
- Investigate the feasibility of using PET as an in-vivo dosimetric and distal edge verification system for patients treated with proton beam therapy (*for participants enrolled at St Jude only*).

### 1.2.3 Pharmacologic Aims

*Responsible Investigator: Clinton Stewart*

*Responsible Biostatistician: Arzu Onar-Thomas*

- To develop a population pharmacokinetic model containing covariates which explain inter- and intra-patient pharmacokinetic variability for high-dose methotrexate in young children with brain tumors, and to explore the relationship between clinical effect (toxicity and antitumor efficacy) and methotrexate pharmacokinetics.
- To assess the extent of inter-patient variability in the pharmacokinetics of intravenous and oral cyclophosphamide and metabolites in young children with brain tumors, and to explore possible associations between cyclophosphamide pharmacokinetic parameters and patient specific covariates (e.g., age, sex, race, weight).
- To assess the ability to achieve the target systemic exposure of intravenous topotecan in young patients with metastatic brain tumors.
- To assess the extent of inter-patient variability in the pharmacokinetics of intravenous and oral topotecan in young children with brain tumors, and explore possible associations between topotecan systemic exposure and patient specific covariates (e.g., age, sex, race, weight).

- To assess the extent of inter-patient variability in the pharmacokinetics of oral erlotinib in young children with medulloblastoma and ependymoma, and explore possible associations between erlotinib pharmacokinetic parameters and patient specific covariates (e.g., age, sex, race, weight).
- For St. Jude patients enrolled on the PGEN5 protocol, to assess the relation between pharmacogenetic variation in drug metabolizing enzymes and drug transporters, and the pharmacokinetics of methotrexate, cyclophosphamide, topotecan, and erlotinib in young children with brain tumors.

#### 1.2.4 Cancer control aims

- To explore possible associations between cerebrospinal fluid (CSF) neurotransmitter concentrations, with emphasis placed upon concentrations of dopamine and its metabolites, and the development of neurocognitive deficits as identified by standardized tests.  
*Responsible Investigator: Clinton Stewart; Heather Conklin*  
*Responsible Biostatistician: Hui Zhang*
- To explore the association between genetic polymorphisms affecting central dopaminergic transmission and specific phenotypes, including CNS neurotransmitter and neurocognitive performance phenotypes.  
*Responsible Investigators: Clinton Stewart; Heather Conklin*  
*Responsible Biostatistician: Hui Zhang*
- To investigate changes in neuropsychological performance among patients enrolled in the study, and examine the impact of the proposed treatment regimen and other disease related factors (e.g., hydrocephalus) on neuropsychological performance.  
*Responsible Investigator: Heather Conklin*  
*Responsible Biostatistician: Hui Zhang*
  - Hypothesis 1: There will be a decline (negative slope) in global cognitive functioning for the group as a whole.
  - Hypothesis 2: Hydrocephalus (correcting for CSF diversion) will be associated with a steeper decline in global cognitive functioning.
  - Hypothesis 3: Higher radiation doses to specified structures (e.g., the frontal lobes) will be associated with steeper declines on related functions (e.g., attention and working memory).

- To assess the impact of changes in quantitative MR measures in the frontal lobe on neurocognitive performance in attention, working memory, and fluency.  
*Responsible Investigator: Eugene Reddick, Heather Conklin*  
*Responsible Biostatistician: Hui Zhang*
- To assess the impact of changes in quantitative MR measures in the right frontal-parietal regions on neurocognitive performance on visual-spatial reasoning and processing speed.  
*Responsible Investigator: Eugene Reddick, Heather Conklin*  
*Responsible Biostatistician: Hui Zhang*
- To assess the incidence of endocrinopathy after radiation therapy using photons or protons (*for participants enrolled at St Jude only*).  
*Responsible Investigator: Thomas Merchant*  
*Responsible Biostatistician: Arzu Onar-Thomas*
- To estimate the rate of longitudinal change in growth hormone secretion after conformal, intensity-modulated and proton beam radiation therapy (*for participants enrolled at St Jude only*).  
*Responsible Investigator: Thomas Merchant*  
*Responsible Biostatistician: Arzu Onar-Thomas*

## 2.0 BACKGROUND AND RATIONALE

### 2.1 OVERVIEW

Medulloblastoma and other embryonal tumors such as ATRT and supratentorial PNET are the most common malignant brain tumors to arise in children.<sup>1</sup> Treatment which includes craniospinal irradiation (CSI) has significantly improved the long-term survival of older children with medulloblastoma,<sup>2-4</sup> however, CSI damages the developing brain resulting in severe neurocognitive<sup>5</sup> and endocrine<sup>6,7</sup> sequelae. Thus, CSI is generally not administered to children less than three years of age. Because of this limitation in therapy, and likely also because of inherent differences in tumor biology, children less than three years old with embryonal tumors generally have a poor prognosis.<sup>8,9</sup> In order to limit the long-term sequelae of CSI, clinical trials in young children have attempted to substitute or delay CSI with intensive adjuvant chemotherapy. This approach has resulted in improved survival for selected groups of patients, most notably those with localized, completely resected medulloblastoma. For the majority of young patients, however, long-term survival rates remain poor, despite very aggressive treatment. Furthermore, these chemotherapeutic regimens may also be associated with significant toxicity, with toxic death rates exceeding 10% in some trials.<sup>10</sup> New treatment strategies will be needed in order to improve survival without further increasing toxicity.

A variety of novel treatments are now being developed which have more specific activity against tumor cells than standard cytotoxic agents, and thus avoid many of the typical toxicities of conventional cytotoxic chemotherapy. Among the most promising are molecularly targeted agents such as the small molecule tyrosine kinase inhibitors,<sup>11</sup> and treatments directed against the tumor vasculature.<sup>12</sup> It is anticipated that such specific therapies may eventually replace conventional cytotoxic agents entirely, but this will be a gradual process, and must be based on a firm understanding of tumor biology. For this transition to occur, molecular targets which are critical for tumor proliferation or survival must be identified first. The use of molecular targeted therapies for the treatment of young patients is likely to be especially challenging since the cell signal pathways that are dysregulated during tumorigenesis often control normal development. Once appropriate targets are identified, drugs with activity against these targets must be developed, and clinical trials then conducted to evaluate the toxicity and efficacy of these agents, and to determine the appropriate means of integrating them with standard therapies.

For medulloblastoma and the other brain tumors of young children, molecularly targeted therapies are in early development, because little is known about the molecular pathophysiology of these tumors. The focus of the current protocol will therefore be a comprehensive study of genome-wide patterns of gene expression and chromosomal alteration in medulloblastomas arising in children younger than three years, with the following goals:

- Increased understanding of molecular pathways implicated in tumor development
- Detection of molecular subtypes of potential biological, clinicopathological, or therapeutic significance
- Identification of potential molecular therapeutic targets

The backbone of the treatment plan will include the therapies that have been the most effective and least toxic in prior clinical trials involving this patient population. The intensity of therapy will be based on the known prognostic factors of stage, extent of resection, and histopathological variant. Novel therapies will be included throughout the treatment regimen for patients with high risk (metastatic) disease, and in a maintenance phase for all patients.

In addition to biological studies involving tumor tissue, a variety of other correlative studies will be included. These studies will help us to optimize the treatment of brain tumors in children younger than 3 years of age by thoroughly examining the factors which affect drug pharmacokinetics and activity, treatment toxicity, and patient outcome.

## 2.2 RESULTS OF CLINICAL TRIALS FOR INFANTS AND YOUNG CHILDREN WITH BRAIN TUMORS

Clinical trials for infants and young children with brain tumors have been characterized by small sample sizes and considerable heterogeneity in both enrollment criteria and treatment strategies, thus making it difficult to directly compare results between studies. Investigators have often used the term “infant” to describe the patient population treated, but in fact these trials vary substantially in the age of patients enrolled, with some including only patients younger than 18 months of age,<sup>13</sup> and others enrolling children as old as 6 years of age.<sup>10</sup> In the following discussion the phrase “infants and young children” will be used to refer generally to children considered too young to receive CSI, keeping in mind that the threshold for routine use of CSI has been defined differently in different trials. The specific age range included in a particular trial will be noted whenever it is pertinent. Treatments have also varied considerably among different trials, as has the terminology used to describe those treatments. CSI consistently refers to irradiation of the whole brain and spine, and implies the use of an additional “boost” dose to the tumor bed or surrounding area. However, the dose of CSI, and the dose and target volume of boost therapy vary considerably between trials and patients. As discussed in detail below, one strategy for avoiding CSI has been to eliminate the RT dose to the whole brain and spine, and administer RT only to the tumor bed and/or surrounding area. This more restricted RT administration has been described variously as “focal (or local) RT,” “tumor bed irradiation,” “posterior fossa irradiation,” or “involved-site RT.” The dose to the tumor bed has varied (generally between 45.0 and 54.0 Gy), as have the clinical target volume (CTV; 1.0 to 2.0 cm), and, for posterior fossa tumors, the dose to the entire posterior fossa (none to 36.0 Gy). In order to simplify the following discussion, “CSI” will be used to refer to any RT regimen which includes the entire brain and spine, regardless of dose, and “focal RT” will refer to any RT regimen which does not include the entire brain and spine, regardless of the dose, CTV, or inclusion of additional RT to the entire posterior fossa.

### 2.2.1 Medulloblastoma results

The results of major clinical trials conducted among infants and young children with medulloblastoma are summarized in Table 1.

TABLE 1 RESULTS OF PROSPECTIVE CLINICAL TRIALS FOR INFANTS AND YOUNG CHILDREN WITH MEDULLOBLASTOMA					
		Event Free Survival (%±SE)		Overall Survival (%±SE)	Reference
Trial	n	1 year	5 year	5 year	
<b>M0 No Residual</b>					
Baby POG	13			69	14,15
CCG 9921	38	66 ± 8	41 ± 8	54 ± 8	16
SFOP	47		29 (95% CI 18-44)	73 (95% CI 59-84)	17
HIT-SKK92	17		82 ± 9	93 ± 6	18
Head Start I+II (age <3)	14		64 ± 13	86 ± 9	ISPNO 2006
PBTC-001	20	70 ± 10			PBTC 2006
<b>M0 With Residual</b>					
CCG9921	23	43 ± 10	26 ± 9	40 ± 11	16
SFOP	17		6 (95% CI 1-27)	41 (95% CI 22-64)	17
HIT-SKK92	14		50 ± 13	56 ± 14	18
Head Start I+II (age <3)	7		29 ± 17	57 ± 19	ISPNO 2006
PBTC-001	7	51 ± 18			PBTC 2006
<b>M0 All Resections</b>					
Head Start I+II (age <3)	21	86 ± 8	52 ± 11	75 ± 10	ISPNO 2006
99703	24		3-yr EFS ~70		COG 2006
PBTC-001	27	66± 9			PBTC 2006
St. Jude (1998 and later)	20	78.7 (95% CI 63-99.7)	67.0 (95% CI 48-93)		
<b>M+</b>					
CCG-9921	31	42 ± 9	25 ± 8	31 ± 9	16
SFOP	15		13 (95%CI 4-38)	13 (95%CI 4-38)	17
HIT-SKK92	12		33 ± 14	38 ± 15	
Head Start II (age< 6)	21		3-yr EFS 49 (95%CI 27-72)	3-yr OS 60 (95%CI 36-84)	19

### 2.2.1.1 Delayed RT trials

Once the severe long-term neurocognitive sequelae associated with the use of CSI in infants and young children became recognized in the 1980s, investigators began to explore alternatives to the immediate postoperative CSI used in older children. Early trials for infants and young children with medulloblastoma attempted to use chemotherapy to delay CSI, with mixed results. Based on encouraging results from a pilot study by van Eys, et al.,<sup>20</sup> the Pediatric Oncology Group (POG) implemented the delayed CSI approach in the POG 8633/34 (Baby POG-1) trial.<sup>14,15</sup> In Baby POG-1, children younger than 3 years of age were treated with chemotherapy consisting of vincristine, cyclophosphamide, etoposide, and cisplatin, followed by CSI at one or two years post-diagnosis, depending on age. CSI was successfully delayed in only 40% of patients, 5-year PFS for all medulloblastoma patients was 31.8±8.3% and 5-year OS 39.7±6.9%. Medulloblastoma patients treated on

Baby POG-1 with M0 disease and gross total resection had a relatively good prognosis, with a 5-year OS of 69%. The subsequent POG study 9233/34 (Baby POG-2) attempted to improve survival by intensifying the chemotherapy regimen; patients were randomly assigned to receive either standard Baby POG-1 treatment or an intensified version of Baby POG-1 therapy in which the drugs were administered at higher doses and more frequently. There was no difference in event free or overall survival between patients receiving standard or intensified Baby POG therapy (POG 9233/34 final progress report, spring 2003 COG meeting).

A delayed RT approach was also used in the Children's Cancer Group CCG-921 trial, which ran concurrently with Baby POG-1. The CCG-921 treatment plan called for children younger than 18 months of age to receive the "8 drugs in 1 day" chemotherapy regimen, followed by delayed CSI or focal RT. In fact, only 9 of 91 patients < 18 months of age at diagnosis received RT as planned. Three-year PFS was 22% for all posterior fossa PNET (medulloblastoma) patients, and posterior fossa PNET patients with completely resected M0 disease had a 5-year PFS of 30%.<sup>13</sup> As in the POG, the subsequent CCG trial (CCG-9921) attempted to improve survival through the use of more intensive chemotherapy. CCG-9921 enrolled children younger than 3 years of age; patients were randomly assigned to receive one of two 5-cycle induction chemotherapy regimens, followed by a uniform regimen of maintenance chemotherapy for 56 weeks. Patients with residual or metastatic disease were to receive CSI or focal RT at the end of maintenance or at 3 years of age, whichever came earlier. As in the prior CCG trial, only a minority (40%) of patients received RT as planned. There was no difference in EFS or OS between chemotherapy arms, and outcomes were similar to Baby POG-1, with a 5-year EFS for all medulloblastoma patients of 32±5% and 5-year OS for completely resected M0 medulloblastoma of 54±8%.<sup>16</sup>

The above studies demonstrated that CSI can be delayed without compromising survival in a subset of medulloblastoma patients younger than 3 years of age at diagnosis. It has subsequently become apparent, however, that delaying CSI does not result in substantially better neurocognitive outcomes than administering CSI immediately after resection. During the 1980s and early 1990s, medulloblastoma patients younger than 3 years of age were treated at St. Jude with delayed CSI according to the Baby POG trials or very similar St. Jude institutional protocols, with OS and EFS comparable to that achieved on the POG and CCG trials.<sup>8,9</sup> In a review of the St. Jude experience with delayed radiation, median IQ for survivors of infant medulloblastoma fell from a baseline median of 88 (range 50 to 111) to a median 62 (range 44 to 86) at a median follow up time of 4.8 years (range 2 to 10.6 years).<sup>9</sup> The suboptimal survival and neurocognitive results attained with delayed CSI have prompted investigators to look for alternative approaches.

### *2.2.1.2 Alternatives to CSI*

One potential alternative to delayed CSI is to administer RT only when chemotherapy is ineffective. This approach was used in the French BBSFOP trial,

in which medulloblastoma patients younger than 4 years of age received chemotherapy for one year, with RT only in cases of recurrence or progression. The BBSFOP chemotherapy regimen was relatively mild, and failures were frequent; the 5-year PFS was 29% (95% CI 18-44) for completely resected M0 medulloblastoma. Many patients were effectively salvaged with RT (either CSI or focal RT, depending on the extent of disease) and high-dose chemotherapy. The salvage regimen resulted in 5-year OS for completely resected M0 medulloblastoma which was similar to Baby POG-1 (73%, 95% CI 59-84).<sup>17</sup>

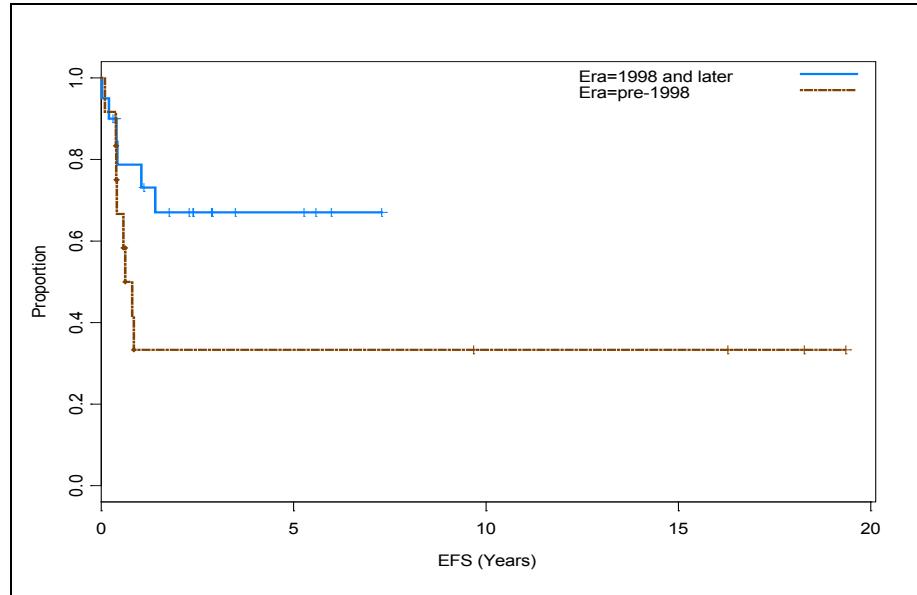
Another strategy to avoid CSI is to include methotrexate in the chemotherapy regimen. Two small trials incorporating methotrexate-based chemotherapy as have demonstrated improvements in long-term survival compared to the POG and CCG trials. The best published results for medulloblastoma patients without macroscopic metastasis come from the German HITSKK92 trial, which included a combination of intraventricular and high-dose intravenous methotrexate, in addition to standard chemotherapy.<sup>18</sup> Patients aged less than 3 years old with completely resected M0/M1 medulloblastoma (n=17) had a 5-year PFS of 82±9%, and 5-year OS of 93±6%. Outcomes in patients with residual disease (n=14, 5-year PFS 50±13%, and 5-year OS of 56±14%) and macroscopic metastasis (n=12, 5-year PFS 33±14%, and 5-year OS of 38±15%) were also somewhat better than in prior series. However, 19 of 23 evaluated HITSKK92 patients had evidence of leukoencephalopathy on MRI. These results suggest that the apparent improvement in survival gained through the incorporation of both intravenous and intraventricular methotrexate came at the cost of neurotoxicity. The best published results for young children with metastatic medulloblastoma were generated by the Head Start II trial, which used five cycles of induction chemotherapy including IV methotrexate (400mg/kg), followed by a consolidation regimen of myeloablative chemotherapy with autologous stem cell rescue (ASCR). The majority of patients (82%) had a CR to induction chemotherapy, and 3-year EFS and OS were 49% (95%CI 27-72) and 60% (95%CI 36-84), respectively. While encouraging, interpretation of these results is limited by the small size of the study and inclusion of older children (only 9 patients under age 3 years at diagnosis were enrolled).<sup>21</sup> The use of high-dose chemotherapy with ASCR has been advocated as an alternative to craniospinal irradiation. The Head Start I and II trials treated patients with localized medulloblastoma with 5 induction cycles very similar to regimen A of CCG-9921, followed by a single consolidation course of high-dose carboplatin, thiotepa, and etoposide with ASCR. The toxicity associated with the Head Start regimen was substantial, with toxic death occurring in 6% of patients during induction; of the patients who went on to receive consolidation with ASCR, an additional 8% died from treatment complications.<sup>10</sup> Final results from the Head Start I and II trials were reported in abstract form at the Spring 2006 International Symposium on Pediatric Neuro-Oncology (ISPNO); for the 14 patients with completely resected medulloblastoma, 5-year EFS was 64±13%, and 5-year OS 86±9%. The Head Start regimen became the basis for the recent CCG 99703 trial, in which patients received three cycles of intensive standard chemotherapy followed by three cycles of high dose chemo with ASCR. Preliminary results



suggest an improvement in 3-year EFS and OS for patients with medulloblastoma when compared with CCG-9921, but final assessment of the relative efficacy of 99703 versus 9921 will depend on longer follow-up and formal analysis of prognostic factors. An important limitation of this trial is that the use of RT was left to the discretion of the investigator. Data regarding which patients received RT, how long RT was delayed, and the dose and extent of RT (focal vs. CSI) were not collected as a component of this trial, thus making it difficult to draw conclusions about the effectiveness of this chemotherapy regimen.

One additional alternative to CSI is the use of focal RT for patients with localized disease. This strategy has been implemented in two recent trials. The COG P9934 study included focal RT after 16 weeks of induction chemotherapy for patients with localized, completely resected medulloblastoma. Survival results from this trial have not yet been reported. In PBTC-001, patients received standard chemotherapy plus intrathecal mafosfamide for twenty weeks, followed by focal RT, and then twenty weeks of maintenance chemotherapy. Early outcome results were reported at the PBTC Fall 2006 meeting, and are encouraging, with 1-year EFS for 20 patients with completely resected M0 medulloblastoma of  $70 \pm 10\%$ . These preliminary results suggest no difference in EFS between patients who received mafosfamide and those who did not. Additional support for the use of focal RT comes from the French BBSFOP trial, in which focal RT was used successfully as salvage therapy for patients with local recurrence, in combination with high-dose chemotherapy. On that trial, 25 of 34 patients with local relapse were given busulfan/thiotepa and focal RT, 5 received CSI, and 4 progressed quickly with no further therapy. Three-year overall survival after local relapse or progression was 61% (95% CI 45-76).<sup>17</sup> The St. Jude experience provides further evidence of the efficacy of focal RT. Between 1983 and 1997, St. Jude patients with M0 medulloblastoma were treated with the Baby POG-1 regimen or similar treatment plans utilizing delayed CSI. A total of 12 patients with M0 medulloblastoma were treated in this early cohort; extent of resection prior to treatment was gross total (n=8), near-total (n=1) or subtotal (n=3), and 1-year and 5-year EFS were both 33.3% (95% CI 15.0-74.2) (Figure 1). Since 1998, all St. Jude patients with M0 medulloblastoma have been treated with a regimen based on focal RT, including 3 enrolled on the PBTC-001 pilot study BB98, 10 enrolled on PBTC-001, 3 treated as per PBTC-001, and 4 enrolled on P9934. Extent of resection prior to treatment was gross total (n=15), near total (n=3), or subtotal (n=2). Results have been very encouraging for these 20 patients, with a 1-year EFS of 78.7% (95% CI 62.2-99.7%), and 5-year EFS 67.0% (95% CI 48.4-92.8%).

Figure 1: Event Free Survival for St. Jude Patients with M0 Medulloblastoma by Treatment Era



Although other changes have occurred between the two eras, including improvements in imaging and surgical technology and better ability to diagnose ATRT, these results suggest that focal RT may play an important role in the treatment of children younger than 3 years of age with localized medulloblastoma.

### 2.2.1.3 Rationale for chemotherapy and focal RT in medulloblastoma patient's $\geq 3$ - $< 5$ years of age

Age at diagnosis and dose of neuraxis irradiation are key determinants of neurocognitive outcome in medulloblastoma survivors. Children younger than 7 years of age are particularly vulnerable to side effects of whole brain irradiation. Published data from the SJMB 96 protocol has documented significant decline in standard risk medulloblastoma patients ( $\geq 3$ -7 yrs of age) treated with 23.4 Gy craniospinal irradiation (CSI) across the following domains: Intelligence Quotient (IQ); Reading; Spelling, and Math as compared to older children treated with identical therapy (Table 1a). With longer follow up of this patient cohort, therapy inflicted neurocognitive deficits have impaired these children from completing high school and seeking higher education.

In our current study SJMB03, 23 patients between the ages of 3-5 have been treated. The 1 and 2 year EFS for this cohort with chemotherapy and neuraxis irradiation are  $86 \pm 7\%$  and  $76 \pm 10\%$ , respectively. Thus in order to preserve the neurocognitive function we propose to treat this group of young children between 3-5 years of age, who are particularly vulnerable to irradiation induced damage, with chemotherapy and focal irradiation to the tumor bed and avoid irradiating the whole brain and spinal cord.

This cohort of patients will be limited to average risk medulloblastoma patients defined as those having a gross total resection with no metastatic disease. The outcome data will be closely monitored for early progressive disease failures as outlined in the statistical section.

*Table 1a: Estimated intercepts and slopes of Intellect and Achievement for Age and Risk Groups*

Outcome	No. of Patients	No. of Observations	Older AR Patients		Older HR Patients		Younger AR Patients		Younger HR Patients	
			Intercept* (points)	Slope (points/year)	Intercept* (points)	Slope (points/year)	Intercept* (points)	Slope (points/year)	Intercept* (points)	Slope (points/year)
IQ	104	244	97.08	-0.42	97.00	-1.56	93.73	-2.41†	94.05	-3.71†
Reading	91	208	97.24	-2.05†	99.26	-1.05	100.25	-4.81†	95.94	-3.90†
Spelling	90	207	95.65	-2.62†	94.30	1.02	90.68	-2.60†	97.14	-5.31†
Math	93	210	94.12	-1.84†	92.91	0.37	87.80	-0.77	96.00	-3.73†

Abbreviations: AR, average risk; HR, high risk; IQ, intelligence quotient.  
\*Expected population average = 100 (standard deviation = 15).  
†Statistically significant departure from 0 slope.

## 2.2.2 Supratentorial PNET results

		Event Free Survival (%±SE)		Overall Survival (%±SE)	Reference
Trial	n	1 year	5 year	5 year	
<i>All Stages and Resections</i>					
CCG 9921	46	26 ± 6	17 ± 6	31 ± 7	16
HIT-SKK-87 and -92	29		3 year 14.9	3 year 17.2	22
<i>M0 No Residual</i>					
CCG 9921	20	25 ± 10	15 ± 8	30 ± 10	16
PBTC-001	2	50 ± 25			PBTC 2006
<i>M0 With Residual</i>					
CCG9921	17	29 ± 11	18 ± 9	33 ± 12	16
PBTC-001	7	71 ± 16			PBTC 2006
<i>M+</i>					
CCG-9921	9	22 ± 14	0	0	16

PNET arising outside the posterior fossa is rare in children less than 3 years of age, and has generally connoted a poor prognosis (Table 2), especially in patients with pineal tumors (pineoblastoma). CCG-921 included 8 patients with pineoblastoma and 11 with non-pineal supratentorial PNET. Pineoblastoma patients had a dismal prognosis, with a 3-year PFS of 0%, while those with other supratentorial PNETs had a 3-year PFS of 55±16%.<sup>13</sup> In the subsequent CCG-9921 trial, 46 patients with supratentorial PNET, including pineoblastoma, had a 5-year EFS and OS of 17±6% and 31±7%, respectively. Extent of resection did not appear to be prognostically important, as M0 patients with completely resected disease (n=20, 5-yr EFS 15±8%, 5-yr OS 30±10%) had similar outcomes to those with residual disease (n=17, 5-yr EFS 18±9%, 5-yr OS 33±12%). No patient with M+ supratentorial PNET survived 5 years (n=9).<sup>16</sup> Similar results were reported by the German cooperative group for patients less than 3 years of age treated on the HITSKK87 and HITSKK92 trials, which included intravenous and combined intravenous/intraventricular methotrexate, respectively. A total of 29 patients with supratentorial PNET were enrolled on the two trials, with no significant difference in outcome detected between trials; 3-year OS for the combined cohort was 17.2%, and 3-year PFS 14.9%. Administration of RT was the only significant positive predictor of survival.<sup>22</sup> Preliminary results from PBTC-001 have demonstrated less difference in outcome between medulloblastoma and supratentorial PNET. As discussed at the fall 2006 PBTC Meeting, the 9 M0 supratentorial PNET patients on that trial had a 1-year PFS of 67±15%, and the 27 M0 medulloblastoma patients had a 1-year PFS of 66±9%. Longer follow-up will be necessary to see if this apparent improvement in survival for patients with PNET persists.

### 2.2.3 ATRT results

<i>TABLE 3 RESULTS OF PROSPECTIVE CLINICAL TRIALS FOR ATRT</i>					
		<i>Event Free Survival (%±SE)</i>		<i>Overall Survival (%±SE)</i>	<i>Reference</i>
<i>Trial</i>	<i>n</i>	<i>1 year</i>	<i>5 year</i>	<i>5 year</i>	
<i>M0 No Residual</i>					
<i>CCG 9921</i>	<i>13</i>	<i>31 ± 13</i>	<i>15 ± 10</i>	<i>38 ± 13</i>	<i>16</i>
<i>PBTC-001</i>	<i>10</i>	<i>33 ± 16</i>			<i>PBTC 2006</i>
<i>M0 With Residual</i>					
<i>CCG9921</i>	<i>5</i>	<i>20 ± 18</i>	<i>0</i>	<i>0</i>	<i>16</i>
<i>PBTC-001</i>	<i>6</i>	<i>17 ± 11</i>			<i>PBTC 2006</i>
<i>M+</i>					
<i>CCG-9921</i>	<i>10</i>	<i>40 ± 15</i>	<i>20 ± 13</i>	<i>30 ± 14</i>	<i>16</i>

ATRT is a distinct and highly aggressive neoplasm which has been recognized fairly recently.<sup>23,24</sup> These tumors have a typical histologic pattern which includes cells with “rhabdoid” features such as eosinophilic cytoplasm, eccentric nuclei, and

prominent nucleoli. The histologic findings within a particular ATRT can be quite heterogeneous, however, and the typical histology may not be present. Areas of a tumor, and in some cases entire tumors, may be morphologically indistinguishable from medulloblastoma or PNET. It is likely that in the past, many ATRTs were misclassified as medulloblastoma or PNET. ATRT is characterized by alterations in the *hSNF5/INI1* gene on chromosome 22q11, also known as *SMARCB1*. Eighty-five percent of ATRTs contain homozygous deletions of the *SMARCB1* gene, or loss of one allele and mutation of the other copy.<sup>25,26</sup> More frequent utilization of molecular diagnostic techniques such as FISH or immunohistochemistry in recent years has permitted more accurate diagnosis of ATRT based on the detection of *SMARCB1/hSNF5/INI1* abnormalities.<sup>27</sup> ATRTs occur primarily in children younger than 2 years of age, and are frequently metastatic at presentation. While older children may be successfully treated with complete resection, CSI, and high-dose chemotherapy with ASCR, outcomes for children younger than 3 years of age are dismal (Table 3).<sup>28</sup> In the St. Jude experience reported by Tekautz, et al. 2-year EFS for the 22 children under 3 years of age at diagnosis was 11±6%. Similarly, 2-year EFS for 28 children < 3years old treated on the CCG-9921 trial was 14±7%.<sup>16</sup> Use of sarcoma-type therapy based on the IRS-III study has resulted in long-term survival in a few cases, but includes a combination of early radiation, intrathecal chemotherapy and doxorubicin with substantial potential for acute and long-term toxicity.<sup>29,30</sup> Results of PBTC-001 suggest no improvement over prior studies, with a 1-year EFS for M0 ATRT patients of 17±11%. Among the 12 St. Jude ATRT patients on PBTC-001, 10 have experienced recurrence or progression to date, and all relapses occurred by 20 weeks of therapy. Because of the dismal prognosis associated with this diagnosis, patients with ATRT are good candidates for novel treatment approaches.

#### 2.2.4 Clinical trial results for other diagnoses

High-grade gliomas are rare in children < 3 years of age.<sup>1</sup> To date, three small prospective studies have described the outcome of young children with high-grade glioma.<sup>31-33</sup> They reported long term OS rates from 50% to 59%, and PFS rates from 31% to 43%. Several children in each series survived without ever receiving RT, and the extent of surgical resection was not a significant prognostic factor in any of these studies. These results suggest that young children have a more favorable prognosis than older patients with histologically identical neoplasms, perhaps due to differences in tumor biology.<sup>31-33</sup> We recently reviewed the St. Jude experience with high-grade astrocytoma in 16 children younger than three years, and documented survival rates similar to the prior publications, with a 5 year OS of 66.3% and 5 year EFS of 28.6%.<sup>34</sup> No specific treatment regimen that is particularly effective for high-grade glioma in young children has been identified, but in the St. Jude experience, as in the other published reports, patients were treated with the same chemotherapy regimens as young children with other brain tumors such as medulloblastoma. Three St. Jude patients have survived at least 2 years without receiving RT, and all three received chemotherapy including carboplatin, cyclophosphamide, and etoposide.<sup>34</sup> Infants and young children with

high-grade gliomas who progress on chemotherapy will be offered radiation therapy per section 4.0.

While rare overall, ependymoma represents one of the more common CNS malignancies in very young children.<sup>35</sup> Early clinical trials treated ependymomas with the same delayed chemotherapy approach used for medulloblastoma, with some success. The Baby POG 1 trial resulted in a 66% 5 year OS for patients younger than 3 years of age with completely resected ependymoma.<sup>15</sup> Patients with subtotal resections fared poorly, however, with a 5 year OS of 25%. For the older patients on the trial (24 to 36 months of age), radiation was only delayed by 1 year, and 5 year OS was 62.3%. Patients younger than 24 months received RT after a delay of 2 years, with a 5 year OS of only 25.7%, prompting the authors to conclude that delaying RT more than 1 year adversely affected survival.<sup>15</sup> The CCG 9921 trial also utilized delayed RT and achieved similar results; with 5 year OS of 67% for patients with completely resected M0 disease. Five-year PFS for this group was only 32%, however, again emphasizing the importance of RT for this disease.<sup>16</sup> Among best results for localized ependymoma treated with RT have been recently reported by Merchant et al.<sup>36</sup> The 7 yr event free survival was 69.1% (95% CI 56.9 – 81.3). Among the factors that impacted local disease control were extent of resection [gross total vs. near total or subtotal: hazard ratio (HR) 0.16 (0.067-0.38); p<0.0001], sex [male vs. female: HR 3.85 (1.10-13.52); p=0.035]; and age [<3 years vs. ≥3 years: HR 3.25 (1.30 – 8.16); p=0.012]. The study included patients with a median age of 2.9 yrs (range 0.9 – 22.9 yrs). Infants < 1 yr of age, irrespective of the extent of resection are treated with chemotherapy and hence will continue to be eligible for the current protocol. Since several series have demonstrated that extent of resection is a key prognostic factor it is essential for achieving a gross total resection (GTR) in newly diagnosed patients. The addition of chemotherapy has post operatively facilitated a GTR in patients with residual disease. This strategy was used successfully in the recently closed COG trial (ACNS 121) and in our institutional experience (personal communication – F.A Boop). Adjuvant post-operative chemotherapy devascularizes the tumor and makes surgical resection much easier and less risky for the patient. Recent data from a closed trial (CCG – 9942) has demonstrated that 4 cycles of pre-irradiation chemotherapy (including cisplatin, vincristine, cyclophosphamide and etoposide) in patients with residual disease after surgery may improve the cure rate for this subset of children.<sup>36a</sup> Of the 41 patients enrolled on the study – 34 patients were fully evaluable for response to chemotherapy: 14 (42%) had a complete response (CR) and 6 (18%) had a partial response (PR); 9 had a minor response or stable disease (SD) and 5 (15%) patients had progressive disease (PD) prior to radiation. Interestingly the 3 yr EFS for all patients with incomplete resections and chemotherapy was 58%, which was not significantly different than the 62% 3 yr EFS for the completely resected patients who received radiation therapy alone. The patients who achieved a CR to chemotherapy had a 3 yr EFS of 86% and those with a PR had a 3 yr EFS of 83%. These data suggest that the use of pre-radiation chemotherapy may in fact have a beneficial effect on the EFS of this high risk group of patients with residual disease after the initial surgical resection. Hence

infants and young children < 3 years of age with residual disease after initial surgical resection will be eligible to be enrolled on the trial.

Patients with metastatic disease are not amenable to focal RT alone and are treated with chemotherapy regimens similar to what is being proposed on this trial and thus infants and young children < 3 years of age with metastatic ependymoma will be eligible for this trial.

Rare tumors like choroid plexus carcinoma (CPC) and the PNET variants (CNS neuroblastoma, ependymoblastoma, etc.) occurring in very young children have traditionally been treated like medulloblastoma, with maximal resection followed by chemotherapy and sometimes radiation. Wolff, et al. performed a meta-analysis of CPC trials and case reports, and found that complete resection and radiation were significantly associated with prolonged survival in patients of all ages, although these associations were not statistically significant for very young children, probably because of the small number of patients included.<sup>37,38</sup> In the absence of a standard treatment approach for these rare tumors, it is reasonable to continue to treat them on trials designed for patients with embryonal tumors which include surgical resection and radiotherapy. Enrolling these patients in the trial will also facilitate evaluation of the biology of these rare tumors.

#### 2.2.5 Summary of clinical trials

Trials which have attempted to delay or avoid CSI in children younger than 3 years of age with embryonal tumors, ATRT, PNET, high-grade glioma, CPC and ependymoma have demonstrated that some children can be cured with fairly limited adjuvant therapy. Patients with medulloblastoma whose tumor is completely resected and non-metastatic have a relatively good prognosis, even with less intensive chemotherapy, such as that used in the CCG-921 and BBSFOP trials. Small studies suggest that high-dose and intraventricular methotrexate may improve outcomes, but at the risk of neurotoxicity. Early results also suggest that focal RT may be an effective component of treatment for patients with localized disease. The efficacy of high-dose chemotherapy with ASCR has not been conclusively established, but the toxicity associated with this approach has been substantial in most studies. For patients with residual or metastatic disease of any histology, even the best outcomes to date leave much room for improvement.

### 2.3 RATIONALE FOR TUMOR BIOLOGY STUDIES

#### 2.3.1 Rationale for examination of genetic abnormalities in tumor samples from young children with medulloblastoma

No molecular alterations are used in current clinical practice to predict disease risk or treatment response among children with brain tumors. This lack of knowledge reflects the relatively limited characterization of pediatric brain tumors that has been conducted to date. Brain tumors arising in very young children (< 3 years old) are relatively rare, and have hence been especially poorly characterized.

Medulloblastoma (MB) has been associated with several molecular abnormalities.<sup>39</sup> These include gains and losses of large chromosomal regions (e.g. loss of 8p, loss of 9q22, loss of 17p, gain of 17q), amplifications of specific genes (e.g., *MYCC* and *MYCN*), and point or frameshift mutations (e.g., *TP53*, *CTNNB1*, *PTCH1*). Inactivating mutations in *PTCH1* and *SUFU* activate the sonic hedgehog (SHH) cell signal pathway, while mutations in *CTNNB1* promote signaling through the canonical WNT pathway. Certain abnormalities have been associated with prognosis of medulloblastoma, such as high expression levels of *ERBB2* and *MYCC* and *MYCN* amplification (poor prognosis) and nuclear accumulation of the *CTNNB1* gene product,  $\beta$ -catenin (good prognosis).<sup>40-43</sup> However, these data have been derived mainly from retrospective analyses of tumors removed from children older than three years of age; the incidence and prognostic significance of these molecular characteristics in tumors arising in patients less than 3 years is not known.

The SJYC07 protocol offers an excellent opportunity to collect and study medulloblastoma samples from young children. Generated data will increase our understanding of the molecular development of these tumors and can be correlated with key clinicopathological variables, such as histopathological subtype and outcome. Obtaining and storing freshly frozen tissue from these tumors will allow isolation of high quality RNA (including microRNA), DNA, and protein for subsequent analysis. Tumor RNA will be used for gene expression studies, as discussed further in section 2.3.2. Tumor DNA will be analyzed using array technology (methylation profiling), and protein extractions will be used for western blotting or proteomic analysis. This broad strategy will be augmented by an analysis of MBs for molecular abnormalities recognized to have specific associations with clinicopathological variables, particularly prognosis. This can be accomplished both by using data from the microarray studies and by using methods, such as interphase fluorescence *in situ* hybridization (iFISH), gene sequencing and immunohistochemistry (IHC), to demonstrate specific abnormalities, such as *MYC* amplification in clones of cells, *SMARCB1* mutation or WNT pathway activation, respectively. All of these methods can be applied also to the analysis of formalin-fixed paraffin-embedded (FFPE) tissue. FFPE tissue is obtained for diagnostic purposes in virtually all brain tumor resections, unlike frozen tumor material, which is obtained at the discretion of the neurosurgeon. Surplus FFPE tissue is therefore frequently available for research purposes. The efficiency of molecular assays that analyze intact tissue sections is increased markedly by the incorporation of multiple samples of FFPE tumors into tissue microarrays (TMA). We will construct and analyze TMA from FFPE material collected from patients treated on SJYC07.

### 2.3.2 Rationale for analysis of gene expression profiles in young children with medulloblastoma

The limited molecular data that are available regarding medulloblastoma in young children have been obtained from traditional molecular biological approaches which usually evaluate a single gene or cell signal pathway at one time. The advent of



microarray technology now permits the simultaneous evaluation of thousands of genes in each tumor.<sup>44</sup> This approach can therefore interrogate genes previously implicated in the biology of medulloblastoma as well as identifying potentially new causative alterations. Gene expression profiles have been generated for a variety of adult and pediatric tumor types. These profiles have unmasked subtypes of histologic tumor types that provide important insight into the various molecular alterations that can generate these diseases. Best studied are the acute leukemias including pediatric ALL<sup>45,46</sup> and AML.<sup>47</sup> In studies of renal cell carcinoma, gene expression profiles were found to be distinct in patients with aggressive disease versus those with more indolent tumors. When the use of this molecular distinction as a prognostic tool was simulated in the clinical setting, the prediction was accurate in 96% of the cases tested, exceeding the accuracy of prediction by staging.<sup>48</sup> Additional studies involving renal cell carcinoma identified a gene signature associated with metastatic disease,<sup>49</sup> and other studies in patients with ovarian cancer identified a gene signature associated with response to chemotherapy.<sup>50</sup> More recently, Liu and colleagues identified an “Invasiveness Gene Signature” (IGS) based on analysis of breast tumor samples. Interestingly, the IGS was also found to be associated with prognosis in lung cancer, prostate cancer, and medulloblastoma.<sup>51</sup> Taken together, these results provide support for the general approach of using gene expression profiling to identify genes associated with the clinical behavior of tumors and patient survival. This approach must be used cautiously, however, as the analysis of large numbers of genes will always yield apparent distinctions among groups of tumors or patients.

Gene expression analysis of high-grade gliomas has revealed the value of this approach for building new hypotheses and identifying new paths for research in brain tumor patients. Such studies have shown that expression profiles may provide better prognostic information than features such as histology, and may facilitate the development of novel therapeutic targets. For example Freije, et al. found that gene expression profiles were strongly predictive of survival in adults with infiltrating gliomas, independent of histologic subtype.<sup>52</sup> In the largest glioma gene expression study to date Phillips, et al. evaluated 115 high-grade astrocytoma samples from adults, and identified previously undescribed prognostic subclasses which resemble stages in neurogenesis. A tumor class displaying markers of neuronal differentiation showed longer survival, while tumor classes exhibiting markers of proliferation or angiogenesis/mesenchymal differentiation were associated with shorter survival.<sup>53</sup> Associations between gene expression and survival were also demonstrated by Rich, et al. They examined subsets of genes associated with survival in patients > 50 years old with glioblastoma, and identified three particular genes (*SPARC*, *Doublecortex*, and *Semaphorin3B*) which were dominant contributors to regression models associating gene profiles and survival. These genes are involved in cellular migration, and suggest a new set of therapeutic targets which are distinct from more established targets involving cell proliferation and apoptotic pathways.<sup>54</sup> Faury, et al. conducted a similar study with pediatric glioblastomas. They found that a gene expression profile associated with Ras and Akt pathway activation was also associated with poor prognosis. In addition, they

demonstrated that *YB1*, a gene involved in brain embryogenesis which contributes to oncogenesis in epithelial cancers, was overexpressed in a majority of pediatric, but not adult, glioblastomas.<sup>55</sup>

The few medulloblastoma gene expression studies performed to date have evaluated small numbers of tumor samples using microarray platforms which assess relatively few genes compared to the platforms now available. Pomeroy, et al. obtained expression profiles from 99 patient samples using a microarray which included 6817 genes, and were able to distinguish medulloblastomas from other brain tumors including PNETs, ATRTs, and malignant gliomas. They were also able to identify genes that are differentially expressed between classic medulloblastomas and those with desmoplastic histology, and found evidence to support the association between desmoplastic medulloblastoma and abnormalities of the SHH/PTCH pathway.<sup>56</sup> A subsequent analysis of the same patient samples found that gene expression profiles were the only significant predictor of outcome in a multivariate analysis that included standard clinical prognostic markers such as stage and age at diagnosis.<sup>57</sup> While these results are intriguing, the patient population evaluated (60 patients total) included only 10 patients younger than three years of age at diagnosis. A recent publication from Richard Gilbertson's laboratory described gene expression analyses of 46 medulloblastoma samples, including 11 from patients less than three years old, using a microarray that analyzes the expression level of 18,400 transcripts and variants. In that study unsupervised analysis of gene profiles partitioned medulloblastomas into 5 subgroups, two of which were enriched for specific genetic alterations in pathways known to be involved in medulloblastoma (WNT and SHH). The majority of tumors (n=6) from young children were in the subgroup associated with *SHH* abnormalities, while no tumors from young children were in the subgroup associated with *WNT* abnormalities.<sup>58</sup> A similar analysis performed by investigators in the Netherlands also found that MBs clustered into 4 'gene expression' subgroups. In that analysis, the 14 tumors from children < 3 years old clustered into two of these subgroups: 9 tumors in the subgroup associated with SHH pathway abnormalities and 5 tumors in a "retinal gene" subgroup. As in the Gilbertson group's study, none of the tumors from young children was in the subgroup associated with WNT pathway abnormalities (personal communication, Marcel Kool).

The above studies suggest that gene expression profiling may be a valuable tool for examining the biology of medulloblastoma in young children. While very few tumor samples from patients in this population have been analyzed to date, results from other patient populations demonstrate that this type of analysis can identify molecular signatures that predict tumor behavior better than stage or histology. Such analyses may also provide new insights into the molecular pathophysiology of these tumors, and may yield novel therapeutic targets. This analysis will therefore be an integral component of the current trial. Gene expression studies of medulloblastoma samples will be complemented by comprehensive analysis of chromosome gain and loss through the use of 500K single nucleotide polymorphism (SNP) gene mapping arrays, as well as other emerging techniques for

comprehensive molecular characterization of tumor samples, including expression analysis of microRNAs.

In addition to expression arrays that can only be conducted from snap frozen tissue, DNA methylation profiling can be done from DNA extracted from FFPE tissue blocks. DNA methylation profiling in cancer has prognostic potential. DNA extracted from FFPE material will be used for methylation profiling. The current platform available at SJCH for methylation profiling is the Illumina Infinium Human Methylation 450 Bead Chip which will be used according to the manufacturer's recommendations. How methylation subgroups may correlate with RNA expression subgroups is an area of great interest especially given the numerous mutations to epigenetic modifying genes found amongst the current molecular subgroups.

### 2.3.3 Additional tumor biology studies

Although the focus of the SJYC07 trial is the treatment and molecular characterization of medulloblastoma in young children, patients with other diagnoses will be eligible for enrollment on this trial. Very little is known about the biological features of these tumors. ATRT is the second most common CNS embryonal tumor of infancy, and is recognized to have a poor prognosis. Monosomy 22 and mutations of the *SMARCB1* (*INI1*) gene located on 22q set ATRTs apart from other embryonal tumors,<sup>59</sup> but any specific associations between these two genetic abnormalities and tumor morphology, age at presentation or other molecular alterations have yet to be determined. In addition, it appears that mutation of genes other than *SMARCB1* can cause the ATRT phenotype.<sup>60</sup> ATRTs and choroid plexus carcinomas (CPCs) are associated as part of a syndrome of familial posterior fossa tumors of infancy.<sup>61</sup> CPCs may also demonstrate *SMARCB1* mutations,<sup>26</sup> but their frequency and nature in infant CPCs and whether distinct *SMARCB1* mutations occur in CPCs and ATRTs have not been determined. Ependymomas arise from radial glia, and exhibit different profiles at the levels of genome and transcriptome depending on site and histopathological features.<sup>62</sup> However, little is known about the relationship between molecular abnormalities and biological behavior. Gain of 1q has been associated with a poor outcome in pediatric posterior fossa tumors,<sup>63</sup> but whether this or undiscovered molecular abnormalities will be useful to stratify patients for therapy is yet to be determined. For rarer tumors like the non-cerebellar PNETs and high-grade gliomas, few molecular data exist.<sup>64</sup>

While molecular characterization of medulloblastomas in very young children is the primary objective of the SJYC07 trial, this trial provides an excellent opportunity to characterize the molecular features of other brain tumors in this patient population. It is important to note that the density of gene expression and SNP arrays can allow for the identification of novel gene alterations even when analyzing relatively small numbers of tumor samples.<sup>62</sup> The same comprehensive series of molecular studies

proposed for medulloblastoma samples will therefore also be performed for the other tumor samples obtained through this trial.

## 2.4 RATIONALE FOR TREATMENT

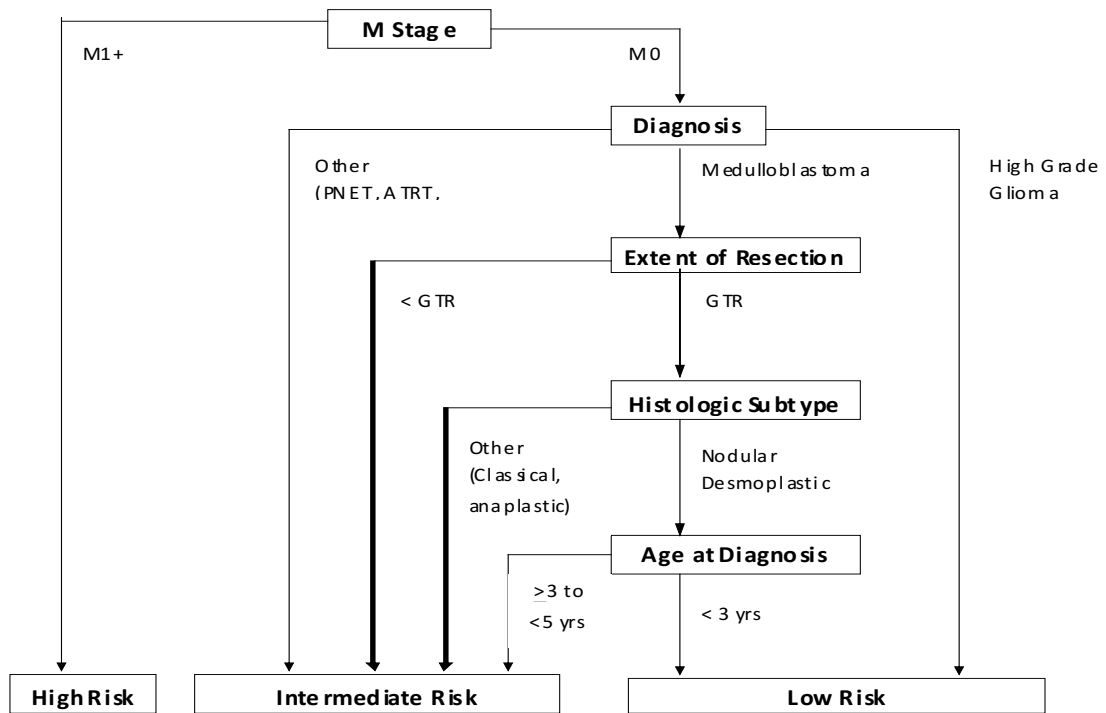
### 2.4.1 Rationale for risk stratification

The risk stratification schema for this trial is demonstrated in Figure 2. Clinical trials have consistently demonstrated better event-free and overall survival for medulloblastoma patients with localized disease (M0). Among these patients, those with completely resected tumors (GTR) have a particularly good prognosis. In the Baby POG 1 trial,<sup>15</sup> patients with GTR/M0 medulloblastoma had a 5-year OS of 69% with standard chemotherapy and delayed craniospinal irradiation. On the more recent SFOP trial, despite less intensive chemotherapy and reduced PFS in all strata compared to other trials, 29% of GTR/M0 medulloblastoma patients remained progression-free at 5 years without irradiation.<sup>17</sup> These results suggest that a subgroup of GTR/M0 medulloblastoma patients can be cured without radiotherapy. Results of other trials suggest that desmoplastic histology is associated with a much better prognosis than other histologic subtypes of medulloblastoma (Table 4). In the German HIT-SKK'92 trial, 20 patients with desmoplastic medulloblastoma had a 5-year PFS of 85±8%, compared to 23 patients with classical medulloblastoma (5-year PFS 34±10%, p<0.001).<sup>18</sup> Similarly, in the combined results of the Head Start I and II trials, 5-year EFS for desmoplastic (n=9) and classical (n=12) medulloblastoma patients with localized disease were 67±17% and 42±14%, respectively. (Abstract MB-03, ISPNO 2006). On the British UKCCSG 9204 trial, 1-year EFS was 82.2% (95% CI 72.9-100%) for 17 patients with desmoplastic medulloblastoma of all stages, and 18.2 (95% CI 0–41.0%) for 11 patients with classical or large cell anaplastic histology (personal communication, Richard Grundy). Finally, a recent combined analysis of data from 5 national groups included 253 patients with early childhood medulloblastoma, and found that desmoplasia was an independent favorable prognostic factor in a multivariable analysis (p<0.001, personal communication, Stefan Rutkowski). In the current study, patients with GTR/M0 medulloblastoma and nodular desmoplastic histology will be considered low-risk, and treated without RT. Patients with all other histologic subtypes of localized medulloblastoma will be considered intermediate risk.

While high-grade gliomas are very rare in children younger than 3 years of age, all published reports suggest that these patients have a better prognosis than older children and adults with the same tumor types.<sup>31-34</sup> Neither complete resection nor treatment with RT has been demonstrated to improve survival in these series. In recent years, clinical practice at St. Jude has been to treat children < 3 years of age with high-grade glioma with chemotherapy (typically cyclophosphamide, carboplatin, and etoposide), and to administer RT only in cases of disease progression or recurrence. Using this approach, 3 patients have avoided RT and survived more than 2 years without disease progression. Based on this experience

and the available literature, we will enroll all M0 high-grade glioma patients on the low-risk arm, regardless of the extent of surgical resection.

Figure 2 Risk Stratification Schema



<i>TABLE 4 EFFECT OF DESMOPLASTIC HISTOLOGY ON PROGNOSIS</i>				
	<i>n</i>	<i>5 year EFS (%±SE)</i>	<i>5 year OS (%±SE)</i>	<i>Reference</i>
<b>HIT-SKK92 (All Stages)</b>				
Classical	23	34 ± 10	41 ± 11	
Desmoplastic	20	85 ± 8	95 ± 5	
	<i>p</i>	<0.001	<0.001	<sup>18</sup>
<b>Head Start 1 + 2 (M0 only)</b>				
Classical	12	42 ± 14	75 ± 13	
Desmoplastic	9	67 ± 16	78 ± 14	ISPNO 2006
<b>UKCCSG 9204 (All Stages)</b>				
Classical/Large Cell Anaplastic	11	18.2 (95% CI 0-41)	18.2 (95% CI 0-41)	
Desmoplastic	17	35.3 (95% CI 13-58)	52.9 (95% CI 29-77)	*
*Personal Communication, Richard Grundy				

All patients on the intermediate risk arm will receive focal RT. Medulloblastoma patients with incompletely resected M0 tumors have a higher rate of relapse than those with GTR, with 5-year PFS rates of less than 50% in published trials.<sup>16,18</sup> Patients with residual medulloblastoma will therefore undergo re-resection prior to treatment whenever possible. Those patients with residual disease which cannot be completely resected by the start of treatment will also be considered intermediate risk, regardless of histologic subtype. All patients between  $\geq 3$ - $< 5$  years of age diagnosed with non-metastatic medulloblastoma having a gross total resection will be considered as intermediate risk irrespective of histology. Patients with M0 supratentorial PNET and ATRT have a poor prognosis compared to those with desmoplastic medulloblastoma, and will be treated on the intermediate risk arm. Finally, although there is no standard treatment for patients with rare CNS tumors such as choroid plexus carcinoma or the PNET/medulloblastoma variants, these patients have often been treated with therapy similar to that used for medulloblastoma, and complete resection and irradiation have been associated with better outcomes in some series.<sup>37,38</sup> Patients with these diagnoses and no evidence of metastasis will therefore be assigned to the intermediate risk arm. Patients with ependymoma have been included in prior infant brain tumor chemotherapy studies.<sup>14,16,65</sup> Results of these studies have documented that approximately 1/3 of infants with ependymoma can be cured with chemotherapy alone. At the current time we do not feel proton beam RT alone is the best way to treat these patients because anecdotal evidence of recurrent tumors in the anterior 3<sup>rd</sup> ventricle. Hence we will treat all infants up to 3 years of age on the protocol based on their risk stratification.

Patients with metastatic brain tumors have a poor prognosis, and will be treated on the high risk arm of this study. High-risk patients who reach the age of 3 years by

week 17 of treatment will be offered craniospinal irradiation, with the neuraxis dose based on response to induction chemotherapy. Patients whose parents choose not to pursue RT and those younger than 3 will undergo intensive consolidation with topotecan and cyclophosphamide.

## 2.4.2 Rationale for use of high-dose intravenous methotrexate

### 2.4.2.1 Clinical experience with methotrexate in medulloblastoma

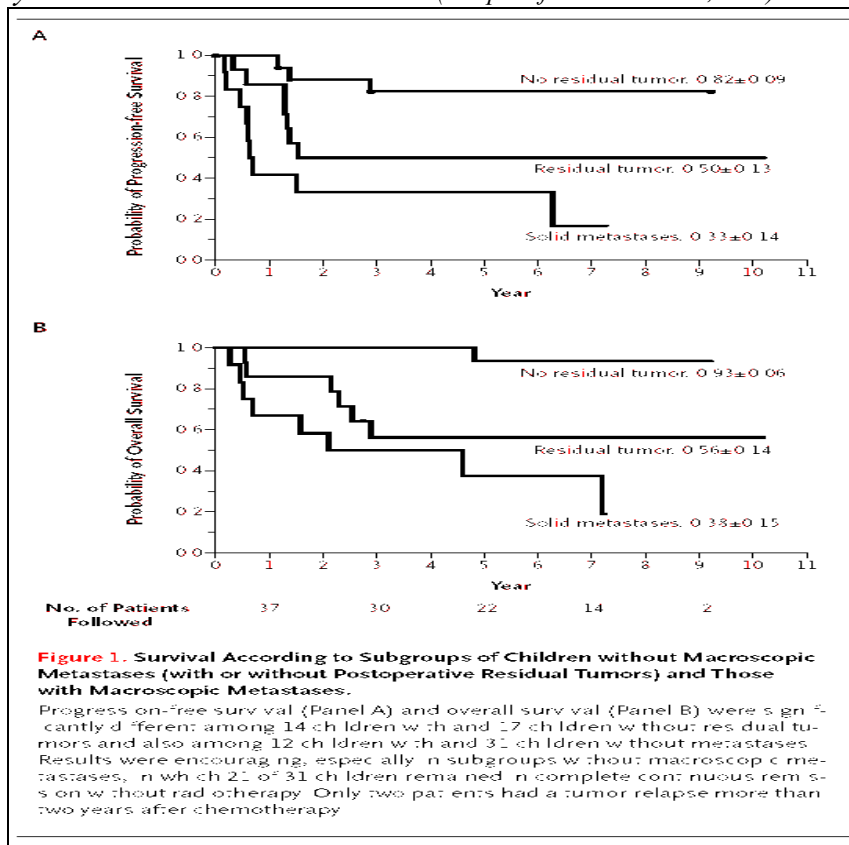
*NOTE: Readers are urged to exercise caution when comparing methotrexate doses between trials. Some published clinical trials have dosed methotrexate by patient weight (doses given in mg/kg), while others have used body surface area (doses given in mg/m<sup>2</sup>). Doses have traditionally been converted via the “rule of 30,” in which 1 mg/kg is approximately equivalent to 30 mg/m<sup>2</sup>. In the literature, BSA doses of methotrexate are typically given in grams per square meter, while weight-based doses are given in milligrams per kg.*

The first clinical experience with methotrexate in medulloblastoma was reported by Rosen, et al. in 1977.<sup>66</sup> Seven patients with recurrent medulloblastoma were treated with 300 mg/kg to 500 mg/kg IV methotrexate, and 5 of these patients had objective responses to 2 or 3 cycles, including one CR. Mooney, et al. subsequently treated 5 patients with recurrent medulloblastoma with 1 to 5 doses of IV methotrexate at 2500 mg/m<sup>2</sup>/dose, with no objective responses.<sup>67</sup> Allen et al. treated two adult patients and one 10 year-old child with newly diagnosed medulloblastoma or pineoblastoma with 8000 to 11,000 mg/m<sup>2</sup> iv methotrexate. One patient had a CR, one a PR, and one stable disease after 2 to 4 cycles.<sup>68</sup> These early series provided the rationale for the use of high-dose methotrexate in the Head Start II trial, in which 400 mg/kg methotrexate was given in conjunction with each of 5 cycles of induction cisplatin/cyclophosphamide/ vincristine/etoposide to patients with metastatic (M1+) medulloblastoma. Although limited by small patient numbers and the inclusion of patients up to 6 years of age, this trial has achieved the best outcome for patients with metastatic disease, with 3-year EFS of 49%. Detailed neurocognitive outcomes have not been reported, but patients who “avoided irradiation retained normal intellectual function and quality of life.”<sup>19</sup> The German HITSKK92 treatment regimen included six doses of intravenous methotrexate at 5000 mg/m<sup>2</sup>/dose, in addition to 36 doses of intraventricular methotrexate at 2 mg/dose. Survival was better than in prior studies across all risk categories; 5-year EFS was 82%, 50%, and 33% for medulloblastoma patients with M0/1 completely resected, M0/1 incompletely resected, and M2+ disease, respectively (Figure 3). Leukoencephalopathy was common, however (19 of 23 evaluated patients), and long-term neurocognitive outcomes were significantly worse for patients treated with this approach than for healthy age-matched controls. A significant correlation between the grade of leukoencephalopathy and the cumulative dose of intraventricular methotrexate was found (correlation coefficient 0.53, p<0.01), but there was no correlation between leukoencephalopathy grade and intravenous methotrexate dose.<sup>18</sup> The Head Start II and HITSKK92 trials resulted in the best



reported survival for young children with medulloblastoma to date, and provide evidence that methotrexate may play an important therapeutic role in this population. These studies were small, however, and many important questions about the use of methotrexate in this population remain unanswered. In order to maximize therapeutic benefit and limit toxicity, it will be important to gain a better understanding of the pharmacokinetics of methotrexate in very young children, and to evaluate the relationships between dose, antitumor effect, and toxicity.

*Figure 3 Survival with Methotrexate-Based Therapy on the German HIT-SKK92 Trial for Children <3 years old with Medulloblastoma (Adapted from Rutkowski, et al)<sup>18</sup>*



#### 2.4.2.2 Clinical experience with methotrexate in other tumors

The published experience with high-dose methotrexate in other CNS tumors in very young children is quite limited. Results for ATRT patients treated on the Head Start II trial were presented in abstract form at the 2004 ISPNO meeting, and suggest that methotrexate has some activity in this disease. On Head Start II, ATRT patients were generally treated with high-dose methotrexate 400 mg/kg/dose x 5 in conjunction with the Head Start chemotherapy regimen described above. Three of 6 ATRT patients had no evidence of disease at 12, 24, and 46 months from diagnosis, including two patients who received methotrexate and one patient who did not. This contrasts with the results from Head Start I, in which none of the 6 ATRT patients received methotrexate; they were otherwise treated identically to

those on Head Start II, and none of the 6 patients in the Head Start I trial survived.<sup>69</sup> The Head Start regimen was generally not very effective for the treatment of ependymoma, but results for patients with metastatic disease suggest that methotrexate may have some activity. Five patients with metastatic ependymoma received the methotrexate-containing Head Start II regimen, with one toxic death during induction, as well as one disease progression and three complete responses detected at the end of induction. The patient with progressive disease was effectively treated with CSI, so that all 4 patients who completed induction have survived, with follow-up durations of 2.1 to 7.8 years; survivors include one patient who was 2.4 years old at diagnosis and has not received RT.<sup>65</sup> The German cooperative group reported results for patients with supratentorial PNET treated on HITSKK87 and HITSKK92. Both regimens contained intravenous methotrexate at 5 g/m<sup>2</sup>/dose, and results were similar to those from contemporary trials without methotrexate, with a 3-year PFS of 14.9%.<sup>22</sup>

High-dose methotrexate (8g/m<sup>2</sup>) given in combination with cyclophosphamide and vincristine resulted in an objective response rate of 47% in a series of children with high-grade glioma.<sup>70</sup>

#### *2.4.2.3 Rationale for methotrexate dosing*

The current trial, as well as planned future medulloblastoma trials in the Children's Oncology Group, will utilize only intravenous methotrexate. Administration of intraventricular methotrexate in this patient population is problematic, as many patients (~40% of patients on PBTC-001) have hydrocephalus requiring placement of a ventriculoperitoneal shunt. This prevents the effective administration of intraventricular chemotherapy, unless the shunt can be temporarily turned off. While placement of a shunt with an on/off valve is technically possible, life-threatening elevation in intracranial pressure can result if the shunt is turned off inadvertently or for too long. Additionally, there is some evidence to suggest that the risk of neurocognitive dysfunction associated with combined intravenous and intrathecal methotrexate administration is greater than that associated with high-dose intravenous administration alone.<sup>71,72</sup> As discussed above, the severity of leukoencephalopathy on the HITSKK92 trial was significantly correlated with cumulative intraventricular, but not intravenous, methotrexate dose.<sup>18</sup>

A standard intravenous methotrexate dosing regimen for medulloblastoma in young children has not been established. Given the potential for neurotoxicity, particularly in patients who go on to receive RT, it is important to avoid using more of this agent than necessary, while still giving enough to achieve a therapeutic benefit. Although clinical evidence of therapeutic efficacy has been documented at methotrexate doses of 400 mg/kg (~12,000 mg/m<sup>2</sup>, on Head Start II) and 5,000 mg/m<sup>2</sup> (~167mg/kg, given in addition to intraventricular methotrexate on HITSKK92), no prospective comparison of different methotrexate doses has been conducted, and substantial differences between the patient populations studied in Head Start II and HITSKK92 make it difficult to compare their results directly.

Comparison of these results is also complicated by the use of different methotrexate infusion lengths. The Head Start II regimen utilized a 4 hour infusion, as is the common practice in the treatment of osteosarcoma.<sup>19</sup> In HITSKK92, each methotrexate dose was infused over 24 hours, as is typically done in the treatment of childhood ALL. In ALL, HDMTX is an effective component of CNS-directed therapy, and it therefore seems reasonable to use the same administration approach for brain tumor patients. The relationship between plasma and CSF methotrexate levels has been studied extensively for a variety of ALL treatment regimens, and investigators have shown that methotrexate penetration into the CSF is slow,<sup>73</sup> requiring up to 8 hours to reach a steady state concentration.<sup>74</sup> A prolonged infusion may therefore result in better methotrexate delivery to the CNS. Hence, for the current protocol, methotrexate will be administered as a 24 hour infusion.

While ALL studies have demonstrated that 24-hour methotrexate infusions are well tolerated in very young children at doses as high as 33,600 mg/m<sup>2</sup>,<sup>75,76</sup> investigators have shown that increasing the dose beyond 8.0 g/m<sup>2</sup> does not result in a proportional increase in CSF levels.<sup>77</sup> These and other results<sup>78</sup> suggest that there is a saturable carrier system for methotrexate and its metabolite 7-OH methotrexate between serum and CSF. If this is the case, increasing the dose of methotrexate beyond the doses that are typically used clinically may provide little additional therapeutic benefit. Indeed, ALL patients treated with very high dose methotrexate (33.6 g/m<sup>2</sup>) on the CCG trials had a higher than expected rate of CNS relapse, although these results may be at least partially explained by excessive leucovorin rescue and decreased intensity CNS therapy in other components of the protocol.<sup>79</sup> As a methotrexate dose of 5 g/m<sup>2</sup> over 24 hours was administered safely and effectively in the German HITSKK92 infant medulloblastoma trial,<sup>18</sup> and there is limited rationale for exceeding this dose, the 5 g/m<sup>2</sup> dose will be used for all patients in the current trial.

#### *2.4.2.4 Rationale for reduced MTX dosage for patients enrolled at ≤ 31 days of age*

Currently, the methotrexate dosage for all patients enrolled on SJYC07 is 5 g/m<sup>2</sup> administered over 24 hours. Recently we have studied two children that were less than one month of age when they received methotrexate therapy. Since no data were available regarding methotrexate disposition in children this young, we empirically reduced their dosage to 2.5 g/m<sup>2</sup> over 24 hours. This was primarily because patients ≤ 1 month of age have reduced renal function, and in particular a reduced glomerular filtration rate. Both infants tolerated therapy well with methotrexate concentrations similar to what we have observed in our other patients treated at full dosage and without any significant toxicities.

Since no data are published regarding methotrexate clearance in these very young infants, we contacted Dr. Stefan Rutkowski, the lead author of the study upon which the use of methotrexate is based for his guidance in dosing methotrexate in very young infants. Dr. Rutkowski indicated that they have not used methotrexate in children with brain tumors that young, so he was unable to provide any insights. So the only experience we have is as noted above, two infants enrolled on SJYC07

have been  $\leq 1$  month of age when starting induction therapy. Their methotrexate clearances were 13.2 and 54.8 L/h/m<sup>2</sup>, compared to a mean (SD) of 96.9 (32) L/h/m<sup>2</sup> for patients older than 1 month.

Although these patients will be older than 1 month by the start of the second course of methotrexate, we propose to keep the methotrexate dosage at 2.5 g/m<sup>2</sup> in subsequent courses. Interestingly, in a preliminary analysis of all children enrolled on SJYC07 we noted a decrease in the MTX clearance between course 1 (median = 101.6 L/h/m<sup>2</sup>) and course 2 (median = 86.3 L/h/m<sup>2</sup>). We hypothesize that the reason for the drop in clearance after course 1 is the administration of cisplatin, which is nephrotoxic. Although clearance did not drop further in subsequent courses, we have very little experience in administering MTX to patients this young. We therefore will adopt a conservative approach and administer the reduced methotrexate dosage (2.5 g/m<sup>2</sup> over 24 hours) for all 4 courses.

#### 2.4.2.5 Rationale for leucovorin rescue

**Table 5 Comparison of Leucovorin Rescue on Current Trial and Other Recent HDMTX Trials**

<i>Trial</i>	<i>MTX Dose</i>	<i>Infusion (hours)</i>	<i>LV dose</i>	<i>First LV</i>	<i>Last LV</i>	<i>Cumulative LV Dose*</i>
SJYC07	5 g/m <sup>2</sup>	24	15 mg/m <sup>2</sup> q6h	Hour 42	Hour 66	45 to 75 mg/m <sup>2</sup> †
Proposed COG High-risk Infant MB	400 mg/kg	4	10 mg (fixed dose) q6h	Hour 24	MTX level <0.1µM	50 mg (fixed dose)
CCG-191P, -134P, 144P (Infant ALL)	33.6 g/m <sup>2</sup>	24	200 mg/m <sup>2</sup> bolus, then 12 mg/m <sup>2</sup> q 3h x 6, then 12 mg/m <sup>2</sup> q 6h	Hour 36	MTX level < 0.08 µM	~300 mg/m <sup>2</sup>
P9754 (Osteosarcoma)	12 g/m <sup>2</sup>	4	15 mg/m <sup>2</sup> q6h	Hour 24	MTX level <0.1 µM	75 to 135 mg/m <sup>2</sup>
AOST0331 (Osteosarcoma)	12 g/m <sup>2</sup>	4	15 mg/m <sup>2</sup> q6h	Hour 24	MTX level <0.1 µM	75 to 135 mg/m <sup>2</sup>
HITSKK'92	5 g/m <sup>2</sup>	24	15 mg/m <sup>2</sup> q6h	Hour 42	MTX level <0.25 µM	90 mg/m <sup>2</sup>
Interfant99 (Infant ALL)	5 g/m <sup>2</sup> **	24	15 mg/m <sup>2</sup> q6h	Hour 36	Hour 48	45 mg/m <sup>2</sup>
IFNT06 (Infant ALL)	5 g/m <sup>2</sup> **	24	15 mg/m <sup>2</sup> q6h	Hour 42	Hour 54	45 mg/m <sup>2</sup>
POG 9917 (NHL)	5 g/m <sup>2</sup>	24	75 mg/m <sup>2</sup> initial, then 15 mg/m <sup>2</sup> q6h	Hour 36	MTX level <0.1 µM	105 to 165 mg/m <sup>2</sup>
POG 9404 (T-cell ALL)	5 g/m <sup>2</sup>	24	75 mg/m <sup>2</sup> initial, then 15 mg/m <sup>2</sup> q6h	Hour 36	MTX level <0.1 µM	105 to 165 mg/m <sup>2</sup>
NHL-BFM 95 (NHL)	5 g/m <sup>2</sup>	4 vs. 24	30 mg/m <sup>2</sup> initial, then 15 mg/m <sup>2</sup> q6h x 2	Hour 42	Hour 54	60 mg/m <sup>2</sup>
TOTAL XV (ALL)	5 g/m <sup>2</sup>	24	15 mg/m <sup>2</sup> q6h	Hour 42	MTX level <0.1 µM	45 to 75 mg/m <sup>2</sup>

LV, leucovorin; \*Assumes normal clearance, leucovorin continued until hour 48 to 72 without dose modifications;  
\*\* doses reduced for children < 12 months of age;

As experience with the use of intravenous HDMTX in very young children with medulloblastoma is limited, there is no standard regimen for leucovorin rescue in this setting. Similar doses of HDMTX have been used extensively in other pediatric malignancies, however, including infant ALL. For comparison, leucovorin regimens from several recent clinical trials are presented in Table 5. The leucovorin regimen for the current protocol is the same as that used at St. Jude Children's Research Hospital for patients with ALL receiving an identical dose and infusion length.

#### 2.4.3 Rationale for conventional chemotherapy

In order to allow consistent dose calculations within the trial, and facilitate comparisons with other studies, all drug doses will be based on body surface area (BSA). While many past trials have utilized weight-based dosing or a combination of weight and BSA-based dosing for very young children, BSA-based dosing has been utilized safely and effectively in some recent trials, including the trial with the best outcome for medulloblastoma in this population to date, the German HITSKK92 study.<sup>18,81</sup> BSA-based dosing has also been utilized safely and effectively in infant leukemia trials.<sup>75</sup> The optimal conversion between weight and BSA-based doses is not well established for any drug, and likely varies by a number of drug and patient factors. In selecting the doses for this treatment plan, we have relied on published BSA doses from trials including patients < 3 years old whenever possible. When no such doses were available, we selected BSA-based doses from trials involving older children. In those cases, we attempted to choose doses that would result in similar weight-based doses (as calculated by the “rule of 30”) to those used in other infant brain tumor trials.

Cisplatin, cyclophosphamide, and vincristine have been included in almost all successful chemotherapeutic regimens for infants with embryonal tumors.<sup>14-16,18,19</sup> These drugs will form the backbone of chemotherapy for all patients. The Baby POG-2 trial randomized patients to receive chemotherapy regimen A, which included 1 dose of cyclophosphamide per cycle (65 mg/kg/dose, ~2 g/m<sup>2</sup>/dose), or regimen B, which included 2 doses of cyclophosphamide per cycle (65mg/kg/dose), and revealed no difference in overall survival between the two groups. Our regimen will therefore only include a single 1.5 g/m<sup>2</sup> dose of cyclophosphamide per cycle. Similarly, Baby POG-2 showed no advantage to increasing the cisplatin dose from 4 mg/kg to 5 mg/kg, and subsequent studies<sup>16,19</sup> have achieved reasonable outcomes using a dose of 3.5 mg/kg. A cisplatin dose of 75 mg/m<sup>2</sup> will consequently be used in this study. This dose has been used safely and successfully in two recent medulloblastoma trials.<sup>2,4</sup> Because of the potential for nephrotoxicity and ototoxicity with cisplatin, some groups have replaced cisplatin with carboplatin, with mixed results.<sup>17,18</sup> In the current trial, all patients will receive four induction cycles of high-dose methotrexate, vincristine, cisplatin, and cyclophosphamide. In low-risk patients this will be followed by consolidation consisting of two cycles of carboplatin, cyclophosphamide and etoposide. The low-risk consolidation doses are

very similar to those administered on the St. Jude CNS-14 trial. This combination was well tolerated, and has been used extensively for very young brain tumor patients receiving non-protocol treatment at St. Jude in recent years.

Etoposide has been included in all of the large prospective chemotherapy trials for very young children with medulloblastoma, but the use of this agent in other solid tumors has been associated with the occurrence of second malignancies,<sup>82</sup> and it is therefore important that this drug be used judiciously, especially in the low-risk group of patients with a high likelihood of long-term survival. Furthermore, recent data suggest that inclusion of this agent may not be absolutely necessary for treatment of these diseases. For example, the recent St. Jude (SJMB96) and COG A9961 trials for older medulloblastoma patients treated with craniospinal irradiation and adjuvant chemotherapy resulted in excellent survival rates, and included no etoposide.<sup>2,4</sup> The total dose of etoposide administered on SJYC07 will therefore be quite limited in comparison to prior trials. Patients treated on the most recent COG low-risk infant medulloblastoma protocol (P9934) received a total of 285.6 mg/kg (~8568 mg/m<sup>2</sup>) of oral etoposide, while patients treated on the most recent PBTC infant brain tumor protocol (PBTC-001) received 142.8 mg/kg (~4284 mg/m<sup>2</sup>) of oral etoposide. Patients treated on the recently opened COG high-risk infant medulloblastoma protocol (ACNS0334) will receive a total of 22.5 mg/kg (~675 mg/m<sup>2</sup>) of intravenous etoposide. On SJYC07, by contrast, low-risk patients will receive 400 mg/m<sup>2</sup> (~13.3 mg/kg) of intravenous etoposide during consolidation, and no etoposide during maintenance. Intermediate and high-risk patients with medulloblastoma or ependymoma will receive no etoposide at any time, while intermediate and high-risk patients with other diagnoses will receive no etoposide during consolidation, and a total of 3150 mg/m<sup>2</sup> (~ 105 mg/kg) of oral etoposide during maintenance.

#### 2.4.4 Rationale for use of G-CSF in Induction and Consolidation

Because the drugs used in this treatment plan are expected to cause substantial myelosuppression, G-CSF support will be administered during all induction and consolidation chemotherapy courses. Numerous clinical trials and meta-analyses have now demonstrated that the prophylactic use of G-CSF decreases the duration of neutropenia, incidence of febrile neutropenia, length of hospitalization, and length of antibiotic use, and some, but not all, trials have demonstrated a decrease in documented infections and infection-related mortality.<sup>83-85</sup> The American Society of Clinical Oncology (ASCO) clinical practice guidelines have recently been revised to recommend that prophylactic G-CSF be used in all treatment regimens with an expected incidence of febrile neutropenia of > 20%.<sup>86</sup> During the recent PBTC-001 trial, which used similar drug doses to those used in this trial, patients experienced febrile neutropenia in 29-44% of courses (PBTC-001 progress report, Spring 2006).

#### 2.4.5 Rationale for Consolidation with pharmacokinetically-targeted topotecan and cyclophosphamide in high-risk patients

Topotecan is a topoisomerase I inhibitor with extensive antitumor activity in preclinical studies, including xenograft models of pediatric CNS tumors such as medulloblastoma.<sup>87-89</sup> The antitumor activity of topotecan has been found to be highly dose and schedule dependent. Clinical trials have also demonstrated a high degree of inter- and inpatient variability in topotecan pharmacokinetics.<sup>90</sup> Topotecan has substantial CSF penetration, which is augmented by prolonged infusion. Zamboni, et al. demonstrated that increasing the length of topotecan infusion from 30 minutes to 4 hours results in higher CSF topotecan lactone levels.<sup>91,92</sup>

The recent SJMB96 trial for patients 3 years of age or older with medulloblastoma and other embryonal tumors included an upfront phase II window for high-risk patients consisting of topotecan targeted to an AUC of 120 to 160 ng/mL\*hr, daily for 5 doses in each of two 3-week cycles. Of 36 assessable patients, four patients (11.1%) had a complete response, six (16.6%) showed a partial response, four (11.1%) had a marginal response, and disease was stable in 17 patients (47.2%). Toxicity was mostly hematological, with only one patient experiencing treatment delay. Cyclophosphamide is also highly active against medulloblastoma and other embryonal tumors,<sup>14,93</sup> and the combination of cyclophosphamide and topotecan has been effective in the treatment of metastatic Ewing's sarcoma and other pediatric solid tumors.<sup>94,95</sup> In this protocol, the combination of topotecan and cyclophosphamide will be administered in a consolidation phase for high risk patients who do not receive craniospinal irradiation. Two cycles of this combination will be given. Topotecan will be administered as a 4 hour infusion on days 1-5 of each cycle, with the topotecan dose targeted to achieve an AUC of 140±20 ng/mL\*hr. Cyclophosphamide will be administered on day 4 and 5 at a dose of 600 mg/m<sup>2</sup>/day. Based on the SJMB96 experience with single agent topotecan, count recovery is expected within 21-28 days with this regimen.

#### 2.4.6 Rationale for anti-angiogenic therapy

##### 2.4.6.1 Overview

In the early 1970's, Judah Folkman observed that tumors must have an adequate blood supply in order to grow beyond minimal size, and proposed that targeting the tumor blood supply would provide an alternative means of limiting tumor growth.<sup>96</sup> In the subsequent decades, considerable research effort has been devoted to exploring the mechanisms by which tumors induce angiogenesis, and identifying aspects of tumor angiogenesis which might be targeted for therapeutic benefit.<sup>12,97-100</sup> It is now clear that tumor angiogenesis is a complex process, involving multiple interactions between tumor and endothelial cells, intra- and intercellular signals, and the extracellular matrix.<sup>101,102</sup> There remains considerable interest in the

vasculature as a therapeutic target but the development of effective anti-angiogenic treatments remains in its infancy.<sup>97,98,100,103</sup>

#### 2.4.6.2 Tumor angiogenesis

The underlying mechanisms of tumor angiogenesis have been explored extensively during the past three decades. One component of Folkman's hypothesis which has drawn particular attention is the concept of the angiogenic switch.<sup>96</sup> The development of solid tumors was thought to include an avascular dormant stage, in which tumors remained less than 1-2mm in size, and received adequate oxygen and nutrients through diffusion from adjacent normal vasculature. Tumors could exist in this dormant phase for prolonged periods of time, and evidence for this concept came from the frequent identification of small, non-lethal tumor nodules in autopsy studies.<sup>104</sup> It was only when a tumor "switched on" the angiogenic phenotype that the tumor could induce the development of its own vasculature. Tumors that made the transition to the angiogenic phenotype gained the potential to grow much larger, metastasize, and become lethal.<sup>103,105</sup> This hypothesis required the presence of a soluble factor which allowed the tumor to initiate angiogenesis, and the identification of the first such factor, basic fibroblast growth factor (bFGF)<sup>106</sup> in 1984 helped Folkman's ideas gain wider acceptance.<sup>103</sup> Other pro-angiogenic factors such as vascular endothelial growth factor (VEGF) were soon isolated, and the list of angiogenic activators continues to grow. Eventually a number of endogenous anti-angiogenic factors including thrombospondin-1, endostatin, and angiostatin were also identified. Current models of tumor angiogenesis suggest that the angiogenic phenotype may be turned on or off at various points in the life of a tumor, depending on the tumor type and microenvironment,<sup>101,107,108</sup> and may be induced by changes in gene expression, rather than progressive genetic derangements.<sup>109</sup> The switch to the angiogenic phenotype is thought to be controlled by the balance of activators and inhibitors in a particular tumor at any given time, and is mediated by interactions among tumor and endothelial cells, pericytes, and the extracellular matrix.<sup>102</sup> While a full understanding of the mechanisms and regulation of tumor angiogenesis will take many more years of study, the basic research insights gained to date have led to substantial progress in the development of anti-angiogenic treatments.<sup>12,97-100</sup>

Tumor angiogenesis is an attractive therapeutic target for several reasons. From a theoretical standpoint, dependence on adequate blood supply for progressive growth is a hallmark of cancer,<sup>110</sup> so effective anti-angiogenic therapies can potentially be utilized for a wide variety of tumor types. Also, because many tumors must trigger angiogenesis to grow beyond minimal size or metastasize, anti-angiogenic therapy administered to patients with limited tumor burden could prevent this switch, and maintain the tumor in a chronic dormant state.<sup>111-114</sup> Furthermore, intriguing new research suggests that anti-angiogenic therapy may target tumor stem cells. Like stem cells in normal tissues, cancer stem cells (CSCs) have the capacity to divide and differentiate into more mature tumor cells. Traditional cancer therapies may fail to effectively kill cancer stem cells. A small number of residual CSCs may thus



allow tumor repopulation, and play a role in resistance to therapy and relapse.<sup>62,109,115</sup> The CSC represents an attractive therapeutic target, but no therapies have yet been developed which specifically target these cells. Recent work from the laboratory of Richard Gilbertson suggests that brain tumor stem cells exist in a vascular niche, and that anti-angiogenic therapy may target the CSC population by disrupting this niche. In this series of experiments CSCs were identified in brain tumor cell lines and primary tumors by the expression of Nestin and CD133. Nestin<sup>+</sup> cells were found in close proximity to tumor vasculature, and CD133<sup>+</sup> cells isolated from primary medulloblastoma and ependymoma specimens interacted physically with endothelial cells in culture. Endothelial cells maintained self-renewing and undifferentiated brain tumor cells in culture via secreted factors, and promoted the propagation of brain tumor cells in an orthotopic mouse xenograft model. Perhaps most interestingly, when orthotopic xenografts of ERBB2-transfected Daoy cells were utilized as a model of aggressive medulloblastoma, antiangiogenic therapy with the anti-VEGF antibody bevacizumab or the EGFR/ERRB2 antagonist erlotinib depleted tumor vasculature, ablated the self-renewing tumor cell population, and inhibited tumor growth.<sup>116</sup> In a similar series of studies from Duke University, Bao et. al demonstrated that a glioma stem cell population (stem cell-like glioma cells, or SCLGCs) secretes VEGF and supports tumor angiogenesis, and that this effect is abrogated by bevacizumab. They also demonstrated that bevacizumab therapy limited growth of tumors derived from SCLGCs in a xenograft model.<sup>117</sup> Most recently, investigators in the Kerbel lab demonstrated that the combination of cytotoxic chemotherapy and anti-angiogenic therapy inhibited tumor growth and reduced the tumor stem-cell-like fraction in a nude mouse glioma xenograft model.<sup>118</sup> Taken together, these results suggest that tumor stem cells and tumor endothelial cells are interdependent, and that anti-angiogenic therapy may target both populations.

Anti-angiogenic therapy is also attractive on a practical basis. As endothelial cells are far more genetically stable than tumor cells, it was initially believed that the development of resistance to anti-angiogenic therapy was unlikely.<sup>119</sup> As clinical experience accumulates, it is becoming apparent that resistance based on acquired mutations in endothelial cells is indeed rare, but that tumors can develop resistance to anti-angiogenic therapy by other mechanisms, including the use of alternative angiogenic signaling pathways. Such resistance may, however, be surmountable through the use of combinatorial anti-angiogenic approaches.<sup>12</sup> Another practical advantage of anti-angiogenic therapy is that these treatments have a different pattern of toxicity than standard cytotoxic regimens, and can thus be used in conjunction with standard therapy, or in patients who have been heavily pretreated with cytotoxic drugs.<sup>12</sup> Finally, as will be discussed in detail below, many existing chemotherapeutic agents have been found to have anti-angiogenic activity.<sup>99,120</sup> Some anti-angiogenic strategies can therefore be developed based on alternative scheduling of agents that are readily available and have well-established pharmacologic characteristics and toxicities.

The complexity of tumor angiogenesis allows for a wide variety of potential therapeutic strategies. One general approach is to target receptors or signaling molecules that are specific to an angiogenic pathway. The most successful of these to date has been bevacizumab (Avastin®), a humanized monoclonal antibody to VEGF.<sup>12</sup> As monotherapy, bevacizumab has been efficacious only in metastatic renal cell carcinoma, where it improved progression free, but not overall survival.<sup>121</sup> It has, however, been considerably more effective when used in combination with cytotoxic chemotherapy. The combination of bevacizumab and standard chemotherapy resulted in improved progression free and overall survival in colorectal cancer patients in two large Phase III trials,<sup>122,123</sup> and the drug has consequently been approved by the FDA for combination first-line therapy in colorectal cancer. The combination of bevacizumab and chemotherapy has also been effective in non-small cell lung cancer, metastatic breast cancer,<sup>123</sup> and recurrent high-grade glioma,<sup>124</sup> and is being evaluated in ongoing clinical trials in many other malignancies, including recurrent or refractory pediatric high-grade glioma and diffuse pontine glioma in the PBTC 22 trial. Bevacizumab has generally been well tolerated, with common toxicities consisting primarily of mild proteinuria and hypertension. It has, however, also been associated with rare but severe adverse events including hemorrhage, thrombosis, gastrointestinal perforation and reversible posterior leukoencephalopathy syndrome (RPLS).<sup>122,123,125</sup> While the efficacy of bevacizumab provides substantial support for the concept of anti-angiogenic therapy, we have chosen not to incorporate bevacizumab into this frontline treatment protocol because of the lack of experience with this agent in very young children and the potential for severe toxicity. Other novel agents include receptor tyrosine kinase inhibitors designed to interfere with signaling pathways involved in tumor angiogenesis. A large number of agents in this category are under development, and two of these, sunitinib and sorafenib, have been approved by the FDA for monotherapy of renal cell carcinoma.<sup>126</sup> These anti-angiogenic tyrosine kinase inhibitors offer considerable promise for future applications in pediatric oncology. As with bevacizumab, however, none of these agents have been studied in young children. Furthermore, most of these drugs are only available in oral formulations (e.g., unbreakable tablets or capsules) that cannot be administered to infants. The oral tyrosine kinase inhibitor erlotinib was designed to diminish tumor cell proliferation mediated by abnormal EGFR and ERBB2 signaling,<sup>127</sup> but has also been found to down-regulate VEGF expression *in vivo* and *in vitro* in cells overexpressing ERBB2.<sup>116</sup> As discussed in detail in section 2.4.6, erlotinib has been well tolerated in two pediatric phase I studies, and is commercially available in a tablet formulation which can be crushed for administration to young children. Erlotinib will be administered to all medulloblastoma, high-grade glioma and ependymoma patients in the maintenance phase of this protocol. Although this agent was included in the treatment regimen primarily because of its potential anti-proliferative activity in ERBB2 overexpressing tumors, it may also have anti-angiogenic activity.<sup>116</sup>

### 2.4.6.3 Low-dose (metronomic) chemotherapy

The most feasible strategy for targeting tumor angiogenesis in very young children at the present time is to use standard chemotherapeutic agents at low doses with frequent administration. A growing body of preclinical research has demonstrated that this “metronomic” dosing strategy inhibits tumor angiogenesis.<sup>99,120,128,129</sup> Browder, et al. first showed that cyclophosphamide given at both the standard maximum tolerated dose (MTD) and in frequent low doses induced endothelial and tumor cell apoptosis in an experimental model of drug resistant cancer.<sup>130</sup> The continuous low dose regimen, however, produced a more prolonged anti-angiogenic effect, and suppressed tumor growth more effectively. Subsequent preclinical and early clinical studies have demonstrated the activity of metronomic chemotherapy against a variety of adult and pediatric tumor types (a partial list is provided in Table 6).

<b>TABLE 6 – RESULTS OF CLINICAL TRIALS INVOLVING METRONOMIC CHEMOTHERAPY IN RELAPSED/ REFRACTORY CANCER</b>				
<i>Adult Trials</i>				
<i>Disease</i>	<i>Chemotherapy</i>	<i>n</i>	<i>Results</i>	<i>Ref.</i>
Metastatic Breast Cancer	Cyclophosphamide and Methotrexate	64	2 CR, 10 PR	131
Non Small-Cell Lung Cancer	Etoposide	17	1 PR, 6 SD (8-32 wks)	132
Non-Hodgkin’s Lymphoma	Cyclophosphamide and Celecoxib	35	2 CR, 9 PR Median PFS 4.7 months	133
Melanoma	Treosulfan and Rofecoxib	12	1 PR, 4 SD (24-36 wks)	134
Prostate Cancer	Cyclophosphamide and Dexamethasone	17	Reduced PSA in 74% of patients	135
<i>Pediatric Trials</i>				
<i>Disease</i>	<i>Chemotherapy</i>	<i>n</i>	<i>Results</i>	<i>Ref.</i>
Brain Tumors	Temozolomide dose escalation x 42 days on, 14 days off	28	MTD 85 mg/m <sup>2</sup> 2CR, 2 PR	136
Solid Tumors (Phase I)	Cyclophosphamide 50 mg/m <sup>2</sup> /d and Topotecan 0.8mg/m <sup>2</sup> /d	17	Recommended duration 14 days; 1 PR, 1 SD	137
Solid Tumors (Pilot/PK)	Celecoxib with either Cyclophosphamide 30 mg/m <sup>2</sup> /d or Vinblastine 1 mg/m <sup>2</sup> 3x/wk	33	Anti-angiogenic levels of cyclophosphamide and vinblastine attained, 4 SD >28 wks	138
Solid Tumors (Feasibility)	Celecoxib, Thalidomide, Cyclophosphamide 2.5 mg/kg/d alternating cycles with Etoposide 50 mg/m <sup>2</sup> /d	20	8 patients completed 6 months therapy, 4 SD >123 wks	139
PK – Pharmacokinetics; CR – complete response; PR – Partial Response; SD – Stable Disease; PFS – Progression Free Survival; PSA – Prostate Specific Antigen				

The anti-angiogenic effect often occurs at drug concentrations substantially lower than those achieved by standard tumor cytotoxic doses. Administering drugs in this manner can therefore result in minimal toxicity to normal tissues, and may allow the use of metronomic chemotherapy in conjunction with standard MTD therapy or other anti-angiogenic agents.<sup>140,141</sup> Because metronomic chemotherapy can prevent

repopulation of endothelial cells damaged by MTD treatment, it may actually be most effective when used in combination with MTD chemotherapy, and indeed some preclinical evidence suggests that this is the case. In studies utilizing the RIP1-Tag2 transgenic mouse model of pancreatic cancer, Pietras and Hanahan<sup>142</sup> demonstrated that a “chemo-switch” strategy (MTD followed by metronomic cyclophosphamide) produced more prolonged tumor growth suppression than MTD cyclophosphamide alone. Interestingly, the combination of chemo-switch cyclophosphamide with SU11248, a tyrosine kinase inhibitor with activity against PDGFR, VEGFR-1 and -2, c-Kit, FGFR-1, and EGFR, resulted in complete responses and unprecedented survival for that model. The chemo-switch strategy of intensive cytotoxic treatment followed by prolonged administration of low dose chemotherapy is also strikingly similar to the treatment approach utilized in pediatric acute lymphoblastic leukemia, one of the pediatric cancers with the best rates of long-term survival.<sup>99</sup> Furthermore, as discussed above, recent evidence from glioma xenograft studies in the Kerbel laboratory suggests that a reduction in the tumor stem-cell-like fraction may be achieved through the combined use of cytotoxic and anti-angiogenic treatments, including low dose metronomic cyclophosphamide.<sup>118</sup> The current protocol will utilize metronomic chemotherapy in three components of the treatment plan: 1) For patients with high-risk (metastatic) disease, low-dose vinblastine will be administered between cycles of MTD chemotherapy. 2) Low-dose cyclophosphamide and topotecan will be used in the maintenance phase for all patients. 3) For patients with diagnoses other than medulloblastoma, high-grade glioma and ependymoma, low-dose oral etoposide will be used in place of erlotinib in the maintenance phase.

#### *2.4.6.4 Rationale for use of vinblastine as an anti-angiogenic agent*

Vinblastine was one of the first standard chemotherapeutic agents to have recognized anti-angiogenic activity. Vacca, et al. showed that picomolar concentrations of vinblastine inhibited proliferation, chemotaxis, and adhesion to fibronectin of cultured human endothelial cells. The drug was not cytotoxic to endothelial cells at these concentrations, and did not inhibit proliferation of cultured tumor cells. The same vinblastine concentrations inhibited angiogenesis reversibly *in vivo* in the chick embryo chorioallantoic membrane model.<sup>128</sup> Klement, et al. subsequently confirmed that low dose vinblastine inhibited endothelial cell proliferation in culture, and showed that it inhibited angiogenesis *in vivo* in a subcutaneous matrigel plug assay. They also demonstrated that low-dose vinblastine diminished the growth rate, perfusion, and vascularity of tumors in a CB-17 SCID mouse xenograft model of neuroblastoma, and caused dramatic tumor regression in the same assay when used in combination with an anti-VEGF receptor-2 antibody.<sup>143</sup>

In the clinical setting, weekly low-dose vinblastine was reported to induce a long-lasting remission in 8 of 13 patients with relapsed childhood anaplastic large cell lymphoma.<sup>144</sup> This preclinical and retrospective clinical data prompted two prospective pediatric clinical trials featuring the use of low-dose vinblastine as an

anti-angiogenic agent. In the recently published pilot study by Stempak, et al.,<sup>138</sup> children with recurrent or refractory solid tumors were treated with celecoxib in combination with either intravenous vinblastine 1 mg/m<sup>2</sup> 3x/week (n=17) or metronomic oral cyclophosphamide 30 mg/m<sup>2</sup> daily (n=16). Both regimens were well tolerated. In these heavily pretreated patients, there were only 3 episodes of grade III or IV neutropenia lasting <5 days (2 in the vinblastine arm and 1 in the cyclophosphamide arm). Pharmacokinetic analysis demonstrated that these dosing regimens achieved serum concentrations consistent with those shown to have antiangiogenic effects in preclinical studies. Although clinical outcome was not a primary goal of the study, approximately 20% of patients had stable disease lasting 3 to 6 months, and extended periods of stable disease (7-18 months) were noted in 4 patients. In the ongoing Children's Oncology Group trial AEWS02P1, patients with metastatic Ewing's sarcoma receive celecoxib and intravenous vinblastine 1mg/m<sup>2</sup> 3x/week continuously throughout a treatment regimen consisting of standard MTD chemotherapy (vincristine/doxorubicin/ cyclophosphamide alternating with ifosfamide/etoposide) and focal RT. This regimen has been similarly well tolerated, with rates of myelosuppression comparable to prior Ewing's studies, and no novel toxicities (Children's Oncology Group Fall 2006 Meeting AEWS02P1 Progress Report and personal communication with study principal investigator Judy Felgenhauer, MD). Since these trials began, celecoxib has been associated with an increased risk of fatal cardiovascular events in adults, although no such toxicity has been documented in children. Due to concerns about the continued availability of the drug, the difficulty of administering the available capsule formulation to young children, and the potential for nephrotoxicity when used in conjunction with other nephrotoxic agents in our protocol, we have chosen not to include celecoxib in this treatment regimen.

In summary, low-dose vinblastine has anti-angiogenic effects in preclinical models at non-cytotoxic levels. Administration of the dose to be used in this protocol (1mg/m<sup>2</sup> 3x/week) has been shown to achieve serum levels consistent with anti-angiogenic activity in children with recurrent/refractory solid tumors. This dosing regimen caused minimal myelosuppression when used in combination with celecoxib, and no apparent additive myelotoxicity when used with an intensive MTD chemotherapy/radiation regimen in children with Ewing's sarcoma. Because vinblastine is administered intravenously, it will be an ideal agent for use in infants and young children, who are typically unable to swallow capsules/tablets, and may suffer significant nausea from the other agents used in this protocol. As most MTD chemotherapy has an anti-angiogenic effect of limited duration,<sup>120,130,145</sup> vinblastine will be administered only during the breaks between MTD cycles, rather than simultaneously with MTD chemotherapy, as is being done in the AEWS02P1 protocol. This strategy should also limit the potential for undesirable pharmacokinetic interactions between vinblastine and standard chemotherapeutic agents.

#### *2.4.6.5 Rationale for maintenance therapy with low dose cyclophosphamide and topotecan*

Lower intensity, prolonged duration chemotherapy is an integral component of the successful management of childhood acute lymphoblastic leukemia.<sup>99</sup> The role of maintenance therapy in other tumors is not well established, but several prior infant medulloblastoma protocols have incorporated a maintenance phase. In the era of delayed radiation studies, chemotherapy was continued for up to two years before patients were irradiated.<sup>14,16</sup> Subsequent trials have utilized various strategies, including a shorter maintenance phase (P9934), continued moderate intensity therapy for 6-12 months (HIT-SKK92 and BBSFOP),<sup>17,18</sup> or termination of therapy after an induction phase followed by consolidation with high dose chemotherapy and stem cell rescue (Head Start and 99703).<sup>10,19</sup> In this study, a 24 week maintenance phase will be included for all patients. This will provide an opportunity to evaluate the feasibility and toxicity of administering oral metronomic and molecularly targeted therapy in this patient population. Cyclophosphamide and topotecan are active against medulloblastoma and other embryonal tumors when given as single agents at maximum tolerated doses,<sup>14,93,146</sup> and against metastatic Ewing's sarcoma and other pediatric solid tumors when given in combination.<sup>94,95</sup> Both agents have also been demonstrated to have anti-angiogenic activity,<sup>130,147-149</sup> and the combination of these drugs is therefore a natural choice for the metronomic component of this maintenance regimen.

As described above, cyclophosphamide was one of the first agents shown to have anti-angiogenic activity when administered in frequent low doses,<sup>130</sup> and remains the best-studied metronomic agent.<sup>99,150,150-152</sup> In adult studies, metronomic cyclophosphamide is typically given at a dose of 50 mg per day, (roughly 30mg/m<sup>2</sup>) either continuously or in 21-day cycles, followed by a 7-day rest period. Toxicity consists primarily of mild, reversible myelosuppression.<sup>133,153</sup> In children, daily oral cyclophosphamide has been administered in several regimens. Kieran, et al. tested the feasibility of administering a four drug anti-angiogenic regimen to 20 children with recurrent or progressive pediatric cancer. Patients received continuous oral thalidomide and celecoxib, with alternating 21 day cycles of oral etoposide and oral cyclophosphamide (2.5mg/kg/day to a maximum of 100mg/day). 21 episodes of grade III and no episodes of grade IV neutropenia occurred within the first six months of therapy. During the same interval, 4 episodes of grade III anemia occurred, with no grade IV anemia and no grade III or IV thrombocytopenia. One quarter of patients remained progression free more than 123 weeks from starting therapy, including the single patient in the study with medulloblastoma.<sup>139</sup> In addition, as described above, Stempak et al. administered cyclophosphamide 30mg/m<sup>2</sup>/day in combination with BID celecoxib to 16 pediatric patients with recurrent/refractory solid tumors. Toxicity was minimal, and prolonged stable disease was observed in 1 patient.<sup>138</sup> Heavily pretreated pediatric solid tumor patients have therefore tolerated oral cyclophosphamide in combination with other

agents at doses ranging from 30 mg/m<sup>2</sup>/day (approximately 1 mg/kg/day) to 2.5mg/kg/day (approximately 75 mg/m<sup>2</sup>/day).

Topotecan has activity in medulloblastoma and PNET,<sup>146</sup> and has also been shown to have anti-angiogenic activity when administered in frequent low doses in preclinical models of Wilms tumor and neuroblastoma.<sup>147-149</sup> In a retrospective study from Memorial Sloan Kettering Cancer Center, Kramer, et al. reported that 20 patients with relapsed/refractory neuroblastoma were treated with 21 day cycles of daily oral topotecan, generally at 1 mg/m<sup>2</sup>/d in two divided doses. Toxicity including diarrhea (n=12) and myelosuppression (n=11), both requiring dose adjustment in several cases. Anti-neuroblastoma activity was seen in 7 patients, lasting 4 to 12 months.<sup>154</sup>

In a pediatric Phase I study, Bowers, et al. administered cyclophosphamide 50 mg/m<sup>2</sup>/day in combination with topotecan 0.8mg/m<sup>2</sup>/day to 17 children with recurrent or refractory malignant solid tumors. A partial response was observed in one neuroblastoma patient, and prolonged stable disease was observed in 1 of 3 medulloblastoma patients enrolled in the trial. Myelosuppression was the DLT, and the maximum tolerated duration of combined therapy was 14 days out of a cycle lasting 21 to 28 days.<sup>137</sup> To optimize the anti-angiogenic effect, it will be preferable to administer chemotherapy for a greater proportion of each cycle, and limit the duration of the rest period. In the maintenance phase of this protocol, oral cyclophosphamide will be given at 30 mg/m<sup>2</sup>/day for 21 days, followed by a 7 day rest. Topotecan will be given at 0.8 mg/m<sup>2</sup>/day for the first 10 days of each 28 day cycle containing cyclophosphamide. If patients experience delays in therapy due to myelosuppression, the dose of cyclophosphamide, and if necessary, duration of topotecan will be adjusted in subsequent courses (see section 4.5.4 for details). Cyclophosphamide/topotecan cycles will be alternated with cycles of single-agent erlotinib or etoposide (see below).

#### 2.4.7 Rationale for molecularly-targeted therapy with erlotinib

##### 2.4.7.1 *ERBB Family expression in medulloblastoma and other brain tumors*

The receptor tyrosine kinase I (RTK I), also known as ERBB, family of transmembrane receptors includes EGFR (also known as ERBB1), ERBB2 (also known as HER2), ERBB3 and ERBB4. These receptors participate in cell signaling pathways which control a variety of cell processes including proliferation and apoptosis.<sup>155</sup> Abnormalities of ERBB family proteins have been detected in a number of tumor types, including breast, lung, and pancreatic cancer.<sup>156</sup> Overexpression of ERBB2 results in abnormal receptor homodimerization which causes constitutive receptor activation and stimulates cell proliferation mediated through the PI3K/AKT pathway. Gilbertson, et al. detected ERBB2 expression by immunohistochemistry in 80% of medulloblastoma tumor samples from pediatric patients, and ERBB4 in 66%. ERBB1 and ERBB3 were rarely expressed. Patients with high levels of ERBB2 expression (≥ 50% immunoreactive tumor cells) had a significantly worse prognosis, and co-expression of ERBB2 and ERBB4 was associated with particularly poor survival.<sup>157</sup> Further studies in the Gilbertson

laboratory have shown that ERBB2 overexpression in medulloblastoma is associated with increased proliferative activity, tumor invasiveness, and metastatic potential,<sup>158-161</sup> and other investigators have reported an association between ERBB2 expression and survival.<sup>162-164</sup> As a component of the current SJMB03 trial for medulloblastoma patients  $\geq 3$  years of age, evaluation of ERBB2 protein expression by Western blot is being performed on snap frozen tumor samples. In the pilot study which preceded SJMB03, ERBB2 protein expression was an independent predictor of poor prognosis, and the combination of ERBB2 expression and clinical disease characteristics provided a highly accurate means of risk stratification for children three years of age or older at diagnosis. This pilot study included 15 samples from children younger than three years of age. ERBB2 expression was detected in 8 of 15 tumors, but the small number of samples available precluded survival analysis within the younger cohort. Interestingly, ERBB2 expression and nodular desmoplastic histology were not mutually exclusive, as ERBB2 was expressed in 5 of 9 young children with nodular desmoplastic medulloblastoma, and 3 of 6 young patients with classical or anaplastic histology.<sup>40</sup> The frequent expression of ERBB2 in medulloblastoma of all histologic subtypes, and its association with aggressive clinical behavior, make it an attractive therapeutic target.

The frequency of ERBB family abnormalities in other tumors eligible for this trial has not been investigated as thoroughly. Overexpression of the ERBB family member EGFR has been detected in approximately 80% of pediatric high-grade gliomas,<sup>165</sup> although the expression of EGFR has not been evaluated in high-grade gliomas from children younger than three years of age. ERBB2 and ERBB4 have been found to be co-expressed in approximately 75% of ependymomas, and high-level co-expression has been associated with increased tumor cell proliferation and aggressive clinical behavior.<sup>166</sup> In contrast, one small series demonstrated that EGFR expression was not detected by immunohistochemistry in any of 9 ATRT samples.<sup>167</sup> Similarly, preliminary analysis of gene expression profiles from ATRT samples collected on the St. Jude NBTP01 biology study has revealed no evidence of alterations in expression of ERBB family genes (personal communication, Amar Gajjar, NBTP01 principle investigator). No studies of ERBB family alterations in pediatric supratentorial PNET have been published, to our knowledge.

#### *2.4.7.2 Erlotinib*

The amplification, overexpression and mutation of ERBB family receptors in a number of different tumor types have prompted the development of several new drugs that target these signal proteins. One of the most advanced in clinical development is erlotinib (Tarceva, OSI-774), an orally available small-molecule inhibitor of the ERBB1 and ERBB2 tyrosine kinases. Erlotinib has been approved by the FDA for use in non-small cell lung cancer and pancreatic cancer,<sup>156</sup> and is being evaluated in ongoing clinical trials in a number of other cancer types, including the St. Jude SJHG04 study for children with high grade glioma. While this agent was originally developed because of its activity against EGFR (ERBB1),



more recent preclinical work has demonstrated that erlotinib also blocks signaling by ERBB2.<sup>116,127</sup> A study from Richard Gilbertson's laboratory demonstrated that erlotinib significantly inhibited ERBB2 phosphorylation and expression of the downstream target S100A4 in the Daoy medulloblastoma cell line, and inhibited migration of Daoy.2 cells *in vitro*. Erlotinib also inhibited pro-metastatic gene expression in Daoy.2 cells grown as s.c. xenografts in nude mice.<sup>161</sup>

Erlotinib has been generally well tolerated in adult clinical trials. Toxicity has consisted primarily of rash and diarrhea. Diarrhea has been the dose limiting toxicity in adult studies, and the adult MTD has been established at 150 mg daily. While interstitial lung disease (ILD) was a rare but sometimes fatal adverse event in early clinical trials, the BR-2 study, a randomized, double blinded, placebo controlled study involving 731 adult patients with incurable advanced stage NSCLC, found no difference in the incidence of ILD between the treatment and control groups (0.8% in each group).<sup>168</sup> The Children's Oncology Group ADVL0214 trial was a pediatric Phase I study for patients with selected recurrent/refractory solid tumors, including brain tumors. 46 patients were enrolled, including 23 younger than 12 years of age. Patients received an initial 28 day course of single-agent erlotinib, followed by repeated courses of erlotinib in combination with temozolomide 180 mg/m<sup>2</sup>, administered on the first five days of each course. Myelosuppression was minimal during the single agent erlotinib phase, with only one episode of grade 3 leukopenia. Dose limiting toxicities were rash, diarrhea, and hyperbilirubinemia, and the MTD of erlotinib for both the single agent and combination phases was 85 mg/m<sup>2</sup>. No pulmonary toxicity was reported in this pediatric phase I trial (COG Spring 2006 Meeting Progress Report).

In the SJHG04 trial for children older than three years of age with high grade glioma, erlotinib is given concurrently with focal irradiation and as a single agent thereafter. During the initial phase I component of this trial, the dose of erlotinib was escalated to the maximum planned dose level of 120 mg/m<sup>2</sup> once daily, and no MTD was reached. An amended version of the trial which opened subsequently allowed further dose escalation. While no MTD was reached, pharmacokinetic studies demonstrated that serum erlotinib levels reached a plateau at higher doses. As in prior studies, toxicity consisted primarily of rash and diarrhea (personal communication, Alberto Broniscer). An 8-year old patient on this trial required externalization of a ventriculoperitoneal shunt due to infection, and provided an opportunity for simultaneous evaluation of plasma and CSF erlotinib pharmacokinetics. At a dose of 78 mg/m<sup>2</sup>/day, CSF penetration for erlotinib and its metabolite OSI-420 were 7% and 9%, respectively.<sup>169</sup>

In summary, expression of EGFR or ERBB2 is detectable in a large proportion of medulloblastomas, high-grade gliomas and ependymomas, and is associated with aggressive clinical behavior. Erlotinib is a receptor tyrosine kinase inhibitor with activity against ERBB2 and good CSF penetration. As discussed in section 2.4.5.2, it may also have anti-angiogenic activity in ERBB2 overexpressing tumors. It has been well tolerated in pediatric phase I trials which include brain tumor patients.

Furthermore, unlike many other molecularly targeted agents, erlotinib tablets can be crushed, and can thus be given more readily to very young children. This trial will evaluate the feasibility and toxicity of administering this drug to young children with medulloblastoma, high-grade glioma or ependymoma as a component of maintenance therapy. Erlotinib will be given at a dose of 90 mg/m<sup>2</sup> by mouth daily for 28-day cycles, in alternation with cycles of metronomic cyclophosphamide/topotecan.

ERBB2 expression has not been detected in ATRT, and has not been evaluated in supratentorial PNET or the rarer tumors eligible for this trial. There is therefore minimal rationale for administering erlotinib to patients with tumors other than medulloblastoma, high-grade glioma or ependymoma. Oral etoposide will be substituted for erlotinib for these patients at a dose of 50 mg/m<sup>2</sup> daily for the first 21 days of each maintenance cycle B. This dose of etoposide has been shown to produce clinical responses and prolonged disease stabilization in patients with recurrent medulloblastoma and other brain tumors, as well as non-CNS solid tumors.<sup>170-173</sup> This regimen has also been successfully administered to very young children with medulloblastoma and other embryonal CNS tumors on the recent P9934 and PBTC-001 trials. It has been well tolerated in this population, with the primary adverse effect being manageable myelosuppression (COG Fall 2006 Meeting P9934 Progress Report).

#### 2.4.8 Rationale for RT

RT is an essential component of treatment for medulloblastoma and other CNS tumors in older patients, but investigators have attempted to limit the use of this modality in very young children due to unacceptable toxicity.<sup>174,175</sup> While results have varied by trial, even the most effective chemotherapy regimens have not allowed the omission of RT in all patients. RT therefore remains a necessary component of treatment for many patients in this population. Fortunately, attempts to limit the late effects of RT by reducing the dose to normal brain structures have met with some success. These improvements have been achieved through the use of smaller target volumes and conformal treatment planning. Implementation of these strategies has resulted in effective disease control for ependymoma patients as young as 12 months, with good neurocognitive outcomes.<sup>36</sup> As described in section 2.2.1, preliminary results from treatment regimens utilizing local RT in very young patients with M0 medulloblastoma have also produced encouraging survival rates.

Patients enrolled on this trial will be at risk for neurocognitive sequelae from a variety of insults, including direct tumor effects, hydrocephalus, surgery, and chemotherapy. Accordingly, this treatment plan will attempt to limit the toxicity of RT in several ways. For patients with low-risk disease (those with M0 completely resected nodular desmoplastic medulloblastoma), RT will be omitted entirely. For patients with intermediate risk disease (all other M0 patients), focal RT will be required; in order to minimize damage to normal brain, we will use a clinical target volume margin of 5 mm. Given the high likelihood of neurocognitive dysfunction

associated with CSI in children less than 3 years of age, we will not routinely administer CSI to high-risk patients on this protocol. Some patients with high-risk disease will, however, reach the age of 3 years while on treatment, and as it is a component of standard therapy for children older than 3 years, CSI will be offered to these patients. For patients whose parents do elect CSI, we will use a clinical tumor volume margin of 5 mm for the primary tumor site boost, and adjust the neuraxis dose based on the response to induction chemotherapy. Proton beam RT is an emerging technology which has the potential to reduce the radiation dose to normal structures to a greater extent than is possible with conformal photon therapy techniques.<sup>176</sup> The use of proton RT may ultimately result in decreased neurocognitive toxicity, and this modality is being used with increasing frequency in the treatment of pediatric tumors, including brain tumors. As this technology is not currently available at St. Jude, patients enrolled on this protocol will be allowed to receive proton radiotherapy at other institutions; all such cases should be discussed with the principal investigator or radiation oncology section coordinator prior to the initiation of RT.

## 2.5 RATIONALE FOR PHARMACOKINETIC/PHARMACOGENETIC STUDIES

*NOTE: Methotrexate pharmacokinetic studies will be obtained for all patients at all sites as a component of standard care, and topotecan pharmacokinetics will be evaluated for all patients at all sites who are treated on the high-risk arm and receive consolidation with targeted topotecan and cyclophosphamide. During the maintenance phase, trough levels will be obtained at two time points for all patients at all sites receiving oral cyclophosphamide and erlotinib in order to assess compliance. All other pharmacokinetic studies will be optional, and will be offered only to patients treated at St. Jude.*

### 2.5.1 Methotrexate studies

Methotrexate, a folic acid antagonist, is structurally similar to folic acid and acts by reversibly inhibiting dihydrofolate reductase, ultimately interfering with the synthesis of DNA and cell reproduction. Renal excretion constitutes the major route of methotrexate elimination, with renal clearance accounting for 60-80% of total body clearance. Following an intravenous methotrexate dose, between 40 and 90% of the dose is recovered unchanged in the urine.<sup>177</sup> Considerable evidence has been published that the pharmacokinetic parameters (e.g., systemic clearance) of high-dose methotrexate (HDMTX) are highly variable among children and that this variability can influence event-free survival of pediatric patients with ALL.<sup>178,179</sup> A greater risk of ALL relapse was observed in children with a lower systemic exposure to MTX due to more rapid systemic clearance of HDMTX.<sup>180,181</sup> Seidel and colleagues also noted that higher MTX clearance was associated with a worse outcome in pediatric patients with ALL.<sup>182</sup>

A number of factors may explain at least part of the observed variability in methotrexate pharmacokinetic parameters, particularly systemic clearance.

Different studies have shown that hydration and urinary alkalinization regimens, renal function, as well as emesis and concomitant drug administration can significantly affect HDMTX disposition in children.<sup>183</sup> Aumente and colleagues<sup>184</sup> have published the results of a population pharmacokinetic study using NONMEM analysis involving 49 pediatric patients with newly diagnosed ALL aged 6 months to 17 years receiving HDMTX (3 g/m<sup>2</sup> infused over 24 hours). The investigators propose that age is an important factor that explains part of the inter-individual variability of methotrexate pharmacokinetics. For the same total body weight, children younger than 10 years had a total CL 30% higher than in children older than 10 years. These data supports the previous reports demonstrating that systemic methotrexate CL decreases progressively as a function of age.<sup>185</sup>

At birth and up to approximately 2 months, glomerular filtration rate is approximately 40 ml/min/1.73 m<sup>2</sup>. As methotrexate is primarily eliminated by glomerular filtration, very young infants might be expected to have diminished methotrexate clearance. Of the few data published on methotrexate pharmacokinetics in infants with acute lymphoblastic leukemia, results are mixed. In children receiving high-dose MTX, Donelli and colleagues<sup>186</sup> found no significant differences in MTX pharmacokinetics when comparing infants aged less than 1 year and older children aged up to 10 years in a small series. By contrast, McLeod and colleagues showed that infants (n=4) between 3-12 months had a significantly lower clearance of 80 ml/min/m<sup>2</sup> compared to 103 ml/min/m<sup>2</sup> in 108 children aged 1-19 years (p=0.01), but observed no increased toxicity in the infants.<sup>187</sup> Thompson and colleagues also reported a modest difference in clearance when comparing very young pediatric patients (less than 6 months old, 89 ± 32 ml/min/m<sup>2</sup>) to older infants aged 7-12 months (111 ± 40 ml/min/m<sup>2</sup>).<sup>188</sup> These authors also observed a difference in the average methotrexate clearance when comparing very young infants (0-3 months) to infants between 7 and 12 months old. In this study, very young infants (0 to 3 months) had greater renal toxicity (all grades) than the older infants, but no significant differences were noted in hepatotoxicity or mucositis between the age groups. The authors urged caution in interpretation of the toxicity results since the study was not designed to collect data on other factors that might contribute to methotrexate toxicity (e.g., aminoglycoside usage, etc.).

The intra- and inter-patient variability reported in methotrexate pharmacokinetics remains largely unexplained in patients with normal renal function and sufficient hydration. A well-documented methotrexate efflux transporter is the ABC transporter ABCC2 or MRP2/cMOAT, which is expressed in the luminal membrane of hepatocytes and proximal tubular cells of kidney. The involvement of ABCC2 in methotrexate transport has been documented *in vitro* as well as *in vivo*.<sup>189,190</sup> The methotrexate plasma area under the curve between 36 and 48h (AUC<sub>36-48</sub>) was higher in female patients as well as in patients who carry at least one -24T allele. Moreover, a significant interaction between genotype and gender was found, leading to a hypothesis that a gender-specific effect could exist for the -24C>T ABCC2 polymorphism on high-dose methotrexate pharmacokinetics.<sup>191</sup>

Considering the potential importance of MTX in treating very young children with brain tumors, and the possible influence of infant physiology on MTX disposition, the present study aims to characterize MTX pharmacokinetics and toxicity in infants as important steps toward improving the safety and efficacy of future therapy for this vulnerable patient population. For St. Jude patients enrolled on the institutional PGEN5, the relationship between pharmacogenetic and pharmacokinetic variables will also be explored.

### 2.5.2 Rationale for cyclophosphamide pharmacokinetic and pharmacogenomic studies

Wide inter-patient variability in cyclophosphamide systemic clearance is observed in both adult and pediatric patients. Although many factors may account for this difference, the relatively increased hepatic cytochrome activity in children compared with adults is thought to account for much of the difference in pharmacokinetics parameters between adults and children.<sup>192,193</sup> Pediatric patients have also demonstrated considerable inter-patient variability in their exposures to cyclophosphamide metabolites DCCY and CEPM,<sup>194</sup> but inter-patient variability in systemic exposure to the metabolite HCY has not been evaluated in children. In previous studies, variations in DCCY and CEPM exposure could not be attributed to a child's body surface area, age, gender, glomerular filtration rate, or hepatic function,<sup>195,196</sup> which suggests the contribution of other factors to the variability in cyclophosphamide metabolite exposure. Although a few studies have previously described cyclophosphamide pharmacokinetics in children, none have used recently developed, highly sensitive analytical techniques to assess inter- and intra-patient variability in cyclophosphamide exposure. In fact, intra-patient variability in cyclophosphamide exposure has not been addressed thoroughly by any means in pediatric patients, much less very young children. Thus, in this study we propose to describe the pharmacokinetics of cyclophosphamide and its metabolites (i.e., HCY) in very young children receiving intravenous and oral cyclophosphamide, using a recently developed LC MS/MS analytical method. We will also assess the extent of inter-patient variability in the pharmacokinetics of cyclophosphamide and metabolites, and the effects of patient specific covariates upon the pharmacokinetics.

Cyclophosphamide is a known inducer of its own metabolism, and variable increases in cyclophosphamide clearance have been described after repeated administration.<sup>197</sup> Unfortunately, autoinduction may confound the pharmacokinetic analysis by contributing to intra-patient variation between oxazophosphorine courses, as was observed with ifosfamide, a related isomer of cyclophosphamide. We therefore propose to evaluate intra-patient pharmacokinetic variation by conducting pharmacokinetic studies in selected patients (those receiving consolidation with IV topotecan plus cyclophosphamide 600 mg/m<sup>2</sup>/day IV on 2 consecutive days). The results of these studies will be very interesting to compare with those from a similar study performed in older children with medulloblastoma,

but at a much higher cyclophosphamide dosage. In that study, cyclophosphamide 65 mg/kg/d (~1950 mg/m<sup>2</sup>/day) IV was administered on 2 consecutive days, and results of detailed pharmacokinetic studies showed cyclophosphamide clearance was increased on the second day by an average of 32%.

Concomitant medications, particularly those affecting CYP isozymes, have been shown to influence the half-life of cyclophosphamide,<sup>198</sup> but the effect of these compounds upon the systemic exposure of the metabolites has not been explored. The half-life of cyclophosphamide has been extended by allopurinol and chlorpromazine, while shortened by etoposide, dexamethasone,<sup>199</sup> and enzyme-inducing anticonvulsants.<sup>200</sup> The mechanisms of these interactions have not been evaluated, although dexamethasone may shorten the half-life of cyclophosphamide by induction of cytochrome P450 3A activity,<sup>201</sup> while fluconazole and itraconazole are thought to increase cyclophosphamide half-life by inhibiting CYP2C8, CYP2C9 and CYP3A4.<sup>202,203</sup> Patients receiving inducers or inhibitors of CYP enzymes, such as glucocorticoids, enzyme-inducing anticonvulsants,azole anti-fungals, or macrolide antibiotics (see Appendix I) will be analyzed and the impact of such drugs upon cyclophosphamide / HCY pharmacokinetics will be described. As we expect that very few patients enrolled on SJYC07 will be receiving inhibitors or inducers of metabolizing enzymes, any analysis of the effects of concurrent medications upon cyclophosphamide or metabolite pharmacokinetics will be purely descriptive.

Genetic polymorphisms have been identified as factors influencing variability in drug exposure and response amongst patients, particularly with anti-neoplastic agents.<sup>204-206</sup> Recent *in-vitro* evidence has suggested that polymorphisms in CYP2B6 and CYP2C9 can affect formation of cyclophosphamide metabolites, notably the formation of the active 4-OH metabolite.<sup>207-209</sup> Moreover, polymorphisms in CYP3A4/5, the most abundant cytochrome P-450 isoforms in humans, are suspected of impacting the rates of cyclophosphamide metabolite formation both *in-vitro* and *in-vivo*.<sup>210</sup> Previous studies from Petros et al. have demonstrated an association between polymorphisms in CYP3A4/5, cyclophosphamide disposition, and overall survival in breast cancer patients.<sup>211</sup> Recent evidence has also suggested that alkylating agents are substrates for MRP2/cMOAT, and this transporter is responsible for their distribution in the body.<sup>212,213</sup> These studies suggest that a high-efflux MRP2/cMOAT phenotype could influence systemic HCY exposure, thus affecting anti-tumor efficacy and/or the toxicity profile of cyclophosphamide/HCY. In light of these data, we propose to study the distribution of polymorphisms in cytochrome P450 enzymes and ABC transporters amongst St. Jude patients enrolled on the institutional pharmacogenetic protocol PGEN5. The associations between the selected genetic polymorphisms and pharmacokinetic phenotypes will be investigated in an exploratory manner.

To characterize the pharmacokinetics of cyclophosphamide and its metabolites 4-OH-cyclophosphamide and carboxyethylphosphoramidate mustard (CEPM) in infants with brain tumors, we have performed studies in all consenting patients during the

induction phase (i.e., all risk groups), and also during consolidation for high-risk patients. Thus far cyclophosphamide pharmacokinetic studies have been completed in 23 infants during induction, and in 3 out of 6 infants with high-risk tumors during consolidation (with the other 3 patients going off-study prior to consolidation).

The hepatic metabolism of cyclophosphamide is very complex involving several cytochrome P450 enzyme systems including CYPB6, CYP2C9, and CYP3A4/5, which undergo a rapid maturation process in children less than 3 years of age.<sup>213a,b,c,d</sup> In general, these studies have shown that CYP3A4/5 activity can rapidly increase in the first year of life to a level that is actually higher than adult CYP3A4/5 activity. CYP3A4 activity varies over 3-fold within the three years from birth to 3 years of age.<sup>213a,d</sup> CYP2C9 liver expression and activity also increase rapidly in the first year of life, but remain below adult values.<sup>213b</sup> The developmental changes in hepatic CYP450 expression may lead to a difference in cyclophosphamide pharmacokinetics between induction and consolidation in our patient population. In particular, changes in CYP isoform expression during the four months that the patient matures may lead to different activation of CTX to the 4-OHCTX form, possibly resulting in altered pharmacologic effect (e.g., decreased efficacy or increased toxicity).

Initially, we planned to address the question of whether cyclophosphamide clearance changed between induction and consolidation by studying patients during induction and consolidation for the high-risk group; however, our experience shows that we are unlikely to accrue sufficient numbers of high risk patients for an adequate analysis. Thus, we propose to expand our studies of cyclophosphamide pharmacokinetics to include the group of children in the low-risk arm that receive cyclophosphamide during consolidation. This will enable us to accrue adequate patient numbers as exemplified by the power analysis detailed below.

Total accrual on the protocol is estimated to be ~400 patients, and current estimates as well as SJ historical data suggests that approximately 50% of patients enrolled will be either LR or HR. Hence 200 total LR+HR patients are expected to be enrolled on this protocol. Thirty eight (38) of these have already been enrolled, so we expect to have 162 additional enrollees in these two risk groups. The investigators expect that 50-60% of these patients will be treated at SJ and the rest will be treated at the collaborating sites, which is consistent with prior SJ-initiated multi-institutional brain tumor protocols, such as SJMB03. Based on current accrual, we estimate that 50% of the LR+HR patients will be LR and 50% will be HR. Thus of the approximately 81-98 additional patients treated at SJ, 40-50 are expected be LR and 40-50 to be HR. The EFS rate at 4 months for HR is ~60%. We don't have a good estimate of the EFS rate for LR group (since so few were in the SJ historical cohort) but we may conservatively estimate the EFS rate at 4 months as 90%. Hence 36-45 of the LR patients and 24-30 of the HR patients are expected to reach consolidation without progression. The investigators expect that 90% of patients treated at SJ will consent to these optional PK studies, as was observed in prior studies. Thus we estimate that an additional 32-40 LR and 22-27 HR patients

will be enrolled on the protocol and will have both induction and consolidation samples available.

We do not know *a priori* whether the change in cyclophosphamide clearance between induction and consolidation ( $\Delta\text{CL}$ ) vs. age is linear or even monotonic. If the observed relationship is linear, or can be linearized via a transformation such as log, and the data does not deviate substantially from bivariate normality, then the Pearson’s correlation coefficient will be used to test the existence of a relationship. The following table provides power estimates based on Pearson’s Correlation for various sample sizes and for two population correlation values,  $\rho=0.4$  and  $\rho=0.5$ . The power values provided in the table below are in the acceptable range ( $\geq 80\%$ ) for most cases. If the observed data are grossly non-normal and/or the relationship between  $\Delta\text{CL}$  and age cannot be linearized, then we will use Spearman’s rank correlation instead, which may lead to some loss of power. While it is difficult to estimate the magnitude of the loss in power, it is relatively safe to assume that the power associated with  $n=35$  in the table below would approximately correspond to the power associated with  $n=40$  for the Spearman’s rank correlation.

*Power estimates based on testing  $\rho=0$  vs.  $\rho\neq 0$ , where  $\rho$  is Pearson’s Correlation Coefficient with  $\alpha=0.05$*

Sample Size	Association Value to be detected	Power for Pearson’s Correlation-based Test
35	0.4	68.07%
35	0.5	88.27%
40	0.4	74.15%
40	0.5	92.22%
45	0.4	79.24%
45	0.5	94.91%
65	0.4	91.93%
65	0.5	99.17%
65	0.3	68.99%

This table shows that if we perform cyclophosphamide pharmacokinetic studies during consolidation separately for LR and HR patients, we will have adequate power to address the question of how clearance changes between induction and consolidation and how these changes relate to age. Combining the two cohorts would allow us to have higher power overall, detect smaller correlation values and mediate the loss of power in the event that we may have to use Spearman’s rank correlation due to non-linearity or non-normality in the data. We therefore propose to study the pharmacokinetics of cyclophosphamide during consolidation in LR patients as well as HR patients.

### 2.5.3 Rationale for topotecan pharmacokinetic and pharmacogenomic studies

As described in Section 2.4.5.5, “Rationale for consolidation with pharmacokinetically-targeted topotecan and cyclophosphamide in high-risk



patients”, these two very active agents will be combined and administered for two cycles of consolidation therapy. We have extensive experience with pharmacokinetically guided dosing of topotecan in children with cancer. Most of this experience was to attain a plasma systemic exposure; however, in children with medulloblastoma and other embryonal tumors we used our pharmacokinetically guided approach to topotecan dosing to attain a target exposure duration threshold (EDT) in the vCSF defined as 1 ng/ml for 8 hours. In a subsequent pharmacokinetic analysis of the data from this clinical trial, we demonstrated that a plasma pharmacokinetic model could be used to estimate vCSF topotecan concentrations in children with medulloblastoma.<sup>214</sup> Furthermore, we showed that as the plasma topotecan AUC increased, so did the likelihood of exceeding the EDT. Whereas previous studies have validated the three-compartment pharmacokinetic model used to evaluate the topotecan plasma and vCSF disposition, this was the first study to evaluate the ability of a plasma pharmacokinetic model to provide an estimate of vCSF exposure that was based solely on plasma topotecan data.

We have conducted many individual pharmacokinetic studies of topotecan in children with cancer, and our results have shown wide inter-individual variability in topotecan clearance<sup>215-217</sup>. In order to better understand the inter- and intra-patient variability in topotecan lactone disposition, we have conducted a population pharmacokinetic analysis using NONMEM. This analysis included 220 children divided into a model building and a validation set. The modeling building set included 162 children that participated in four Phase I and three Phase II clinical trials of topotecan, and the validation set, which included 58 children in one clinical trial. The patient covariates that were evaluated included continuous (e.g., age) and categorical variables (e.g., sex). Although our analysis included children from 0.04 to 22.0 years, we noted no continuous relation with age and topotecan clearance. When evaluated as a linear continuous variable, age was not a significant covariate ( $p > 0.1$ ). However, for infants less than 0.5 years (10 studies in 1 patient), BSA-normalized topotecan clearance was 40% less than that observed in patients greater than 0.5 years of age ( $p < 0.001$ ). The results of this larger study would suggest that unless a child is in the first months of life, the topotecan clearance will be similar to older patients. We speculate that maturation of renal function during the first weeks of life, leading to a change in glomerular filtration rate, produces this observed change in topotecan clearance with age (up to 6 months). However, in our population of 162 children, only 4 children were  $\leq 1$  yr of age and  $14 \leq 2$  yr of age. Thus, to gain a better understanding of the variability of topotecan disposition in very young children it is crucial to study the pharmacokinetics and pharmacogenomics of topotecan in this population.

Currently the sampling times for targeting topotecan studies are pre, and 5 min, 1 hour, and 4 hours after the end of a four-hour infusion. With the processing time required for the whole blood topotecan assay used in this protocol, it requires a minimum of ten hours to complete a targeting study (time from start of study to reporting AUC results). Even when patients are started very early in the Medicine

Room this makes for a long day for patients and families. Thus, we have performed simulation studies to assess the effect of moving the last sample time point from 4 hours up sooner. The results of these simulation studies showed that collecting the last sample at 2 hrs post-infusion resulted in a biased estimate of the topotecan AUC, but collecting the last sample at 3 hrs post-infusion allowed us to estimate the topotecan AUC as accurately as the 4-hr time point. Thus, we recommend adjusting the sample time points for the topotecan targeting studies to change the 4-hour sample to a 3-hour sample.

#### *2.5.3.1 Rationale for stratifying topotecan initial dosage by age*

During Consolidation, patients that are high-risk receive two cycles of pharmacokinetically-guided topotecan therapy. For those children who were treated on non-protocol treatment plans as per SJYC07 and for patients enrolled on the study, a topotecan dosage of 3.5 mg/m<sup>2</sup> was selected for day 1 of course 1. With this initial dosage, only three of 12 patients (9 patients treated as per SJYC07 on non-protocol treatment plans and 3 SJYC07 patients) had topotecan AUC values within the desired topotecan lactone AUC range of 140±20 ng\*hr/ml on day 1 of course 1. Out of 3 SJYC07 patients, only 1 has been in the AUC target range. It was noted that the two patients with the highest topotecan AUC values (277 and 234 ng\*hr/ml), were also the youngest (8 months and 11 months, respectively). Topotecan is primarily cleared by renal elimination<sup>213f</sup> and it is known that renal function, and in particular glomerular filtration, develops over the first year of life.<sup>213a</sup> We do not yet have adequate data from SJYC07 patients and as per SJYC07 non-protocol treatment plan patients to determine the relationship between age and topotecan clearance because most patients thus far have been older than 1 year.

We therefore examined data from SJRET5, another clinical protocol in which topotecan is administered intravenously to young children. Preliminary analysis of data from SJRET5 shows that topotecan systemic clearance is linearly related to age for the first year of life, then reaches a plateau after 1 year. The relationship of topotecan systemic clearance and age mirrors renal development.<sup>213a</sup> Thus, we used combined data from SJRET5, SJYC07 and NPTP patients to determine the initial dosage of topotecan that is the most likely to result in patients being within the target AUC range on day 1 of course 1. We recommend that patients ≥12 months of age continue to receive 3.5 mg/m<sup>2</sup>, patients 6-11.9 months of age should receive 2.5 mg/m<sup>2</sup> as the initial course 1 day 1 topotecan dosage, and patients <6 months of age receive 2 mg/m<sup>2</sup> as the initial course 1 day 1 topotecan dosage. With this adjustment in the course 1 day 1 topotecan dosage, we expect a reduction in the number of topotecan targeting pharmacokinetic studies that will be necessary to get patients within the target topotecan AUC range.

#### *2.5.4 Rationale for erlotinib pharmacokinetic and pharmacogenetic studies*

Currently no data are published on the pharmacokinetics of erlotinib and its metabolites in children. We have conducted a clinical pharmacokinetic study of

erlotinib in children with high grade glioma enrolled on SJHG04. These patients received escalating erlotinib dosages (70, 90, and 120 mg/m<sup>2</sup>/d) along with radiation therapy. We measured erlotinib and OSI-420 pharmacokinetics on days 1, 2, 3, and 8 of course 1 (the erlotinib dose on day 2 was held). The preliminary results of the pharmacokinetic studies show at the 70 mg/m<sup>2</sup>/d dosage level, the median (range) erlotinib and OSI-420 C<sub>max</sub> and T<sub>max</sub> were 1405 ng/ml (937 to 2180) and 4.1 hr (2.2 to 8.2) and 158.5 ng/ml (45 to 203) and 4.1 hr (2.2 to 7.9), respectively. The youngest child on that clinical trial was 3 years of age, so that it is crucial to study the pharmacokinetics of erlotinib in this very young patient group to assess the inter- and intra-patient variability in erlotinib disposition.

Erlotinib is extensively metabolized by hepatic CYP3A4 and CYP3A5. It is also metabolized in minor amounts by CYP1A2 and CYP2C8, by pulmonary CYP1A1 and by CYP1B1 in tumor tissue. Because erlotinib is largely metabolized by CYP3A4/5, drugs that induce or inhibit this isoenzyme may interact to decrease the efficacy or increase associated toxicities, respectively (see Appendix I). By evaluating the pharmacogenetics in this patient group (for St. Jude patients enrolled on the institutional protocol PGEN5 only) as well as collecting patient specific data, we can assess for covariates that will explain the variability that we observe in the erlotinib pharmacokinetic parameters.

## 2.6 RATIONALE FOR CNS NEUROTRANSMITTER STUDIES

Morbidity associated with the treatment of brain tumors in young children is well known. Patients receiving standard therapies often demonstrate late neurocognitive deficits, especially in the area of attention.<sup>218-220</sup> Declines in cognitive ability indicated by diminished intelligence quotients often continue for 10 or more years after therapy.<sup>221</sup> Studies have also described an association between late neurocognitive deficits and decreases in normal cerebral white matter volume.<sup>222-224</sup> While the exact mechanism of white matter damage is still unknown, both radiotherapy and chemotherapy have been implicated.<sup>225</sup> It is generally accepted, however, that therapy-induced microvasculature damage may precipitate cerebral white matter injury.<sup>226,227</sup> Ischemic injury, including that resulting from microvasculature damage, has been shown to induce alterations in striatal dopamine distribution and depress dopamine D2 receptor activity in animals.<sup>228,229</sup> It is unclear whether such a phenomenon occurs in young children after therapy for medulloblastoma and other tumors, but accruing evidence suggests perturbations in central dopaminergic transmission may play a role in the cognitive deficits seen after medulloblastoma therapy in older children. The role of dysfunctional dopaminergic transmission has not been investigated in very young children receiving treatment for CNS malignancies.

A recent study by Reddick et al. has demonstrated that the primary consequence of cerebral white matter damage in children treated for brain tumors was a decrease in attentional ability.<sup>230</sup> Other investigators have also described similar attention and working memory deficits in pediatric brain tumor and leukemia patients.<sup>231-233</sup>

Similarly, deficits in sustained attention and working memory are classical findings associated with attention deficit hyperactivity disorder (ADHD): a condition often attributed to defective central dopaminergic transmission. Moreover, patients exhibiting neurocognitive deficits after cancer therapy have responded to methylphenidate, an amphetamine derivative used to treat ADHD.<sup>234,235</sup> Methylphenidate is thought to improve attentional abilities by releasing stored dopamine from the presynaptic vesicular pool, decreasing dopamine reuptake via transporter inhibition, and by inhibiting monoamine oxidase. The increased extracellular dopamine is believed to activate inhibitory autoreceptors in the striatum, thus improving attention. It has also been demonstrated that increasing the availability of dopamine in the prefrontal cortex facilitates performance on working memory tasks known to be dependent on this brain region.<sup>236</sup> These data suggest that neurocognitive deficits observed after cancer therapy may share a pathophysiological basis with ADHD; that is, defective central dopaminergic transmission.

To validate the link between ADHD and central dopaminergic defects, studies have investigated the relationship between CSF monoamine concentrations and behavior in ADHD patients.<sup>237,238</sup> A significant positive correlation between CSF homovanillic acid (HVA), a dopamine metabolite, and inattention has been made.<sup>239</sup> Another study demonstrated that higher CSF HVA concentrations prior to stimulant therapy predicted better drug response in one group of ADHD patients.<sup>240</sup> The results of these studies provide support for the relationship between ADHD and defective central dopaminergic transmission. Similarly, we propose exploring CSF dopamine and metabolite concentrations in young children with medulloblastoma and other brain tumors to determine the role impaired dopaminergic transmission may play in the genesis of therapy-related neurocognitive deficits. We propose to correlate the CSF neurotransmitter phenotype with expression of inattentiveness, working memory deficits and cognitive impairment as quantified by standardized psychological measures. It is our hypothesis that neurocognitive deficits after anticancer therapy in the young child with a central nervous system tumor result from iatrogenically induced disturbances in central dopamine transmission.

#### 2.6.1 Rationale for genotyping for dopamine-related gene polymorphisms in patients enrolled on SJYC07

As mentioned previously, we hypothesize that the neurocognitive deficits observed after cancer therapy are related to defects in central dopaminergic transmission. Such neurocognitive deficits after therapy manifest themselves as an inattentive phenotype similar to that observed in ADHD, a disorder arising from defective dopaminergic transmission. To date, several studies have investigated the dopaminergic hypothesis of ADHD from a genetic perspective, with significant but variable genotype-phenotype relationships described.

The gene encoding the dopamine transporter DAT1 has been studied extensively, and significant correlations between a variant found in the 3' un-translated region of

the gene and ADHD have been made.<sup>241-244</sup> This DAT1 polymorphism is referred to as the 480 bp DAT1 variant or DAT1\*10. In population analyses, the DAT1\*10 allele has demonstrated a high expression frequency of approximately 0.7, depending on ethnicity.<sup>245,246</sup> The DAT1\*10 allele was also associated with poor response to methylphenidate in children with ADHD<sup>247,248</sup>. It is believed that this polymorphism leads to either a hyperactive dopamine transporter<sup>249</sup> or an increased transporter density<sup>250</sup>, both of which ultimately result in decreased extracellular dopamine. The presence of such a polymorphism in patients may identify a predilection towards developing an inattentive phenotype after brain tumor therapy. Therefore, we propose to study the expression of the 10-repeat DAT1 (DAT1\*10) allele amongst young children with medulloblastoma and other brain tumors, and to correlate patient DAT1 genotypes with neurocognitive and CSF neurotransmitter phenotypes.

Polymorphisms in dopamine receptor genes have also been correlated with ADHD in several studies. The most studied polymorphism in dopamine receptors is the 7-repeat DRD4 (dopamine D4 receptor) variant found in exon 3, known as DRD4\*7, which exhibits an allelic frequency of approximately 0.17 to 0.2.<sup>251,252</sup> This polymorphism, which results in a diminished inhibitory response to dopamine,<sup>253</sup> has been correlated with ADHD in numerous investigations.<sup>254-258</sup> On the contrary, other studies have found no association between the 7-repeat DRD4 allele and ADHD.<sup>259-261</sup> Studies also suggest that dopamine D5 and D2 receptor polymorphisms are associated with the ADHD phenotype, specifically the 148 bp DRD5 and *TaqI* poly DRD2 variants.<sup>262-265</sup> Many investigators believe that these polymorphisms in dopamine receptors and DAT1 transporter lead to decreased central dopaminergic transmission, and thus an ADHD phenotype. It is likely that such polymorphisms may somehow be related to the development of neurocognitive deficits that occur after brain tumor therapy, particularly the inattentive phenotype. We hypothesize that specific genetic polymorphisms may result in an increased susceptibility to iatrogenically-induced neurocognitive impairment. Therefore, we propose to study the expression of such polymorphisms in very young children with brain tumors, and to correlate genotypes with neurocognitive and CSF phenotypes. In particular, we have chosen to focus upon two specific polymorphisms in the DAT1 and DRD4 genes, DAT1\*10 and DRD4\*7, for correlative analyses.

## 2.7 RATIONALE FOR NEUROCOGNITIVE STUDIES

### 2.7.1 Neuropsychological evaluation

Young children treated for CNS malignancies experience not only a significantly poorer response to treatment, as evidenced by decreased survival rates,<sup>266-268</sup> but also increased treatment-related toxicities including neuropsychological impairment.<sup>269,270</sup> They may be particularly at risk for neuropsychological deficits due to increased vulnerability of the developing brain to therapeutic interventions. As survival rates improve for infants treated for CNS malignancies, the late effects

of treatment and their impact on quality of life become paramount. Neuropsychological deficits are of great concern as they have been shown to associate with academic failure, high unemployment rates and a reduced quality of life.<sup>271</sup>

The few studies that have investigated neuropsychological outcomes in children treated for CNS malignancies in infancy have been inconsistent in their findings. Several studies have found radiation therapy to be associated with a decline in neurocognitive functioning.<sup>8,17,272-275</sup> Some studies found that any treatment resulted in neurocognitive impairment,<sup>276,277</sup> whereas others found that children treated with chemotherapy but without radiation therapy were within the average range with respect to cognitive outcome.<sup>273-275,278,279</sup> These studies have generally been limited by small sample sizes, heterogeneous treatment approaches, lack of longitudinal cognitive data and short-term follow-up durations.<sup>9,18,36,273-275,278-280</sup> By improving upon these study limitations, we will be better able to assess longitudinal change in neuropsychological functioning following treatment and identify those disease (e.g., tumor type, tumor location and presence of hydrocephalus) and treatment-related (e.g., chemotherapy agents and radiation dosimetry) factors most predictive of cognitive risk. These investigations are essential for identifying treatment interventions that are both effective and associated with the fewest treatment-related toxicities.

For this study, children will be assessed at baseline, six months and yearly following treatment. Therefore, the assessment battery needs to span the years from infancy to 8 years of age. An effort was made to select measures that could assess the widest age range possible. Nonetheless, as no single measure exists that covers this entire age spectrum, separate test batteries are needed for children less than 3 years of age and another for children 3 years of age and older. Children less than 3 years of age will be administered a single comprehensive battery, the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III).<sup>281</sup> All children 3 and older will be administered a separate battery, listed in section 8.4. These batteries are comparable in the domains assessed such that some longitudinal analyses will be possible across batteries.

### 2.7.2 Neurological evaluation

Adverse neuro-cognitive and neurological outcomes are well recognized in patients who were previously treated as infants for CNS cancer. However, a prospective assessment of neurological development in children younger than three years of age treated for embryonal brain tumors, high-grade glioma, CPC, or ependymoma has not been well documented. Better understanding of this developmental process may inform future treatment trials. Based on experience with older children, we expect neurological deficits (including expressive language, oculomotor dysfunction, facial weakness, difficulty with swallowing, motor paresis, motor ataxia, and impaired gait) to be maximally apparent just after surgery and then stabilize or improve over time.

### 2.7.3 Hydrocephalus measurements

Hydrocephalus resulting from tumor or treatment-related complications may affect the biologic, neuroimaging and cancer control objectives of this study.

Hydrocephalus measurements (evans index, cella media index, ventricular angle and frontal horn diameter) corresponding to the time of diagnosis, enrollment, completion of therapy and two years after the initiation of therapy will be recorded for each patient along with clinical information about CNS diversion (presence or absence of ventriculostomy, CSF shunting and shunt revisions). The dynamics of hydrocephalus measurements and their value as a clinical co-variate will be studied.

### 2.7.4 Radiation dosimetry: correlation with failure and toxicity

Variability is expected in the distribution of dose to target and normal tissues resulting from differences in the size and location of the tumor bed, the method and modality of treatment delivery (3-dimensional conformal radiation therapy, intensity modulated radiation therapy, proton beam radiation therapy) and clinical and technical factors including hydrocephalus, post-operative brain shift, immobilization, target conformity and normal tissue dose constraints. The CTV margin of 5 mm was chosen for this protocol to minimize dose to normal tissues and to encompass microscopic disease in continuity with the tumor bed. The variability in the distribution of dose about the targeted volume may both incidentally and therapeutically irradiate normal tissue beyond the CTV. The following technical factors may be investigated in the evaluation of rate and pattern of failure: planning target volume, dose conformity, and modality of delivery. The pattern of failure may be further investigated by registration of the specific imaging sequence demonstrating failure to the treatment planning CT or MR study, volumetric contouring of the recurrent tumor, and dose-volume histogram and conformity assessment of the recurrent tumor volume. Normal tissue dosimetry may be compared across patients and correlated with functional outcomes by mean doses, mean doses weighted for biologic effect (equivalent uniform dose) or discrete dose-volume elements representing low, intermediate and high-doses relative the prescribed doses in this protocol. The cumulative form of the dose-volume histogram, for each normal tissue volume, has a characteristic shape. Without spatial specificity, these histograms describe the proportion of a normal tissue volume that receives a particular dose. The derivative of the cumulative dose volume histogram generates a frequency versus dose plot. The plot may be binned into discrete dose-volume elements and integrated to derive variables for correlative analyses and comparison of treatment techniques. Our goal is to use dose-volume data to estimate the change in functional outcomes as a function of time after radiation therapy. We will contribute dose-volume variables to the proposed primary and specific aims of this protocol, including the analysis of endocrine, cognitive, neurologic and audiometric effects. Dose-volume data will be generated for the functional elements and the lobes of the brain as described in the RT section as well as volume elements segmented for the neuroimaging objectives. We will compare conformal, intensity-modulated and proton beam radiation therapy

methods using the same dose-volume elements and statistical methods employed in prior analyses.

## 2.8 RATIONALE FOR DIAGNOSTIC IMAGING STUDIES

### 2.8.1 Assessing brain structure and developing models relating therapy and neuroimaging

Aggressive CNS therapy is associated with increased risk for neurotoxicity which is often evidenced by cognitive delays or deficits and negatively affect quality of life of survivors.<sup>282-285</sup> These deficits in cognitive processing are thought to be dependent upon the integrity of widely distributed neural networks supported by interhemispheric and intrahemispheric white matter tracts.<sup>286</sup> In very young children, cranial RT is often delayed by first treating with aggressive chemotherapy including methotrexate which is also associated with structural alteration of cerebral white matter.<sup>287-289</sup> Other potential sources of white matter damage include increased intracranial pressure and treatment with steroids.<sup>290</sup> These relationships suggest that white matter damage may represent a useful index of the cumulative impact of multiple sources of CNS insult.

The pathophysiology of late CNS damage induced by radiation or chemotherapy is not fully understood, especially with regard to the vulnerability of white matter to injury. Some hypotheses attribute primary mechanisms of CNS damage to the death of neuronal cells, oligodendrocytes, or endothelial cells and the subsequent microvascular damage. Ultimately, this damage seems to be a primary or secondary effect associated with administration of treatment to the CNS.<sup>291</sup> Secondary processes, such as damage to the myelin membrane as a result of oxidative stress after radiotherapy, have also been proposed as putative mechanisms of CNS damage.<sup>292</sup> Furthermore, study of the adverse effects of irradiation on the microenvironment of neural precursor cells has helped to integrate the findings from these competing hypotheses.<sup>293</sup> CNS damage from methotrexate is hypothesized to induce reversible leukoencephalopathy by inhibiting turnover of myelin lipids and proteins; this inhibition results in intramyelinic splits and intralamellar formation of vacuoles that can accumulate interstitial fluid. Increases in free water content and decreases in myelin would greatly affect both the T1 and the T2 relaxation times of the tissue, and such effects would result in contrast changes in the image.<sup>294</sup>

Most recent protocols containing methotrexate routinely use MR imaging to qualitatively evaluate neurotoxicity with a subjective grading scale. This subjective grading, however, precludes comparison of therapy-induced changes between clinical trials and does not provide any continuous measure of the intensity or extent of changes as a function of other influential factors. Our own experience with children treated for ALL without irradiation have demonstrated higher prevalence, extent and intensity of white matter changes associated with higher doses and more courses of methotrexate.<sup>295,296</sup> A recent study of white matter anisotropy using



diffusion tensor imaging (DTI) has investigated patients post-treatment for childhood cancer compared to age-matched controls to assess myelin damage.<sup>297</sup> This study found significant relationships between percentage difference in white matter fractional anisotropy (FA) for each patient relative to the age-matched control and full-scale, verbal, and performance IQ. These preliminary findings suggested that white matter FA may be a clinically useful biomarker for the assessment of treatment related atypical maturation. The use of dynamic susceptibility contrast perfusion imaging has been central in the evaluation of vascular impact of CRT. One such study demonstrated highly significant reduction of CBV in the first two years post therapy in both gray and white matter of adults treated for astrocytoma.<sup>298</sup> This reduction in CBV was correlated to total dose delivered to a tissue area with higher doses causing the largest changes. A more recent report assessed the changes in the recirculation phase of normal-appearing brain tissue two months post therapy in adults treated for grade III/IV glioma.<sup>299</sup> Their results suggested a dose-dependent reduction in vessel density and increase in permeability. While many of these studies have been performed in adults, few have focused on children and the impact of radiation and chemotherapy on normal myelin maturation.

#### 2.8.2 Susceptibility-weighted MR imaging (SWI)

This technique was originally developed to assess the vascular architecture of brain parenchyma noninvasively. The unique potential of SWI lies in its ability to exquisitely highlight vascular structures, based on differences between the magnetic susceptibility in blood (BOLD effect) and surrounding tissues. Arteriograms and venograms with spatial resolution in the order of a few hundred microns may therefore be obtained by SWI. SWI also has the potential to detect, perhaps even quantify extremely small amounts of magnetically susceptible substances within brain parenchyma therefore detect the presence of microscopic hemorrhages. Potential uses of SWI include therefore tissue characterization through visualization of microvascular architecture within brain lesions (including but not limited to tumors) and monitor possible changes to that as a response to treatment or indicator of disease progression, as well as detection of small, otherwise likely undetectable amounts of blood degradation products within tumor lesions which may be relevant in novel treatment options (e.g. antioangiogenesis drugs) for both patient selection and monitoring under treatment. Additional benefits of the technique may comprise early detection of radiation induced vascular changes within brain, such as venous anomalies and associated cavernous angiomas and prevent hemorrhagic complications of those.

#### 2.8.3 Positron emission tomography for dose verification after proton beam therapy (PBT) (*for participants enrolled at St Jude only*)

Conventional radiation therapy uses x-rays to deposit dose within a patient. Dose deposition occurs predominantly through photon-electron interactions in tissue. Proton beam therapy (PBT) uses a subatomic particle known as a proton. Protons

interact with electrons in tissue to deposit dose; however, because of their physical characteristics, protons also interact with atomic nuclei. During PBT it is possible to convert stable  $^{16}\text{O}$ ,  $^{14}\text{N}$  and  $^{12}\text{C}$  atoms within tissue to the short-lived positron emitters<sup>299a</sup>  $^{15}\text{O}$ ,  $^{13}\text{N}$  and  $^{11}\text{C}$ . These positron emitters (primarily  $^{11}\text{C}$ ) can be imaged using a clinical PET<sup>299b,c</sup> system. A correlation between the dose deposited by protons and the PET image can be modeled via Monte Carlo simulations,<sup>299d</sup> leading to in-vivo dosimetric and distal edge verification of PBT.

## 2.9 RATIONALE FOR GROWTH HORMONE SECRETION TESTING BEFORE AND AFTER RADIATION THERAPY

*(participants enrolled at St Jude only)*

Growth hormone (GH) deficiency (GHD) may occur following irradiation of the hypothalamic-pituitary axis (HPA).<sup>299e,f</sup> We have modeled the effects of radiation dose and time after irradiation on pituitary growth hormone secretion.<sup>299g,h</sup> The data used to derive these models was obtained from serial provocative tests of GH secretion in patients with localized brain tumors treated by photon-based conformal radiation therapy. These models suggest that low-dose irradiation, even in the dosing range that is unavoidable due to internally scattered photon-electron interactions, increases the near-term risk for GHD. Although GHD is treatable, somatotropin (rDNA origin) therapy is expensive and invasive. Moreover, untreated GHD contributes to the cognitive decline observed after irradiation and causes abnormal growth, development and metabolism. The mitogenic effects of somatotropin replacement are potentially adverse in cancer survivors, and the risk/benefit ratio of this therapy remains controversial in the presence of active cancer.

Proton beam therapy (PBT) may be capable of sparing children from radiation-induced GHD because the dose to normal tissues, including the hypothalamus, is often reduced when compared to photon conformal radiation therapy.<sup>299i</sup> The difference between protons and photons is the physical characteristic of dose deposition in normal tissue. Protons display unique stopping power and less internal scatter compared to photons. Investigating whether proton beam therapy will reduce the incidence of growth hormone deficiency requires baseline and serial assessment of GH release in patients treated with PBT. No current models of radiation dose and effect are applicable for patients treated with protons. Comparable low dose data, as low as achievable with proton beam therapy, has not been available from photon-treated patients.

To estimate the rate of longitudinal change in GH secretion by reducing the dose to the hypothalamus, consenting patients on the intermediate risk arm of this protocol will undergo pre- and post-irradiation provocative endocrine testing. These data will be combined with the calculated hypothalamic dose to estimate the effect of radiation dose and relevant clinical variables on GH secretion abnormality in both photon and proton beam therapy intermediate risk patients.

### 3.0 ELIGIBILITY CRITERIA AND STUDY ENROLLMENT

#### 3.1 INCLUSION CRITERIA FOR ALL PATIENTS

##### 3.1.1 Newly diagnosed tumor of the CNS, to include patients with:

- medulloblastoma (all histologic subtypes)
- supratentorial PNET (including CNS neuroblastoma or ganglioneuroblastoma, medulloepithelioma, and ependymoblastoma)
- pineoblastoma
- atypical teratoid rhabdoid tumor (ATRT)
- choroid plexus carcinoma
- high-grade glioma, including anaplastic astrocytoma (WHO grade III), anaplastic oligodendroglioma (WHO grade III), anaplastic oligoastrocytoma (WHO grade III), anaplastic ganglioglioma (WHO grade III), pleomorphic xanthoastrocytoma with anaplastic features (WHO grade III), high-grade astroblastoma, anaplastic pilocytic astrocytoma (WHO grade III), malignant glioneuronal tumor, glioblastoma multiforme (WHO grade IV) or gliosarcoma (WHO grade IV)
- Patients with ependymoma (including all ependymoma histological variants)

Histologic diagnosis has been verified by institutional pathologist and classified according to the WHO (2007) system.

##### 3.1.2 Age < 3 years at time of diagnosis for all histological diagnosis.

Medulloblastoma patients  $\geq 3$  and < 5 years old at diagnosis who have non-metastatic disease (as defined in section 3.2.1.2) with no more than 1cm<sup>2</sup> of residual tumor are also eligible. *Medulloblastoma patients in the  $\geq 3$  and < 5 years old age group with anaplastic or large cell histology or with MYC or MYCN amplification are excluded. Pathology from collaborating institutions must be centrally reviewed **prior** to enrollment for confirmation.*

##### 3.1.3 No previous radiotherapy or chemotherapy other than corticosteroid therapy.

##### 3.1.4 Patients must begin treatment as outlined in the protocol within 31 days of definitive surgery.

##### 3.1.5 Patients with adequate organ function as defined by the following parameters obtained prior to study entry:

- Normal renal function as defined by a serum creatinine concentration < 3X the institutional upper limit of normal (ULN).

- Normal liver function as defined by SGPT (ALT) concentration  $<5X$  the institutional ULN and a total bilirubin concentration  $<3X$  the institutional ULN.
- Normal bone marrow function as defined by a hemoglobin concentration  $>8$  g/dL (with or without support); white blood cell (WBC)  $>2000/mm^3$ ; absolute neutrophil count (ANC)  $>500/mm^3$ ; platelets  $>50,000/mm^3$  (without support).

3.1.6 Adequate performance status as defined by Lansky Score  $\geq 30$  (except for Posterior Fossa Syndrome; see Appendix II).

### 3.2 CRITERIA FOR ASSIGNMENT TO THE LOW-RISK ARM OF THE PROTOCOL

*Note: the low risk medulloblastoma cohort closed to accrual on December 2, 2015 due to lack of efficacy. Please see section 13.1.4*

3.2.1 Low-risk medulloblastoma (patients must meet all of the following criteria):

- Histologic diagnosis of nodular desmoplastic medulloblastoma (includes medulloblastoma with extensive nodularity). Patients with focal areas of anaplasia or other atypical features suggesting a more aggressive phenotype in a tumor which would otherwise be considered nodular desmoplastic should be treated on the intermediate risk arm. In such unusual cases, final risk stratification will be at the discretion of the principal investigator and study pathologist.
- No evidence of CNS metastasis as indicated by MR images of the brain and spine and by cytologic examination of lumbar CSF 7 to 28 days after surgery. If lumbar puncture is medically contraindicated, ventricular CSF from a shunt or Ommaya reservoir may be used to rule out M1 disease. If CSF sampling is not possible and the patient has no other evidence of metastasis, the patient should be assigned to the intermediate risk arm.
- Gross total resection as determined by the intraoperative observations of the neurosurgeon of record and confirmed by postoperative MR imaging. Gross total resection is defined as residual tumor or imaging abnormality (not definitive for residual tumor) whose size is  $<1$  cm<sup>2</sup> on postoperative CT or MR images.
- If there is brain stem invasion by the tumor in the absence of imaging evidence of residual tumor (tumor size  $<1$  cm<sup>2</sup>) and the patient otherwise meets criteria for enrollment on the low-risk arm, the patient will be classified as low-risk.

- Desmoplastic medulloblastoma patients who are  $\geq 3$  - $< 5$  years of age will NOT be eligible for the low risk arm of the protocol.

3.2.2 Low-risk high-grade glioma (*patients must meet all of the following criteria*):

- Histologic diagnosis of high-grade glioma, including anaplastic astrocytoma (WHO grade III), anaplastic oligodendroglioma (WHO grade III), anaplastic oligoastrocytoma (WHO grade III), anaplastic ganglioglioma (WHO grade III), pleomorphic xanthoastrocytoma with anaplastic features (WHO grade III), high-grade astroblastoma (WHO grade III), anaplastic pilocytic astrocytoma (WHO grade III), malignant glioneuronal tumor, glioblastoma multiforme (WHO grade IV) or gliosarcoma (WHO grade IV).
- No evidence of CNS metastasis as indicated by MR images of the brain and spine and by cytologic examination of lumbar CSF 7 to 28 days after surgery. If lumbar puncture is medically contraindicated, ventricular CSF from a shunt or Ommaya reservoir may be used to rule out M1 disease. If CSF sampling is not possible and the patient has no other evidence of metastasis, the patient will be assigned to the low risk arm.

3.3 CRITERIA FOR ASSIGNMENT TO THE INTERMEDIATE-RISK ARM OF THE PROTOCOL

3.3.1 Patients less than 3 years of age at diagnosis must meet one of the two following criteria:

- Histologic diagnosis of nodular desmoplastic medulloblastoma with less than gross total resection (as defined in section 3.2.1), but with no evidence of metastasis (as defined in section 3.2.1).
- Any eligible histologic diagnosis other than desmoplastic medulloblastoma, with no evidence of CNS metastasis (as defined in section 3.2.1).

3.3.2 Medulloblastoma patients who are  $\geq 3$  and  $< 5$  years of age with no more than  $1\text{cm}^2$  of residual tumor and with no evidence of CNS metastasis (as defined in section 3.2.1.2). Medulloblastoma patients in the  $\geq 3$  and  $< 5$  years old age group with anaplastic histology, large cell histology, melanotic differentiation, or myogenic differentiation or tumors with MYC or MYCN gain or amplification are excluded. *Pathology from collaborating institutions' patients must be centrally reviewed **prior** to enrollment for confirmation.*

### 3.4 CRITERIA FOR ASSIGNMENT TO THE HIGH-RISK ARM OF THE PROTOCOL

3.4.1 Any eligible histologic diagnosis, with evidence of CNS metastasis (as defined in section 3.2.1.2).

3.4.2 Patients with extraneural metastasis are eligible for treatment on the high-risk arm.

Extraneural metastasis at initial diagnosis is extremely rare in patients with medulloblastoma or PNET, occurring in none of the 84 newly diagnosed St. Jude patients younger than 3 years of age with these diagnoses, and 1 of 283 older patients (this patient had a spinal cord PNET with metastasis to bone). Accordingly, testing for extraneural metastasis by bone scan or bone marrow biopsy will not be performed routinely on this protocol. In the unlikely event that extraneural metastasis is detected on an evaluation performed at an outside institution prior to referral or because of clinical suspicion, such M4 patients will be eligible for protocol treatment on the high-risk arm.

### 3.5 SYNCHRONOUS EXTRANEURAL ATRT

Although it is uncommon, ATRT may occur at sites outside the CNS. The most frequent site for synchronous ATRT is the kidney, but other sites have been reported. At St. Jude, 4 of 40 patients younger than 3 years of age with ATRT have had synchronous extraneural tumors, including 2 with renal tumors, 1 with a soft tissue mass in the scapular region, and 1 with an adrenal tumor. The patient with the adrenal tumor is currently undergoing treatment with no evidence of disease eight months after diagnosis, but the other 3 patients have died from disease. Patients with synchronous extraneural ATRT are eligible for treatment on this protocol. Treatment will be based on the extent of both CNS and extraneural disease. See Appendix III for details.

### 3.6 ENROLLMENT ON STUDY

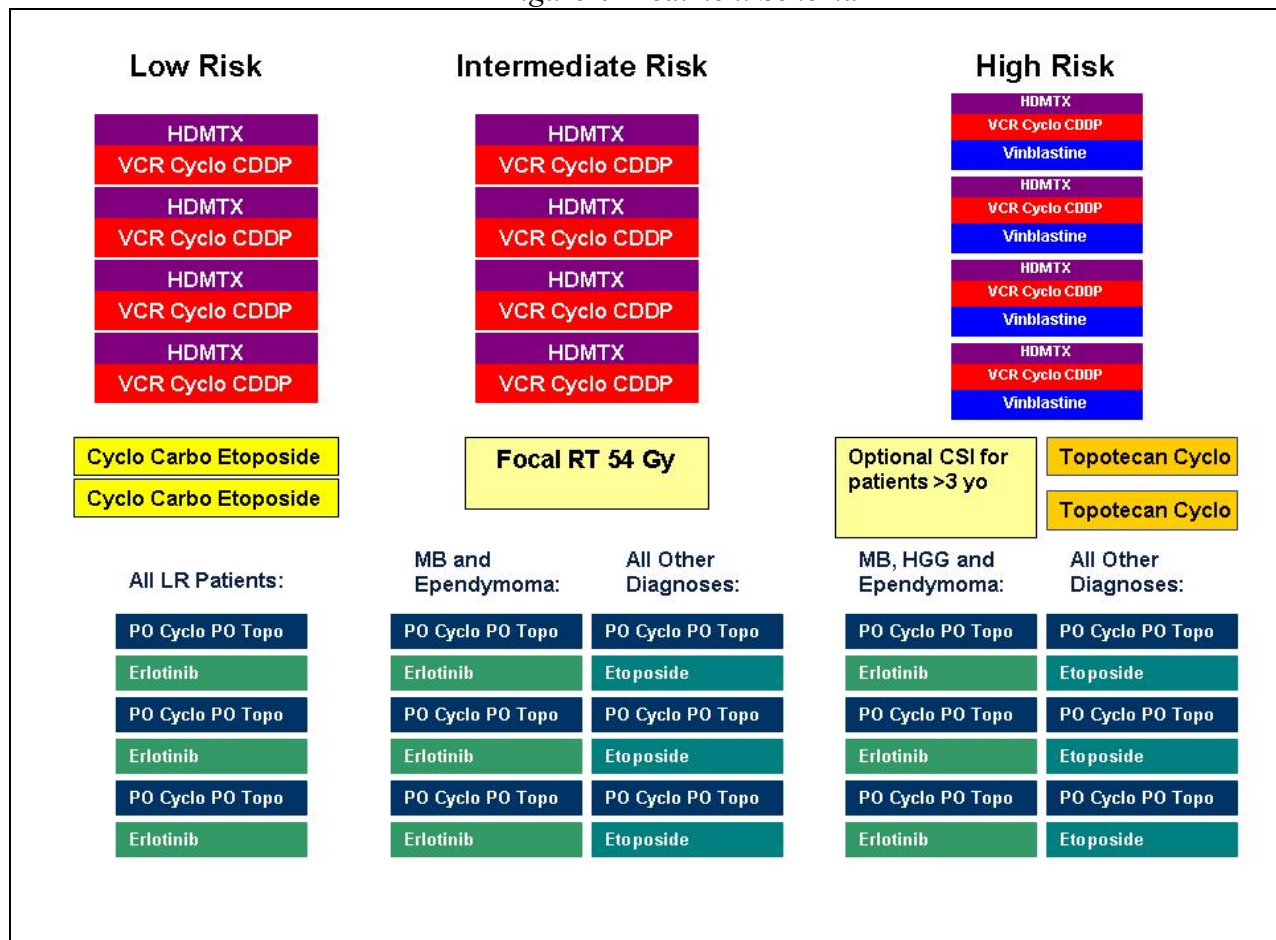
St. Jude enrollments. A member of the study team will confirm potential participant eligibility as defined in Section 3.1-3.2, complete and sign the ‘Participant Eligibility Checklist’. The study team will enter the eligibility checklist information into the Patient Protocol Manager (PPM) system. Eligibility will be reviewed, and a research participant-specific consent form and assent document (where applicable) will be generated. The complete signed consent/assent form(s) must be faxed or emailed to the CPDMO at [REDACTED] to complete the enrollment process.

The CPDMO is staffed 7:30 am-5:00 pm CST, Monday through Friday. A staff member is on call Saturday, Sunday, and holidays from 8:00 am to 5:00 pm. Enrollments may be requested during weekends or holidays by calling the CPDMO “On Call” cell phone [REDACTED] or referencing the “On Call Schedule” on the intranet).

Collaborating site enrollments: Collaborating Site research participants should be registered at St. Jude within 24 hours of enrollment at the site. Email notification should be sent to the PI or to the Neuro-Oncology Research Office [redacted] upon enrollment. The completed eligibility checklist and entire signed consent form should be faxed to the Neuro-Oncology Research Office at [redacted] within 3 working days of obtaining consent and confirming eligibility. Follow with a phone call to [redacted] or email to [redacted] to ensure the fax has been received. The Neuro-Oncology Research Office is staffed 8:00 am to 5:00 pm Central time Monday – Friday, excluding holidays. St. Jude research staff will then complete enrollment with the St. Jude CPDMO.

#### 4.0 TREATMENT PLAN

Figure 4 Treatment Schema



**TABLE 7 - OVERVIEW OF DRUG DOSES**

***Induction***

All Patients:

Methotrexate 5 g/m<sup>2</sup> day 1 over 24 hours (for patient > 31 days at enrollment), or  
2.5 g/m<sup>2</sup> day 1 over 24 hours (for patients ≤ 31 days at enrollment)  
Leucovorin 15 mg/m<sup>2</sup> IV/PO q6hr x 5 doses starting at hour 42 from start of MTX  
Vincristine 1 mg/m<sup>2</sup> IV day 8 and day 15 (±2) days  
Cisplatin 75 mg/m<sup>2</sup> IV day 8  
Cyclophosphamide 1.5 g/m<sup>2</sup> IV day 9  
Mesna 375 mg/m<sup>2</sup> IV x 4 doses  
Filgrastim 5 mcg/kg IV/SQ starting day 10, continue until ANC >2000 post-nadir

High-risk patients only:

Vinblastine 1 mg/m<sup>2</sup> IV days 17, 19, 22, 24, 26

*Repeat for 4 cycles 16 weeks total*

***Consolidation***

Low-risk

Cyclophosphamide 1.5 g/m<sup>2</sup> IV day 1  
Mesna 375 mg/m<sup>2</sup> IV x 4 doses day 1  
Carboplatin (AUC 5) day 2  
Etoposide 100 mg/m<sup>2</sup> IV days 1, 2  
Filgrastim 5 mcg/kg IV/SQ starting  
day 3, continue until ANC >2000  
post-nadir

*Repeat for 2 cycles total*

Intermediate-risk

Focal RT (54 Gy)

High-risk

Topotecan IV over 4 hours targeted to  
AUC 140±20 ng/mL\*hr days 1-5  
Cyclophosphamide 600 mg/m<sup>2</sup> IV days 4, 5  
Mesna 200 mg/m<sup>2</sup> IV x 3 doses day 4, 5  
Filgrastim 5 mcg/kg IV/SQ starting day 6,  
continue until ANC >2000 post-nadir

*Repeat for 2 cycles total*

OR

Optional for patients > 3 years old at end of  
induction only:

CSI (dose based on induction response)

***Maintenance***

Cycle A

Cyclophosphamide 30 mg/m<sup>2</sup> PO daily on days 1-21  
Topotecan 0.8 mg/m<sup>2</sup> PO daily on days 1-10  
Rest day 22-28

Cycle B

Erlotinib 90 mg/m<sup>2</sup> PO daily on days 1-28 (Medulloblastoma, High-Grade Glioma and  
Ependymoma Patients Only)

OR

Etoposide 50 mg/m<sup>2</sup> PO daily on days 1-21 (All other diagnoses)

*Repeat for 3 cycles each (A1-B1-A2-B2-A3-B3) 24 weeks total*



## 4.1 SURGICAL MANAGEMENT

In patients with localized medulloblastoma and other brain tumors, gross total resection results in a substantial survival benefit. For patients with nodular desmoplastic medulloblastoma, the presence of residual tumor will require enrollment on the intermediate risk arm of the study, thereby exposing patients to the additional long-term sequelae of RT. For these reasons, the maximal resection that can be achieved without undue risk to the patient should be attempted prior to trial enrollment. Decisions about initial resectability will be at the discretion of the local neurosurgeon. In rare instances, the feasibility of completely resecting residual tumor may change as a result of induction chemotherapy; in these cases a “second-look” operation may be performed prior to consolidation therapy, after discussion with the principal investigator. Second-look surgery will not affect subsequent treatment; patients who undergo such surgery will continue therapy on the intermediate risk arm.

## 4.2 INDUCTION

Each induction cycle may begin when:

- ANC >500/mm<sup>3</sup> (after G-CSF discontinued)
- Hgb >8 g/dL (with or without transfusion support),
- Platelets >50,000/mm<sup>3</sup> (without support), and
- Total bilirubin < 3X the institutional ULN.

Induction cycles are expected to last 28 days, but cycles may start early (prior to day 29 of the previous cycle) in order to accommodate scheduling concerns, as long as the above criteria are met.

### 4.2.1 High-dose methotrexate (day 1)

#### 4.2.1.1 Methotrexate administration

Hydration for methotrexate should be given according to institutional standard of care; the following are recommended:

- Whenever possible, pre-hydration should start the day prior to HDMTX administration and consist of D<sub>5</sub>W + 40 mEq NaHCO<sub>3</sub>/L + 20 mEq KCl/L to run at 125 mL/m<sup>2</sup>/hour and continue until MTX level is < 0.5 microM.
- If prehydration is not started before admission, pre-hydrate with D<sub>5</sub>W + 40 mEq/L NaHCO<sub>3</sub>/L + 20 mEq KCl/L at 200 mL/m<sup>2</sup>/hr for at least two hours (hours -2 to 0) prior to start of methotrexate. Decrease hydration rate to 125 mL/m<sup>2</sup>/hour when MTX starts and continue until MTX level is < 0.5 microM.



or a history of typhlitis with any chemotherapy, leucovorin should begin at 36 hours with all subsequent MTX; if toxicity recurs, the baseline leucovorin dosage should also be increased.

#### 4.2.2 Criteria for proceeding to day 8 chemotherapy with vincristine and cyclophosphamide

Day 8 chemotherapy may begin when:

- ANC  $>500/\text{mm}^3$
- Hgb  $>8 \text{ g/dL}$  (with or without transfusion support)
- Platelets  $>50,000/\text{mm}^3$  (without support)

Dose modifications for vincristine or cisplatin may be required in the event of hyperbilirubinemia or renal dysfunction, respectively, as detailed in section 4.4. Transient elevations in ALT and AST are expected with high-dose methotrexate administration and do not require delay or modification of day 8 or 9 chemotherapy. Patients who experience mucositis with methotrexate administration should proceed with day 8 chemotherapy as long as severity at day 8 is  $\leq$  grade 2, symptoms are improving, and plasma methotrexate level is undetectable. Day 8 chemotherapy may be given one day early or up to 3 days late at the discretion of the treating physician as long as the above criteria are met.

#### 4.2.3 Vincristine (Days 8 & 15)

- $1 \text{ mg/m}^2$  (max dose 2 mg) given by IV push prior to cisplatin on day 8.
- Repeat vincristine on day 15 ( $\pm 2$  days).

#### 4.2.4 Cisplatin (Day 8)

Hydration for cisplatin should be given according to institutional standard of care; the following are recommended:

- Pre-hydrate with  $200 \text{ mL/m}^2/\text{hr}$  of  $\text{D}_5\text{W}\frac{1}{2}$  NS for at least 2 hours and until UOP  $> 2 \text{ mL/kg/hr}$ .
- Hour 0-0.25: Mannitol  $10 \text{ g/m}^2$  infused over 15 min.
- Hour 0.25-6.25: Cisplatin  $75 \text{ mg/m}^2$  IV over 6 hrs in  $\text{D}_5\text{W}\frac{1}{2}$  NS  $1000 \text{ mL/m}^2$  + mannitol  $10 \text{ g/m}^2$ .
- Hour 6.25-24: Continue hydration with  $\text{D}_5\text{W}\frac{1}{2}$  NS + 10 mEq KCl/L at  $125 \text{ mL/m}^2/\text{hr}$  I.V.

Urine output: Calculate input and output (I/O) q 8 hrs x 24 hrs post completion of cisplatin. Urine output should be at least  $2 \text{ mL/kg/hr}$  x 24 hrs post completion of cisplatin.

If output falls below 2 mL/kg/hr and no fluid deficit, give a mannitol bolus of 6 g/m<sup>2</sup> over 15 min. If no response within 2 hours, call physician.

If negative fluid balance within the previous 8 hrs and not overall fluid positive within the previous 24 hrs, replace any deficits with ½ NS 1 mL: 1 mL over the next 4 hrs.

#### 4.2.5 Cyclophosphamide (Day 9)

Hydration: Patients will be receiving post-cisplatin hydration, which should provide adequate pre-hydration for cyclophosphamide. Monitor electrolytes and make appropriate changes in hydration, if necessary. Cyclophosphamide should not be started until urine specific gravity is ≤ 1.010 and urine RBC <50/HPF.

- -15 min: Mesna 375 mg/m<sup>2</sup> IV in D<sub>5</sub>W over 15 min.
- Hour 0-1: Cyclophosphamide 1500 mg/m<sup>2</sup> IV in D<sub>5</sub>W over one hour (NOTE: Cyclophosphamide dose is lower in cycles with topotecan; see section 4.3.3.1.2 for cyclophosphamide dosing during those cycles).
- Hour 1-24: Continue hydration with D<sub>5</sub> W½ NS + 10 mEq KCl/L at 125 mL/m<sup>2</sup>/hr + KCl 20 mEq/L.
- Hours 3, 6, and 9: Mesna 375 mg/m<sup>2</sup> IV in D<sub>5</sub>W over 15 min.
- Maintain urine output >2 mL/kg/hour to prevent hemorrhagic cystitis.

#### 4.2.6 Filgrastim (beginning Day 10)

Begin filgrastim, 5 mcg/kg/day SQ/IV daily, 24 to 36 hrs after cyclophosphamide infusion is complete. Continue until the absolute neutrophil count is greater than 2000/μL after nadir. Filgrastim must be stopped at least 48 hours before the next cycle of chemotherapy, and may be administered at home after proper instruction.

#### 4.2.7 Vinblastine (*high-risk patients only*)

- 1 mg/m<sup>2</sup> given by IV push on days 17, 19, 22, 24, 26.
- Scheduling of vinblastine may be adjusted as long as all 5 doses are administered and at least 36 hours elapses between doses.

#### 4.2.8 Carboplatin substitution for cisplatin Day 8 for ototoxicity (see section 4.5.5)

- Hour 0-1: Carboplatin; Total dose to be given in D<sub>5</sub>W over 1 hour with the dose based on an AUC of 5.5 mg/mL/min according to the following formula:

$$\text{Dose (in mg/m}^2\text{)} = \text{AUC} \times [(0.93 \times \text{GFR}) + 15]$$

NOTE: GFR is given in mL/min/m<sup>2</sup>

#### 4.3 CONSOLIDATION

Consolidation may begin when:

- ANC >500/mm<sup>3</sup> (after G-CSF discontinued)
- Hgb >8 g/dL (with or without transfusion support)
- Platelets >50,000/mm<sup>3</sup> (without support)

##### 4.3.1 Consolidation for low-risk patients

###### 4.3.1.1 *Cyclophosphamide and Etoposide (Day 1)*

Hydration for cyclophosphamide should be given according to institutional standard of care; the following are guidelines:

- Prehydrate with D<sub>5</sub> W<sup>1</sup>/<sub>2</sub> NS at 125 mL/m<sup>2</sup>/hour for at least 2 hours.
- 15 min: Mesna 375 mg/m<sup>2</sup> IV in D<sub>5</sub>W over 15 min.
- Hour 0-1: Cyclophosphamide 1500 mg/m<sup>2</sup> IV in D<sub>5</sub>W over 1 hour
- (NOTE: *Cyclophosphamide dose is different in cycles with topotecan; see section 4.3.3.1.2 for cyclophosphamide dosing during those cycles*).
- Hour 1-2: Etoposide 100 mg/m<sup>2</sup> IV in NS over 1 hour.
- Hour 2-24: Continue hydration with D<sub>5</sub>W<sup>1</sup>/<sub>2</sub> NS + KCl 10 mEq/L at 125 mL/m<sup>2</sup>/hr x 22 hours, then decrease rate to 65 mL/m<sup>2</sup>/hour .
- Hour 3, 6 and 9: Mesna 375 mg/m<sup>2</sup> IV in D<sub>5</sub>W over 15 min.
- Maintain urine output >2 mL/kg/hour x 24 hours from the start of the cyclophosphamide infusion to prevent hemorrhagic cystitis.

###### 4.3.1.2 *Carboplatin and Etoposide (Day 2)*

- Hour 0-1: Carboplatin; Total dose to be given in D<sub>5</sub>W over 1 hour with the dose based on an AUC of 5 mg/mL/min according to the following formula:

$$\text{Dose (mg/m}^2\text{)} = \text{AUC} \times [(0.93 \times \text{GFR}) + 15]$$

NOTE: GFR is given in mL/min/m<sup>2</sup>

- Hour 1-2: Etoposide 100 mg/m<sup>2</sup> IV in NS over 1 hour

###### 4.3.1.3 *Filgrastim (Beginning Day 3)*

Begin filgrastim, 5 mcg/kg/day SQ/IV daily, 24 to 36 hrs after carboplatin infusion is complete. Continue until the absolute neutrophil count is greater than 2000/μL after nadir. Filgrastim must be stopped at least 48 hours before the next cycle of chemotherapy, and may be administered at home after proper instruction.

#### 4.3.2 Consolidation for intermediate-risk patients

Consolidation for intermediate risk patients will consist of focal irradiation to the tumor bed. (See section 4.6 for RT guidelines). Intermediate risk patients who have not reached 12 months of age by week 17 of treatment should receive consolidation and maintenance therapy according to the low-risk treatment plan in order to delay RT until the age of 12 months. They should then receive focal RT, followed by further maintenance therapy to complete a total of six courses of maintenance therapy. For example, a patient who is 9 months old at week 17 should receive both cycles of low-risk consolidation chemotherapy (carboplatin/cyclophosphamide/etoposide), followed by one cycle of maintenance therapy (PO cyclophosphamide/topotecan), then focal RT, and then the remaining five courses of maintenance therapy, starting with cycle B1. Radiation therapy should begin no later than 6 weeks after the start of the last cycle of chemotherapy. Additional time will be allowed for patients who require surgery following induction chemotherapy and those who experience medical complications.

#### 4.3.3 Consolidation for high-risk patients

##### 4.3.3.1 Topotecan and cyclophosphamide chemotherapy

Topotecan/cyclophosphamide will be administered to all patients <3 years of age at week 17, and patient  $\geq 3$  years of age at week 17 whose parents have elected not to pursue CSI.

##### Topotecan (Days 1 - 5):

Hr 0-4: Topotecan will be given at an initial dose based on the patient's age at the time of receiving chemotherapy in 200 mL D<sub>5</sub>W to infuse over 4 hours at 50 mL/hr.

Age time of receiving chemotherapy	<6 months	6 - 11.9 months	$\geq 12$ months
Topotecan dose	2 mg/m <sup>2</sup>	2.5 mg/m <sup>2</sup>	3.5 mg/m <sup>2</sup>

Topotecan pharmacokinetic studies will be performed for all patients. Day 1 plasma topotecan concentrations will be used to adjust dosage, if needed, to attain a target topotecan lactone AUC of 140 $\pm$ 20ng/mL\*hr. Blood samples (1 to 2 mL) will be obtained prior to the topotecan infusion, and at 5 minutes, 1 hour and 3 hours after the end of the infusion. Dose changes should be made as soon as the results of pharmacokinetic analysis are available. For St. Jude patients, dose adjustments will typically be made for the topotecan doses administered on Day 2 and thereafter. For all patients, pharmacokinetic analysis must be completed and any dose adjustment instituted no later than the Day 4 topotecan dose. Please see Section 8.2.3 for details of pharmacokinetic studies.

*Cyclophosphamide (Day 4 and 5)*

Cyclophosphamide will be given after the topotecan infusion on days 4 and 5 of each consolidation cycle.

Hydration for cyclophosphamide should be given according to institutional standard of care; the following are guidelines:

- Prehydrate with D<sub>5</sub>W<sup>1/2</sup> NS plus 10 mEq KCl/L at 125 mL/m<sup>2</sup>/hr I.V., starting after day 3 topotecan infusion complete and continuing for 24 hours after the completion of day 5 cyclophosphamide.
- Hr 3:45 Mesna 200 mg/m<sup>2</sup> IV in D<sub>5</sub>W over 15 min
- Hr 4-5: Cyclophosphamide 600 mg/m<sup>2</sup> IV in 100 mL D<sub>5</sub>W to infuse over 1 hour on days 4 and 5 only. (*NOTE: cyclophosphamide dose is different in cycles with cisplatin/vincristine or carboplatin/etoposide; see sections 4.2.5 and 4.3.1.1 respectively for cyclophosphamide dosing during those cycles*).
- Hr 7 and 10 (3 and 6 hrs from start of cyclophosphamide): Mesna 200 mg/m<sup>2</sup> IV in D<sub>5</sub>W over 15 min.
- Cyclophosphamide pharmacokinetic studies will be performed for patients enrolled in the optional cyclophosphamide PK component of the trial. For those patients only, PK levels will be drawn immediately prior to the infusion, at the end of the 1 hr infusion, and 3 and 6 hours after the end of the infusion on both days, and 24 hours after the end of the day 5 infusion. See section 8.2.2 for details.

*Filgrastim (Beginning Day 6)*

Begin filgrastim, 5 mcg/kg/day SQ/IV daily, 24 to 36 hrs after day 5 cyclophosphamide infusion is complete. Continue until the absolute neutrophil count is greater than 2000/ $\mu$ L after nadir. Filgrastim must be stopped at least 48 hours before the next cycle of chemotherapy, and may be administered at home after proper instruction.

<b>TABLE 8 TREATMENT PLAN AND TESTING FOR TOPOTECAN AND CYCLOPHOSPHAMIDE CONSOLIDATION</b>						
<i>DOSE DAY</i>	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>	<i>6</i>
Topotecan IV	X	X	X	X	X	
Cyclophosphamide IV				X	X	
Topotecan Blood Samples* (required for all patients)	X*					
Cyclophosphamide Blood Samples** (for St. Jude patients who consent to optional PK study)				X**	X**	
Dose Adjustment		X				
G-CSF						X
<p>* Topotecan PK samples will be taken before infusion begins and at 5 minutes, 1 hour, and 3 hours after the end of the infusion for all patients.</p> <p>** For St. Jude patients enrolled on the optional cyclo PK component of the trial, samples will be taken pre-infusion, at the end of infusion, and 3 and 6 hours after the end of the infusion on days 4 and 5, and 24 hours after the end of the day 5 infusion.</p>						

#### 4.3.3.2 Craniospinal irradiation

Patients  $\geq 3$  years of age at week 17 will be offered craniospinal irradiation (See section 4.6 for RT guidelines).



4.4 MAINTENANCE TREATMENT

Each maintenance cycle may begin when ANC >500/mm<sup>3</sup>, Hgb >8 g/dL (with or without transfusion support), platelets >50,000/mm<sup>3</sup> (without support), total bilirubin <1.5X the institutional ULN, and ALT <3X the institutional ULN. For patients receiving CSI, maintenance should be started 28 (± 3) days after the last RT dose. No break in treatment is required for patients receiving focal RT (intermediate risk patients); these patients should start maintenance within 14 days of the last RT dose, as long as the above criteria are met. Patients receiving consolidation chemotherapy on the low-risk or high-risk arm should start maintenance within 8 weeks of the start of consolidation chemotherapy. If criteria for starting maintenance are not met within 7 days of the planned start date, contact the principal investigator, Dr. Amar Gajjar.

4.4.1 Drug doses for Maintenance

Patient diaries should be maintained for all oral drugs. Caretakers should be interviewed to assess drug compliance when patient drug diaries are unavailable. In such cases, documentation will be provided by the study team in lieu of a diary.

<b>Table 9 Maintenance Therapy</b>	
<b><i>Cycle A</i></b>	
<i>Day</i>	<i>Treatment</i>
1-10	Cyclophosphamide 30 mg/m <sup>2</sup> PO daily PO and Topotecan 0.8 mg/m <sup>2</sup> PO daily
11-21	Cyclophosphamide 30 mg/m <sup>2</sup> PO daily
22-28	Rest
<b><i>Cycle B</i></b>	
1-28	Erlotinib 90 mg/m <sup>2</sup> PO daily (Medulloblastoma, High-Grade Glioma and Ependymoma Patients Only)
1-21	Etoposide 50 mg/m <sup>2</sup> PO daily (All Other Diagnoses)
<i>Maintenance cycles will be repeated for a total of 24 weeks (A1-B1-A2-B2-A3-B3)</i>	

#### *4.4.2 Specific drug administration guidelines*

For all oral drugs, if the patient vomits within 30 minutes of taking the dose, the dose should be repeated. If vomiting occurs between 30 minutes and 5 hours of taking the dose, this should be recorded on the patient diary but the dose should not be repeated.

Oral drug administration represents a particular challenge in young children. In order to assess compliance with the oral treatment regimen, a trough level will be drawn for all patients at all sites during the routine blood draw at week 2 or 3 of cycles A1 (cyclophosphamide) and B1 (erlotinib). See sections 8.2.2 and 8.2.4 for details.

##### *Cyclophosphamide*

For all patients at all sites, required cyclophosphamide trough levels will be drawn to assess compliance during week 2-3 of cycle A1 and again during week 2-3 of cycle A3.

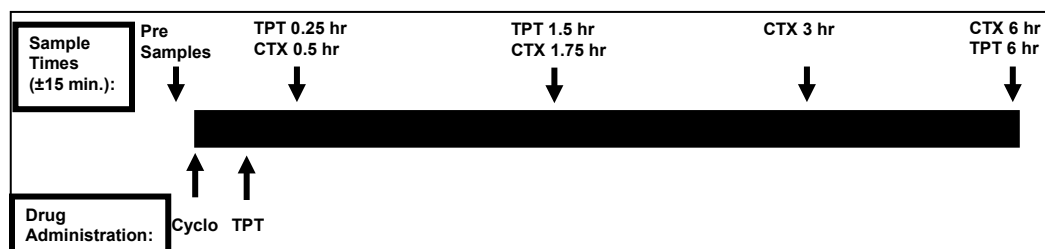
For St. Jude patients enrolled in the optional cyclophosphamide PK component of the trial, cyclophosphamide pharmacokinetic studies will be performed during cycle A1. For those patients only, PK levels will be drawn prior to the dose and at 0.5, 1.75, 3, and 6 hours after cyclophosphamide administration on day 1 of cycle A1. As administering oral medications to very young children can be challenging, flexibility in the timing of drug administration and PK sampling is necessary; every effort should be made to obtain samples within 15 minutes before or after the schedule time point. On the day of PK testing, cyclophosphamide must be administered prior to topotecan administration. See Table 9 and section 8.2.2.

##### *Topotecan*

The IV topotecan formulation will be administered orally during the maintenance phase. The drug may be mixed with a liquid or flavored syrup (e.g., cherry, chocolate) of the patient's choice. The specific diluent used should be recorded on the patient diary.

Topotecan pharmacokinetic studies will be performed during cycle A1 for St. Jude patients enrolled in the optional oral topotecan PK component of the trial. For those patients only, PK levels will be drawn prior to the dose and at 0.25, 1.5, and 6 hours after topotecan administration on day 1 of cycle A1. As administering oral medications to very young children can be challenging, flexibility in the timing of drug administration and PK sampling is necessary; every effort should be made to obtain samples within 15 minutes before or after the scheduled time point. See Figure 6 and section 8.2.3.

Figure 6: Treatment Plan and Testing for Oral Cyclophosphamide and Topotecan



#### 4.4.2.3 Erlotinib

Erlotinib should be preferentially taken with water at the same time each day at least 1 hour before or 2 hours after a meal. Patients who are unable to swallow whole tablets may take crushed tablets dispersed in a small amount of applesauce, flavored syrup (e.g., cherry, chocolate), or other liquid, depending on patient preference. The specific diluent used should be recorded on the patient diary. The erlotinib dose will be calculated based on the body-surface area at the start of each cycle of treatment. The calculated dose should be rounded to the nearest 25 mg.

For all patients at all sites, required erlotinib trough levels will be drawn to assess compliance during week 2-3 of cycle B1 and again during week 2-3 of cycle B3.

For St. Jude patients enrolled in the optional erlotinib PK component of the trial, erlotinib pharmacokinetic studies will be performed during cycle B2. For those patients only, PK levels will be drawn prior to erlotinib administration and at 1, 2, 4, 8 and 24(±2) hours after erlotinib administration on day 1 of cycle B2. Erlotinib should be held until after the 24 hour PK level has been drawn. See section 8.2.4.

## 4.5 DOSE MODIFICATIONS DURING TREATMENT

### 4.5.1 Methotrexate dose modifications

Hepatotoxicity: If total bilirubin is  $>3X$  the institutional ULN, obtain direct bilirubin and proceed with chemotherapy if direct bilirubin is  $<1.5X$  the institutional ULN. If direct bilirubin is  $\geq 1.5X$  the institutional ULN the methotrexate dose may be held or reduced after consultation with the study principal investigator.

Nephrotoxicity: Substantial nephrotoxicity from high-dose methotrexate administration is not expected, but nephrotoxicity due to cisplatin administration may affect methotrexate administration. If serum creatinine is  $>1.5X$  the upper limit of normal by age-appropriate institutional laboratory standards, creatinine clearance should be measured by a Technetium 99 plasma clearance study, and dose adjustments made as follows:

- Creatinine clearance  $>75\%$  of institutional lower limit of normal (LLN): proceed with methotrexate without dose adjustment. Monitor plasma MTX levels and clinical status closely and consider additional hydration and/or early leucovorin.
- Creatinine clearance 50-75% of institutional LLN: Reduce the calculated methotrexate dose by 50% until GFR  $>75\%$  of institutional LLN and then resume full dose.
- Creatinine clearance  $<50\%$  of institutional LLN: withhold methotrexate until the creatinine clearance rises above 50% of institutional LLN. If methotrexate dose is delayed  $>7$  days due to creatinine clearance  $<50\%$  of institutional LLN omit methotrexate dose and proceed with vincristine/cisplatin/cyclophosphamide with appropriate cisplatin dose adjustment when creatinine clearance is adequate (see section 4.5.5).

### 4.5.2 Vincristine toxicity

Seizures: hold one dose, then reinstitute at  $0.5 \text{ mg/m}^2$  (1 mg maximum) while anticonvulsants are continued. If seizures do not recur, return to full dosage at the time of next scheduled dose.

Neurotoxicity (grade 3/4, foot drop, severe paresis, disabling paresthesias) or ileus: Hold one dose, resume vincristine at  $0.5 \text{ mg/m}^2$  (1 mg maximum), and then return to full dosage when symptoms resolve.

Jaw Pain: Treat with analgesics (NOT salicylates). Do not hold or reduce vincristine.

Hepatotoxicity: If total bilirubin is  $>1.5X$  the institutional ULN, hold vincristine dose. If total bilirubin is  $>$  institutional ULN but  $\leq 1.5X$  institutional ULN, administer vincristine at  $0.5 \text{ mg/m}^2$ .

4.5.3 IV cyclophosphamide administered during induction or low-risk consolidation:

Hematopoietic toxicity: Delay cyclophosphamide until the ANC is  $\geq 500/\text{mm}^3$  and platelet count  $\geq 50,000/\text{mm}^3$ .

Hemorrhagic cystitis: The patient should be taken off treatment if patient has Grade 4 toxicity.

4.5.4 PO cyclophosphamide administered with topotecan during maintenance:

Check CBC weekly during first cycle. If  $\text{ANC} < 250/\text{mm}^3$  or platelets (unsupported)  $< 50,000/\text{mm}^3$ , hold cyclophosphamide until counts recover to  $\text{ANC} > 500/\text{mm}^3$  and platelets  $> 50,000/\text{mm}^3$ , then restart at 75% of prior dose. Missed doses should not be made up, and dose reduction should be maintained for the next cycle of oral cyclophosphamide/topotecan. If no significant myelosuppression as defined above occurs during first maintenance course, CBC may be checked every two weeks during subsequent courses. If myelosuppression as defined above recurs during a subsequent course, the duration of oral topotecan should be reduced to 7 days for all remaining courses.

4.5.5 Cisplatin toxicity

Ototoxicity: A decrease in auditory acuity at frequencies above the normal hearing range (4000-8000 Hz) is expected. For Chang Grade 1a or 1b ototoxicity, do not modify dose. For Chang Grade 2b ototoxicity, reduce cisplatin dose by 50% for all subsequent courses. For Chang Grade 3 ototoxicity hold cisplatin and do not restart unless follow-up audiograms show improved hearing function. See Appendix IV for Chang ototoxicity grading. Although we have changed the grade levels for dose modification in amendment 5.0, we are dose reducing based on approximately the same hearing loss as measured at the specified frequencies as indicated in the previous grading scale. A comparison of the CCG and Chang scales is provided in Appendix IV.

Carboplatin may be substituted for cisplatin during induction for patients having Grade 4 ototoxicity or bi-lateral hearing loss after having prior cisplatin dose reduction. The PI must approve of the substitution on a case by case basis. Carboplatin should be administered as per section 4.2.8.

Nephrotoxicity: If serum creatinine is  $>1.5X$  the upper limit of normal by age-appropriate institutional laboratory standards, creatinine clearance should be

measured by a Technetium 99 plasma clearance study, and dose adjustments made as follows:

- Creatinine clearance >75% of institutional lower limit of normal (LLN): Proceed with cisplatin without dose adjustment.
- Creatinine clearance 50-75% of institutional LLN: Reduce the calculated cisplatin dose by 50% until GFR >75% of institutional LLN and then resume full dose.
- Creatinine clearance <50% of institutional LLN: withhold cisplatin until the creatinine clearance rises above 50% of baseline value – repeat Tc99 plasma clearance weekly until clearance is adequate to start cisplatin.

If the cisplatin dose is delayed >14 days due to creatinine clearance <50% of institutional LLN delete cisplatin. If cisplatin is deleted from therapy then carboplatin may be substituted at an AUC of 5.5 after consulting with the PI.

#### 4.5.6 Vinblastine toxicity

Peripheral neuropathy: Hold until resolution for grade 3 or 4 foot drop, severe paresis, disabling paresthesias. Resume at 50% dose and then escalate to full dosage when symptoms resolve.

Hepatic toxicity: If total bilirubin is >1.5X the institutional ULN, hold vinblastine dose. If total bilirubin is > institutional ULN but  $\leq 1.5X$  institutional ULN, administer vinblastine at 0.5 mg/m<sup>2</sup>.

Fever/neutropenia: Vinblastine should be given through periods of febrile neutropenia or infection, as long as there is no evidence of life-threatening consequences (e.g., acidosis, hypotension, septic shock) and signs of local infection are well-controlled with appropriate treatment.

Myelosuppression: As blood counts are expected to drop due to the effects of cisplatin and cyclophosphamide, vinblastine should not be held for myelosuppression. If chemotherapy is delayed more than 7 days due to myelosuppression, the vinblastine dose should be reduced by 25% for subsequent cycles.

#### 4.5.7 Carboplatin toxicity

Hold carboplatin for a serum creatinine compatible with a grade 3 renal toxicity (>3X -6X the age-appropriate institutional ULN). When creatinine returns to pre-treatment values, repeat Tc99 DTPA plasma clearance and recalculate carboplatin dosing.

#### 4.5.8 IV etoposide administered during consolidation (for low-risk patients only):

Hypersensitivity reactions of grade 3 or less which are preventable using antihistamine and/or premedication are not dose limiting for etoposide. Patients

who develop an anaphylactic reaction to etoposide can be switched to etoposide phosphate. The dose of etoposide phosphate is the same as the dose of etoposide.

4.5.9 Oral etoposide administered during maintenance (for patients with any diagnosis other than medulloblastoma, high-grade glioma or ependymoma):

Check CBC weekly during first cycle. If ANC < 250/mm<sup>3</sup> or platelets (unsupported) <50,000/mm<sup>3</sup>, hold etoposide until counts recover to ANC >500/mm<sup>3</sup> and platelets >50,000/mm<sup>3</sup>, then restart at 75% of prior dose. Missed doses should not be made up, and dose reduction should be maintained for all subsequent cycles of oral etoposide. If no significant myelosuppression as defined above occurs during first maintenance course, CBC may be checked every two weeks during subsequent courses.

4.5.10 Erlotinib

Hepatotoxicity:

If total bilirubin is >1.5X the institutional ULN, obtain direct bilirubin and proceed with chemotherapy if direct bilirubin is <1.5X the institutional ULN. If direct bilirubin is ≥ 1.5X the institutional ULN, reduce erlotinib dose by 50%. If direct bilirubin does not improve to < 1.5X the institutional ULN within 7 days of dose reduction, hold erlotinib for the remainder of the cycle and restart at 45 mg/m<sup>2</sup> with the subsequent erlotinib cycle. If hyperbilirubinemia recurs, contact the PI, Dr. Amar Gajjar.

If ALT ≥3X the institutional ULN, reduce erlotinib dose to 45 mg/m<sup>2</sup>. If ALT does not improve to <3X within 7 days, hold erlotinib for the remainder of the cycle and restart at 45 mg/m<sup>2</sup> with the subsequent erlotinib cycle. If ALT elevation ≥ 3X the institutional ULN recurs, contact the PI.

Diarrhea and rash:

Grade 2 diarrhea and skin rash often resolve despite continued treatment with the drug. Therefore, supportive care should be instituted for grade 2 diarrhea or rash (see Section 4.7) and erlotinib should be continued at the same dose. If the toxicity persists for >7 days and the patient finds the toxicity intolerable despite supportive care, erlotinib should be held until the toxicity improves to ≤grade 1, then restarted at 75% of the original dose. If grade 2 intolerable toxicity lasting >7 days recurs after 2 dose reductions, contact the study PI. In the event of grade 3 or higher diarrhea or rash, hold erlotinib until resolution to ≤grade 1; re-start erlotinib at 75% of the original dose if resolution ≤7 days. Contact the study PI if grade 3 toxicity persists for >7 days or if grade 3 toxicity recurs after 1 dose reduction.

Interstitial Lung Disease (ILD):

Although quite rare, ILD can be life threatening. Therefore, patients should be monitored closely for symptoms consistent with ILD, such as new onset dyspnea

without an obvious cause. In the event that ILD is suspected, erlotinib treatment should be discontinued and the patient should receive appropriate medical management. Although there is no proven therapy, systemic corticosteroids are often provided. Erlotinib should not be restarted in those patients suspected of having drug-related ILD. On the other hand, erlotinib therapy can be resumed in patients with improved symptoms where ILD has been ruled out.

#### 4.6 RADIOTHERAPY GUIDELINES

##### 4.6.1 Overview

This protocol includes very young children with CNS embryonal tumors who receive multi-agent chemotherapy prior to the administration of radiation therapy. The guidelines for radiation therapy have been developed to ensure target volume coverage and reduce the side effects of treatment. Unique to this protocol is primary site irradiation using a 5mm clinical target volume (CTV) margin for intermediate and high-risk patients. Intermediate risk patients receive only primary site irradiation. High risk patients, older than the age of 3 years after induction chemotherapy, will be offered craniospinal irradiation (CSI) using response-based dosing. CSI will be followed by primary and metastatic site irradiation, when applicable. The primary site dose will be 54Gy for all patients. CSI doses will be 23.4Gy after complete response and 36-39.6Gy for less than complete response. Risk-classification has been defined as follows: intermediate-risk – non-metastatic and either residual primary site disease at enrollment or non-desmoplastic medulloblastoma (0 - <3 yrs of age); all medulloblastoma histological subtypes ( $\geq 3$  - <5 yrs of age), ATRT, PNET, CPC, or ependymoma; high-risk: metastatic M<sub>1-3</sub> tumor regardless of primary tumor location.

##### Electronic Data Transfer and Submission

For quality assurance, participating institutions will be required to submit radiation therapy planning data. Efforts will be made to streamline this process and to protect patient identity. After the completion of treatment, the final or composite treatment plan will be submitted electronically to St. Jude for clinical and research purposes.

The CSI component of treatment will be planned and administered in a conventional manner; however, irradiation of the primary site is amenable to a spectrum of conformal planning and delivery methods available at the collaborating institutions. Proton beam radiation therapy will be allowed; if this treatment modality is not available at another collaborating site, patients may receive such treatment at other institutions depending on family preference. For patients at collaborating sites who chose to receive proton beam irradiation it is recommended (but not required) that these patients get treated at the University of Florida Proton Therapy Institute (UFPTI). Participants from collaborating sites may be referred to other radiation oncology facilities that have the capability to deliver PBT. The guidelines for this study are generalized to provide consistency in the targeting, dosimetry and reporting. Investigator discretion will be used in various minor



aspects of treatment including the spinal dose for M<sub>2</sub> patients, cranial dose for M<sub>3</sub> (spine disease only), treatment of metastatic sites and residual pineal region tumor after 54 Gy; such treatment decisions should comply with the guidelines detailed in Table 10.

#### Guidelines and requirements for the use of proton beam therapy (PBT)

*As of October 29, 2015 the option to proton beam therapy was withdrawn from the protocol due to safety reasons. Please see section 12.4*

Proton beam therapy (PBT) will be allowed for intermediate-risk patients treated on this protocol at all participating sites. Patients enrolled at St. Jude may receive PBT by referral to the University of Florida Proton Therapy Institute (UFPTI) in Jacksonville, Florida. Only patients on the intermediate-risk arm will be referred for PBT at UFPTI. At the present time, investigators using PBT will be required to comply with the guidelines for the use of protons in National Cancer Institute sponsored cooperative group trials which specify that (1) only passively or actively scattered proton beams will be used; (2) the International Atomic Energy Agency IAEA TRS 398 protocol shall be used for beam calibration; (3) dose reporting will be in Cobalt Gy equivalent ( $1 \text{ CGE} = 1 \text{ proton Gy} * 1.1$ ) which is the same as ICRU 78 DRBE (RBE-weighted absorbed dose)<sup>300a</sup>; and that (4) radiation doses shall be prescribed to protocol specified definitions for gross and clinical target volumes. Throughout this protocol, CGE is to be used instead of Gy when referring to proton dose. For set-up uncertainties and target motion, additional margin, smearing, range of modulation will be added on a per beam basis. Whereas dose is to be specified in centigray (cGy)-to-muscle for photons, for PBT, the absorbed dose is specified with respect to water. In the event that scanning PBT becomes available, the guidelines will be amended after consideration by participating investigators.

The use of proton beam therapy remains experimental and as such, is offered as an optional, special research treatment modality as an alternative to standard photon therapy. Moreover, information is lacking about the combined effects of PBT and chemotherapy in young children. PBT may reduce side effects in children with brain tumors by sparing normal tissue compared with conformal or intensity modulated radiation therapy. Of note, we have seen changes in the MRI images of about half of the patients who have received proton beam radiation on this protocol to date (intermediate risk arm only<sup>367</sup>). These MRI changes are mostly transient signal abnormalities and enhancement in brain parenchyma, consistent with radiation-induced effects on normal-appearing tissue. There is a risk these changes could progress to radiation necrosis which is a radiation form of cell damage that results in the early death of those cells in living tissue. We are not sure what the changes on MRI mean. In many cases those changes resolve. In general, the affected patients did not manifest symptoms; however, in the case of one patient, those changes progressed to radiation necrosis and were manifested clinically based on the involved area of the brain.

Patients sent to UFPTI for PBT may have a component of their treatment simulated at St. Jude prior to referral. This procedure may include imaging using CT, MRI and PET, and the contouring or creation of target volumes and normal tissue structures. This may facilitate the treatment planning process at UFPTI and will create protocol specified target and normal tissue volumes to be used for research. Imaging data and target and normal tissue contours in electronic form will be provided to UFPTI collaborators with relevant clinical information and supportive imaging.

#### 4.6.2 Target volume definitions

The volumes that will be targeted and treated are defined in this section. The definitions for the 3-dimensional target volumes and treatment dosimetry should adhere as closely as possible to the ICRU Report-50<sup>300</sup> and Report 62<sup>301</sup> definitions whenever possible. Examples of target volumes for supratentorial and infratentorial tumors are provided in Figure 7.

Craniospinal Irradiation (CSI): The CSI volume includes the entire subarachnoid volume with special attention to identification and inclusion of the cribriform plate and temporal fossae; the full width of the spinal subarachnoid space and the inferior aspect of the thecal sac.

Gross Tumor Volume (GTV): The GTV includes all gross residual tumor and/or the tumor bed at the primary site based on the initial pre-operative imaging examination that defines the tissues initially involved with disease anatomically and the post-operative and pre-irradiation neuroimaging examinations that identify residual disease and/or the tumor bed. The GTV in most cases will be a contracted or collapsed tumor bed. Tissue defects resulting from surgical approaches will not be included as part of the GTV when not previously involved by tumor. *Investigators are strongly encouraged to register pre-operative MR imaging sequences that demonstrate tumor to assist in the delineation of the GTV.*

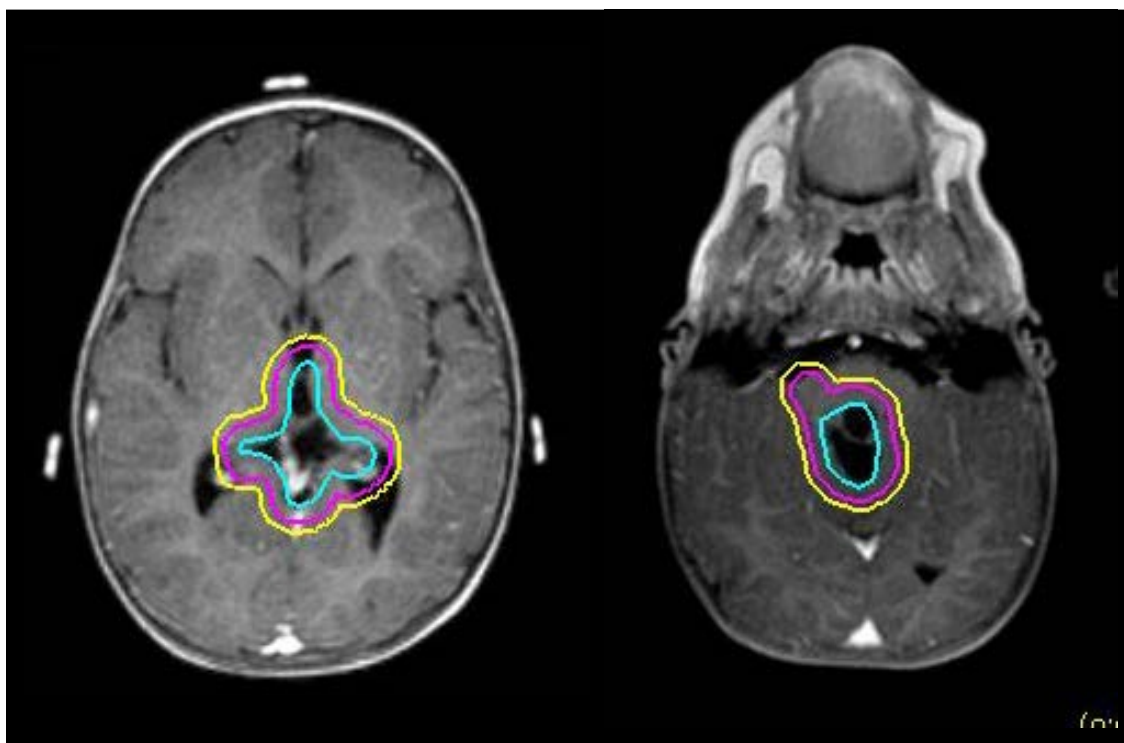
Clinical Target Volume (CTV): The CTV includes the GTV with an added margin that is meant to treat subclinical microscopic disease and is anatomically confined (i.e., the CTV is limited to the confines of the bony calvarium, falx and tentorium where applicable or extends up to but not beyond neuroanatomic structures through which tumor extension or invasion is certain not to have occurred); the CTV margin will be 5mm for all patients. When the GTV approaches the boundary of an anatomic compartment, the CTV will extend up to and include the boundary. *The CTV margin chosen for this study requires treatment planning MR and/or diagnostic MR imaging data with image section thickness  $\leq$  5mm.*

Planning Target Volume (PTV): A margin is added to the CTV in 3-dimensions to create the PTV. The PTV is geometric and not anatomically defined. The purpose of the PTV is to account for uncertainty in immobilization, image registration and daily variability in patient positioning. For this study the PTV margin is 3-5mm.

Given that the CTV is generally confined to the intracranial space, the PTV may extend into or beyond bone but is unlikely to extend beyond the surface of the patient. The GTV, CTV and PTV for patients treated with protons will be identical to those contoured or created for treatment using photons. The PTV margin chosen by the treating investigator requires treatment planning MR and/or diagnostic MR imaging data with imaging section thickness  $\leq$  the chosen PTV margin.

Metastatic Target Volume (MTV): Overt metastatic disease  $>5\text{mm}$  in maximal diameter at the time of treatment planning will define a volume or volumes for potential boost irradiation. The MTV will include the contoured lesion(s) with a geometric margin of 5mm. The treating investigator should be concerned about excessive irradiation of the intracranial or spinal volume and consider measures to minimize the aggregate volume of the combined MTV+PTV. The investigator has the option to evaluate the brain or spine with MR imaging near the completion of CSI and exclude metastatic sites that measure  $\leq 5\text{mm}$ .

**Figure 7 Target Volume Examples**



Post-contrast transverse T1-weighted MR images. Gross tumor (GTV=blue), clinical target (CTV=magenta) and planning target (PTV=yellow) volumes defined for post-operative, post-induction tumor/tumor bed in patients with supratentorial (left) and infratentorial (right) CNS tumors. Protocol specified CTV and PTV margins are 5mm and 3mm, respectively.

#### 4.6.3 Dosimetry and logistics

Craniospinal dosimetry: Dose will be measured at the midplane of the cranial volume and at the posterior border of the vertebral body the spinal field(s) including the dose at the central ray. Effort should be made to minimize inhomogeneity at the craniocervical junction when possible and the gradient of dose across the spinal cord.

*3-D dosimetry – target coverage:*

Photons: The prescription point for each 3-dimensional target volume is at or near the center of the volume and may be a point other than the central axis. The goal is to prescribe to the highest isodose surface that encompasses the PTV with the least inhomogeneity. The entire PTV should be encompassed within the 100% isodose surface, although 95% is acceptable (*i.e.*, 100% of the PTV receives at least 95% of the prescription dose). When inhomogeneity of the treatment plan delivers more than 105% of the prescription dose to more than 10% of the protocol defined spinal cord, optic chiasm or optic nerve, a reduction in the targeted volume or avoidance of the critical structure should be considered after 50.4 Gy even when coverage of the PTV, CTV or GTV is compromised. It has been observed that the dosimetry of superficial tumors can be problematic. Treatment planning systems demonstrate poor PTV coverage based on proximity to the skin surface. In such cases, bolus is not required and efforts should be made to verify coverage of the targeted volumes by other means.

Protons - The PTV, CTV and GTV will be used for dose prescription and reporting; however, dose coverage of the PTV using protons will differ compared to treatment using photons. The goal will be to deliver 100% of prescribed dose to 95% of the PTV (D95% = 54Gy). The PTV will be used to select the appropriate beam size and beam arrangements to achieve lateral coverage of the targeted volume and to minimize heterogeneity. The PTV will not be used to determine the distal range for the individual proton beams. The proton distal target margin will be determined per beam based on following distances and margins:

Proton Distal Target Margin = CTV + Range Uncertainty + Internal Margin

- CTV -the distal aspect of the CTV
- Range uncertainty - 1.5% of the water-equivalent range of the CTV at maximal depth ( $\geq 1\text{mm}$ )
- Internal margin - compensates for all variations in site, size and shape of the tissues contained in or adjacent to the CTV ( $\geq 1\text{mm}$ )

The proton distal range may be adjusted at the discretion of the treating radiation oncologist based on normal tissue dose concerns.

Motion management

Motion of the target volume is not anticipated for patients treated on this protocol; however, if target volume change is anticipated based on tumor cyst formation or expansion, post-operative brain shift, subdural fluid collections or hydrocephalus, MR or CT imaging will be prescribed during the six week course of treatment.

#### Field shaping and beam configuration

Field shaping for photons will be done with either customized cerrobend blocking or multileaf collimation. Field shaping for PBRT will rely on customized brass apertures. Every attempt should be made to minimize dose to organs at risk and limit heterogeneity of the high-dose volume without compromising coverage of the target volume. Three-dimensional conformal therapy (coplanar or non-coplanar), IMRT and PBT are required to minimize dose to normal surrounding structures. Due to uncertainties in the distal range of the proton beam in which the RBE may be greater than 1.1, single proton beam plans which stop in a critical organ will not be allowed. Individual proton beams which are a component of a multi-field proton beam, which stop within such an organ, may be allowed. Beam angles will be chosen to minimize range uncertainty; however, this will not be the sole criteria for beam angle selection.

3-D dosimetry - uniformity: Tissue inhomogeneity corrections are required. No more than 10% of the PTV should receive greater than 110% of the prescription dose. Whenever possible, treatment dosimetry should spare the spinal cord, brainstem, optic chiasm, and optic nerves from receiving more than the prescribed daily dose when not intimately associated with the target. It has been observed that the dosimetry of superficial tumors can be problematic. Treatment planning systems demonstrate poor target coverage based on proximity to the skin surface. In such cases, superficial compensators are not required and efforts should be made to verify coverage of the targeted volumes by other means.

Dose fractionation: Patients will receive one fraction of 1.8 Gy per day, five days per week. Exceptions will include logistical considerations owing to holidays, weather, equipment failure and the need to complete the course of treatment in the least number of elapsed days. This is typically  $42 \pm 2$  days.

**Table 10: Radiation Doses (Gy) by Treatment Volume**

<i>Disease Location And Risk Classification</i>	<i>CSI Dose</i>	<i>Primary Site Dose (†)</i>	<i>Brain/Spine Metastases</i>
<i>INFRATENTORIAL</i>			
Intermediate Risk	0	54	NA
High Risk (CR)	23.4	54	NA
High Risk (<CR)			
M <sub>1</sub>	36	54	NA
M <sub>2</sub>	36-39.6 (brain) 36-39.6 (spine)	54	54
M <sub>3</sub>	36-39.6 (brain) 36-39.6 (spine)	54	54 (brain) 50.4 (spine)
<i>SUPRATENTORIAL</i>			
Intermediate Risk	0	54	NA
High Risk (CR)	23.4	54	NA
High Risk (<CR)			
M <sub>1</sub>	36	54	NA
M <sub>2</sub>	36-39.6 (brain) 36-39.6 (spine)	54	54
M <sub>3</sub>	36-39.6 (brain) 36-39.6 (spine)	54	54 (brain) 50.4 (spine)
(†) Optional local boost for patients with residual pineal region disease after external beam radiation therapy (section 4.6.4.1)			

Treatment interruptions: CSI may be interrupted as necessary for medical status (e.g., ANC <500, fever/neutropenia, VP shunt surgery, neurologic or general medical problems). Treatment may be interrupted for platelet count <20,000 if the count cannot be supported to a level of  $\geq 40,000$ . Interruption shall be defined as missing >4 consecutive days during the course of irradiation.

Sequencing contingencies: When required, CSI will generally be given first, followed by supplemental irradiation (“boost therapy”) to the primary and metastatic sites (when applicable). Boost therapy may be initiated prior to craniospinal irradiation if required by the medical condition. In all cases when CSI is deferred, it should be initiated as soon as medically feasible, before the completion of boost therapy if feasible.

#### 4.6.4 Treatment planning and delivery techniques

Craniospinal irradiation: Patients may be simulated and treated in the prone or supine position using customized immobilization devices that allow for verification of cranial and spinal field junctions. Treatment will be given using standard techniques, encompassing the entire subarachnoid volume. The technique should assure coverage at the cribriform plate and temporal fossae; the full width of the spinal subarachnoid space (dosimetrically to include the medial aspect of the neural foramina) down to the bottom of the thecal space (as indicated by sagittal spinal MR).

Craniospinal irradiation-CT planning: CT studies obtained uniquely for the CSI planning process are allowed and may also serve as the basis for planning boost treatment. CT studies will be performed whenever possible and the information used for craniocaudal intensity modulation. For the cranial component of treatment, the external contours of the skull and neck are viewed in the AP direction and points are chosen to divide the cranial field into 2-4 fields to achieve a field-within-a-field plan and lateral homogeneity over the craniocervical junction. For the spinal component of treatment, the spinal cord and canal are contoured and viewed laterally and points are chosen to divide the spinal field into 4-6 fields to achieve field-within-a-field plan and antero-posterior homogeneity over the spinal cord and thecal sac. The following parameters are suggested: volume - cranial vertex to perineum; section thickness  $\leq 5\text{mm}$ ; image matrix -  $512^2$ . The administration of IV contrast is not required.

Craniospinal irradiation-prescribed doses: As noted in Table 10 high-risk patients who have a complete response to induction chemotherapy will receive 23.4Gy and others 36-39.6Gy. Investigator discretion will be used to determine the spinal dose for M<sub>2</sub> patients and cranial dose for M<sub>3</sub> patients. For example, a patient with M<sub>2</sub> disease might receive 39.6 Gy to the cranial volume and 36Gy to the spine; a patient with M<sub>3</sub> disease (spine only) might receive 36Gy to the cranium and 39.6Gy to the spine. The decision to decrease the dose from 39.6Gy to 36Gy for a portion of the craniospinal volume in either case may be driven by patient age or other factors. In the M<sub>3</sub> example, primary site irradiation should commence with the first fraction after 36Gy.

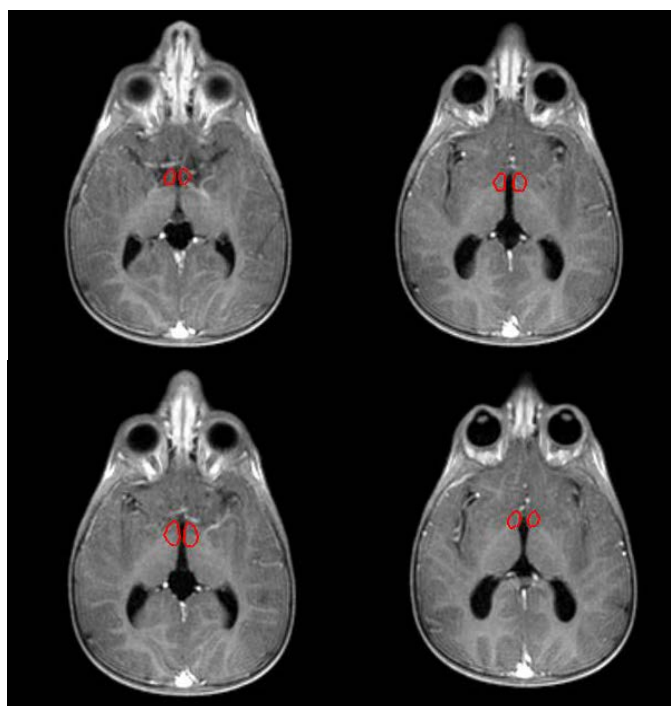
Primary site irradiation: The treatment planning objectives for the primary site component of treatment are to ensure target volume (PTV) coverage, minimize inhomogeneity, respect normal tissue tolerances and minimize dose to the supratentorial brain, cochleae and hypothalamic-pituitary unit. The full spectrum of conformal treatment techniques may be used including forward or inverse planned beam's eye view conformal radiation therapy and step and shoot or dynamic MLC intensity modulated radiation therapy. Proton beam radiation therapy is allowed.

Primary site irradiation – CT and MR planning: Patients may be simulated and treated in the supine or prone position. A treatment planning CT is required (contrast is optional) and should be performed as close to the start of primary or boost treatment as possible and will typically occur during the last week of CSI in those cases that require CSI. The following parameters are suggested: volume -

cranial vertex to thoracic inlet; section thickness – 2mm; image matrix 512<sup>2</sup>. Registration of MR to CT is encouraged using a 3-dimensionally acquired post-Gd T1-weighted data set or T2-weighted MR imaging data set formatted in the transverse plane and matching as closely as possible the section thickness of the CT study. Other data sets representing alternative MR sequences may be registered and used as needed. Whenever feasible the MR studies for RT planning should be obtained as close as possible to the start of treatment and about the time of simulation to account for changes in ventricular volumes, the operative site and extra-axial fluid collections. CT ONLY PLANNING - When MR registration is not available the following structures will be contoured: GTV, CTV, PTV, the outer (skin) surface of the head and neck regions, entire brain, eyes, optic nerves, optic chiasm, pituitary, hypothalamus, temporal lobes, spinal cord, cochleae.

CT AND MR PLANNING - When MR registration is available the following structures will be contoured on both CT and MR: outer (skin) surface of the head and neck regions, eyes and spinal cord. The outer surface volumes will be available for dose and unspecified tissue calculations, the ocular volumes may be used to verify laterality and the spinal cord volumes can be compared to assure similar flexion and extension which is critical in planning treatment for infratentorial tumors. For reporting purposes, only the cochleae, spinal cord and outer (skin) structures will be contoured on CT. It is preferred that the remainder of the structures will be contoured on MR including the GTV, CTV, PTV, entire brain, eyes, optic nerves, optic chiasm, brainstem (entire brainstem, brainstem surface and brainstem core) pituitary, hypothalamus, hippocampi and temporal lobes. An example of the hypothalamus is given in Figure 8. Examples and normal tissue tolerances for other critical structures are discussed in section 4.6.5.

**Figure 8** Transverse post-contrast T1-weighted MR images. The hypothalamus is contoured on four successive MR images as a bi-lobed structure bordering the anterior III<sup>rd</sup> ventricle (red).





Infratentorial primary site irradiation: The GTV is the post-operative residual tumor and/or tumor bed as defined in section 4.6.2, the CTV includes an anatomically confined 5mm margin in adjacent brain as defined in section 4.6.2 and the PTV includes a 3-dimensional geometric margin as defined in section 4.6.2. If the GTV is not anterior to the posterior aspect of the brainstem, the anterior border of the CTV may be limited to the center of the brainstem in axial extent. The primary site PTV will receive a cumulative dose of 54Gy.

Supratentorial primary site irradiation: The GTV is the post-operative residual tumor and/or tumor bed as defined in section 4.6.2, the CTV includes an anatomically confined 5mm margin in adjacent brain as defined in section 4.6.2 and the PTV includes a 3-dimensional geometric margin as defined in section 4.6.2. The primary site PTV will receive a cumulative dose of 54Gy; an additional local boost will be considered at the investigator's option for patients with residual pineal region tumor after external beam radiation therapy.

#### *Radiosurgery options*

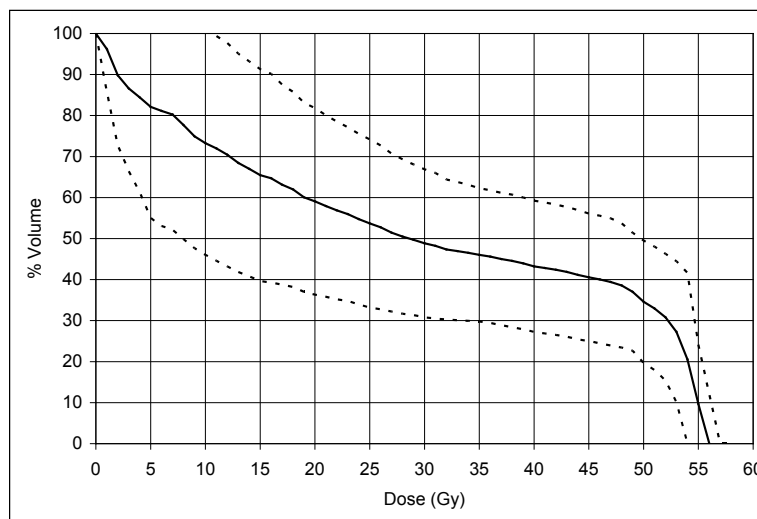
Metastatic site irradiation: Intracranial metastatic disease meeting the size criteria for treatment (> 5mm at the time of irradiation) should be treated concurrently with the primary site and when feasible joined to the primary site volume. The decision to treat separate or combined primary and metastatic boost volumes should be based on normal tissue irradiation. Spinal metastatic disease meeting the size criteria for boost treatment should be treated concurrently with the primary site treatment. As noted previously, aggregate intracranial boost volumes (MTV+PTV) should be minimized.

#### 4.6.5 Normal tissue tolerances

Spinal cord: For the purposes of this study, the upper aspect of the spinal cord begins at the inferior border of the foramen magnum and should be contoured on the treatment planning CT. For purposes of comparison and consistency with dose volume data, the spinal cord should be contoured on a number of images to be determined by the image section thickness (CT section thickness, n=number of images; 2mm, n=30; 2.5 mm, n=24; 3 mm, n=20; 4 mm, n=15). The treatment should be planned without compromising the prescription guidelines, to minimize the dose to the spinal cord and to avoid inhomogeneity that would have the spinal cord receiving > 1.8 Gy per day. An example of the spinal cord defined for image section thickness of 3.0 mm is included in Figure 10.

Dose guidelines based on Figure 9:

- $D_{90\%} \leq 2\text{Gy}$ ,  $D_{50\%} \leq 28\text{Gy}$  and  $D_{10\%} \leq 55\text{Gy}$  - Goal
- $D_{90\%} \leq 16\text{Gy}$ ,  $D_{50\%} \leq 50\text{Gy}$  and  $D_{10\%} \leq 56\text{Gy}$  – Maximum



*Figure 9: Spinal Cord Dose-Volume Histogram: Average brainstem dose volume histogram ( $\pm$ SD) corresponding to a low-risk of spinal cord myelopathy. These data are derived from the treatment of children with infratentorial ependymoma using a prescribed dose of 54Gy.*

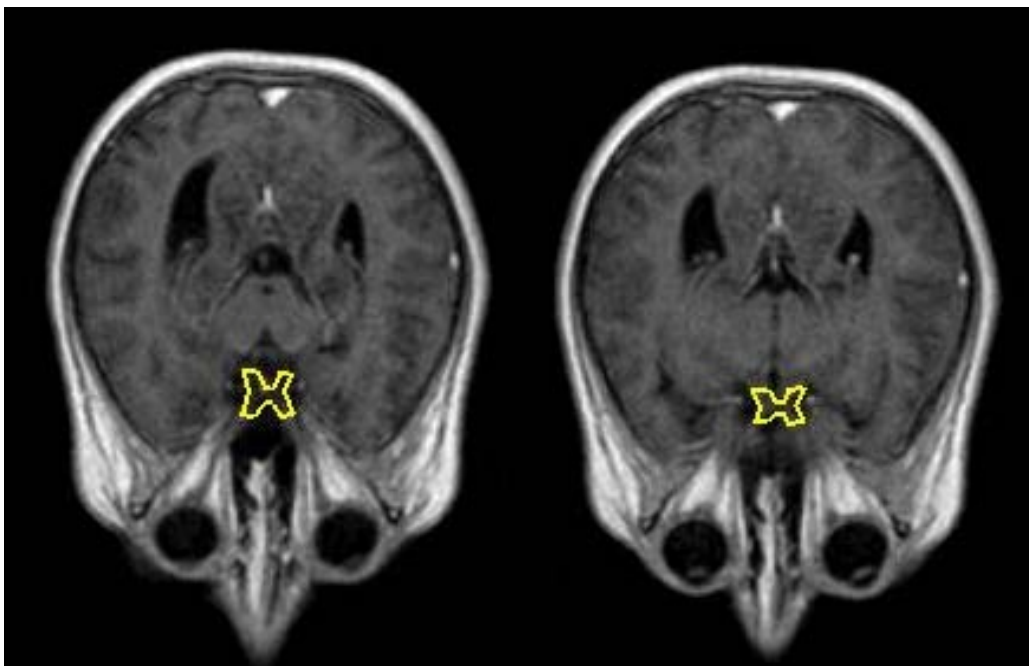
*Figure 10: Spinal Cord: Sagittal radiograph digitally reconstructed from treatment planning CT scan. Spinal cord (red) is contoured on 30 successive CT slices with superior aspect at the level of the foramen magnum. Protocol specified number of CT slices is dependent on slice thickness: 2mm = 30 slices, 3mm = 20 slices.*



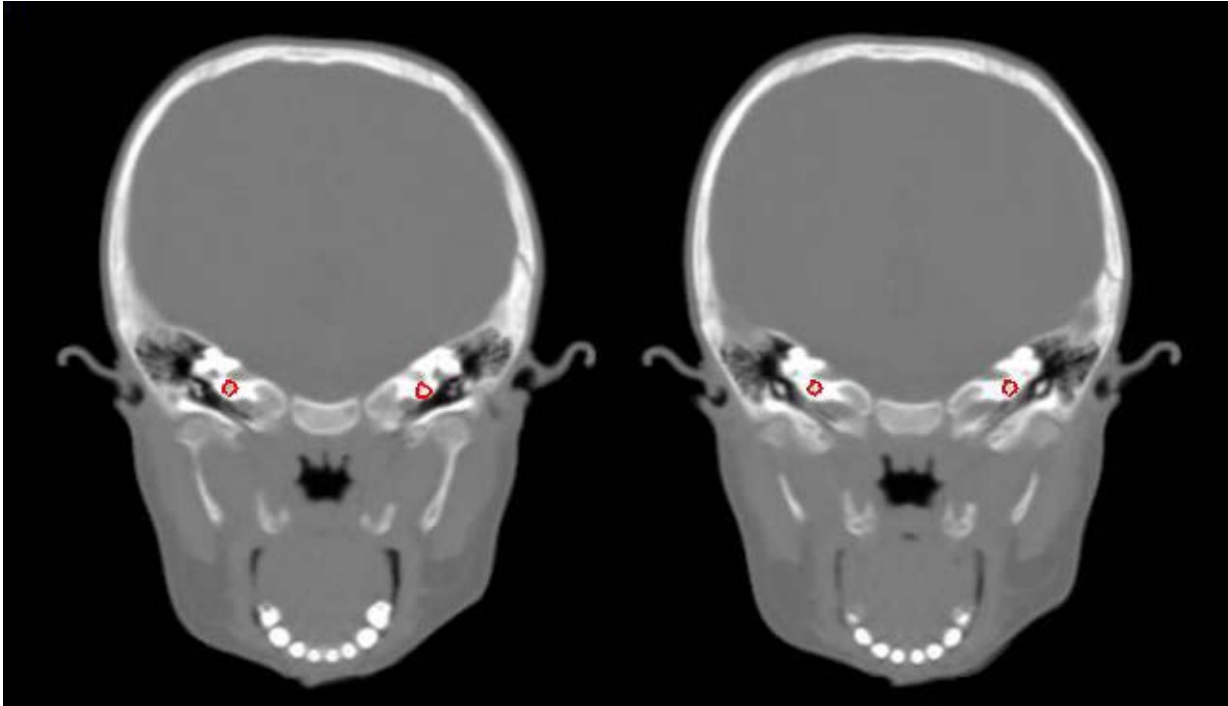
Optic chiasm: The mean dose to the optic chiasm should not exceed 56.7Gy and should be defined on CT or MR appearing on at least two consecutive images. If the mean dose may exceed 56.7Gy, the chiasm should be excluded from the treatment after 52.2Gy and receive no more than 1.25 Gy per fraction for the final treatment. These guidelines allow for the coverage of the target volumes to be compromised in selected cases. An example of the chiasm is included in Figure 11a.

Cochleae: Each cochlea will be contoured on the CT data as a circular structure within the petrous portion of the temporal bone. The size and position of the contoured cochlea will be confirmed by viewing the structures in three dimensions using the treatment planning system. The contour should appear on at least two successive CT images. The mean dose to the cochleae should be limited to 35Gy for intermediate risk patients and 45Gy for high-risk patients. An example of the cochleae is included in Figure 11a.

*Figure 11a: Optic Chiasm and Cochleae*



A) Post-contrast transverse T1-weighted MR images. The optic chiasm is contoured for the protocol with portions of the optic pathway on two successive MR images (yellow).

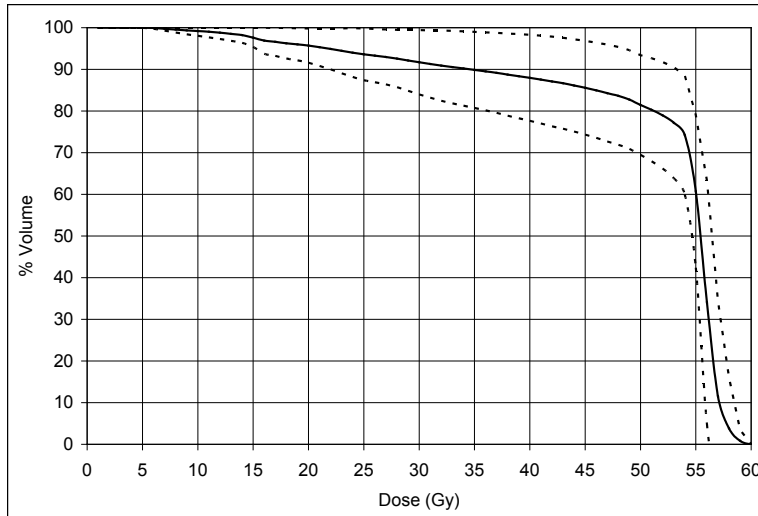


The brainstem will be contoured on the treatment planning CT and will include the midbrain, pons and medulla. The cranial extent will be inferior to the 3rd ventricle and optic tracts. The caudal extent will end at the foramen magnum. For photon planning the entire brainstem will be contoured. For proton planning the entire brainstem, brainstem surface and core will be contoured. The surface will include the superficial aspect of the brainstem to a depth of 3mm. The brainstem core will be the brainstem volume with the surface removed.

Dose guidelines based on Figure 11b:

- $D_{90\%} \leq 35\text{Gy}$ ,  $D_{50\%} \leq 54\text{Gy}$  and  $D_{10\%} \leq 56\text{Gy}$  - Goal
- $D_{90\%} \leq 54\text{Gy}$ ,  $D_{50\%} \leq 56\text{Gy}$  and  $D_{10\%} \leq 58\text{Gy}$  – Maximum

*Figure 11b: Brainstem Dose-Volume Curves: average brainstem dose volume histogram ( $\pm$ SD) corresponding to a low-risk of brainstem-related side effects for a 30 fraction treatment regimen.*<sup>300b</sup>



#### 4.7 CONCURRENT TREATMENT AND SUPPORTIVE CARE GUIDELINES

Hypomagnesemia: Can occur as a result of renal tubular wastage of magnesium caused by cisplatin. Symptoms include paresthesias, muscle cramps, weakness, and occasionally disorientation and seizures. If this occurs, give magnesium IV or PO.

Magnesium: Start supplementation 24 hrs after patient starts cisplatin therapy. Recommended doses for magnesium sulfate are 0.5-1.0 mEq/kg/day, starting with the first course of cisplatin (1 gm of magnesium sulfate equals 8.0 mEq). Alternative oral forms of magnesium (magnesium gluconate 90-150 mg/kg/day and magnesium oxide 10-30 mg/kg/day) are allowable by investigator's preference. Close monitoring of magnesium levels and magnesium supplementation are recommended throughout therapy.

Blood product support: All blood products will be irradiated with 1500-3000 cGy to prevent graft-versus-host disease. Filters to remove leukocytes should be used to prevent WBC sensitization. CMV-seronegative patients should receive CMV-negative blood products.

- *Platelets*: Transfuse as necessary to maintain the platelet count at  $>30,000/\text{mm}^3$  following chemotherapy. This threshold may be adjusted based on individual patient factors (e.g., history of hemorrhage, subdural fluid collections, expected imminent count recovery).
- *Red blood cells*: Therapy-induced anemia and reticulocytopenia are expected. Patients will be transfused as necessary with irradiated packed red blood cells to maintain a hemoglobin  $>8$  g/dL. This threshold may be adjusted based on institutional practice and individual patient factors (e.g., symptomatic anemia).

Pneumocystis prophylaxis: All patients will receive trimethoprim/ sulfamethoxazole (Septra). The following schedule is recommended, with doses given b.i.d. on Mon, Tues, and Weds. Septra prophylaxis should start during the first round of chemotherapy. Patients allergic to Septra may be treated either with Dapsone, monthly aerosolized pentamidine or monthly intravenous pentamidine or investigator preference.

<i>TABLE 12 SEPTRA DOSE</i>		
<i>BSA (m<sup>2</sup>)</i>	<i>Num of Single Strength Tablets*</i>	<i>Liquid Suspension (ml)**</i>
< 0.3	---	2.5
0.3 to 0.79	0.5	5
0.8 to 1.39	1	10
1.4 to 1.89	1.5	15
> 1.89	2	20
	*(sulfamethoxazole 400 mg and trimethoprim 80 mg)	** (sulfamethoxazole 40 mg/ml and trimethoprim 8 mg/ml)

Neutropenia/fever: Patients with fever (>38°C) and ANC <500 will be evaluated promptly, cultured, and treated with broad-spectrum antibiotics with an attempt to avoid nephrotoxic drugs. Antifungal treatment will be considered in patients who have had at least 5-7 days of IV broad spectrum antibiotics in the face of persistent fevers or with documented fungal colonization or CT evidence of systemic fungal infection.

Venous access: All patients will have a central line placed prior to starting chemotherapy. Placement of a double-lumen Hickman line or equivalent venous access is highly recommended.

Nutritional management: Any patient with greater than 10% weight loss over pretreatment baseline should begin enteral or parenteral nutritional support. Enteral support is preferred, and consideration of gastrostomy tube placement is encouraged for patients requiring more than transient nutritional support. For patients with weight loss < 10% compared to pretreatment baseline, the use of cyproheptadine (Periactin) as an appetite stimulant is encouraged. Megestrol acetate (Megace) may cause adrenal suppression when used for more than very brief intervals; the use of this drug as an appetite stimulant is strongly discouraged.

Antiemetics: The preferred antiemetic for cisplatin and cyclophosphamide is ondansetron (0.15-0.20 mg/kg) given 1/2 hr prior to infusion and every 6-8 hours thereafter for at least 3 doses. The use of dexamethasone as an antiemetic is strongly discouraged during chemotherapy. The preferred antiemetic regimen for radiation therapy is ondansetron (0.15-0.20 mg/kg) given prophylactically every 8 hours during radiation therapy and beginning before the first treatment. The prophylactic use of dexamethasone during radiation therapy is discouraged.

Anticonvulsants: Non-enzyme-inducing anticonvulsant use is allowed at any point during this therapy. Preferentially, patients should not receive CYP3A inducers or inhibitors during therapy. A list of all CYP3A inhibitors and inducers is provided in Appendix I. Every effort should be made to avoid placing patients on any drug listed in Appendix I during therapy. However, one of these medications may be used if no effective alternative drug is available, after discussion with the principal investigator.

Histamine-receptor 2 (H2) antagonists, proton-pump inhibitors and antacids for patients on erlotinib: H2 antagonists should be used only when deemed clinically necessary, but their administration should be at least 4 hours apart from that of erlotinib. Proton-pump inhibitors and antacids can be used only if symptoms are not controlled by H2 antagonists at least 4 hours apart from erlotinib administration.

Anti-diarrheal medications: Diarrhea is one of the most common adverse events described with the use of erlotinib. Loperamide or other anti-diarrheal medication should be considered for grade 1 diarrhea, and definitely used for any diarrhea  $\geq$  grade 2. The recommended doses of loperamide are shown in Table 13 below.

*Table 13 – Weight-Specific Guidelines for Therapeutic Use of Loperamide*

Weight	Initial (loading) loperamide dose	Subsequent daytime loperamide dose	Subsequent nighttime loperamide dose
8-10 kg	1 mg	0.5 mg q 3h	0.75 mg q 4h
10-20 kg	1 mg	1 mg q 3h	1 mg q 4h
20-30 kg	2 mg	1 mg q 3h	2 mg q 4h
30-43 kg	2 mg	1 mg q 2h	2 mg q 4h
> 43 kg	4 mg	2 mg q 2h	4 mg q 4h

Rash: Skin toxicities will be monitored by routine physical examination and managed symptomatically. Because secondary bacterial infections are common and can lead to more serious complications, topical or systemic antibiotics may be considered. Anecdotally, topical corticosteroids or a short course of systemic corticosteroids can be helpful for rash secondary to erlotinib.

RSV: RSV treatment should be initiated as per institutional guidelines. Please see Appendix VIII for recommendations.

## 5.0 DRUG INFORMATION

### 5.1 METHOTREXATE

Source and pharmacology: Methotrexate is a folate analogue that acts by inhibiting dihydrofolate reductase. Dihydrofolate reductase is an enzyme important in the conversion of folic acid to tetrahydrofolic acid, which is necessary in the synthesis of purine nucleotides and thymidylate. By inhibiting the production of tetrahydrofolic acid, methotrexate interferes with DNA, RNA and protein synthesis. Methotrexate is poorly and variably absorbed orally, with an average of  $\approx$  40% for

doses of  $\leq 30$  mg/m<sup>2</sup>. At higher dosages, the extent of absorption decreases. Methotrexate is approximately 50% protein bound. It distributes widely into body tissues and fluids with sustained concentrations in the kidney and the liver. Methotrexate undergoes metabolism by cytosolic aldehyde oxidase to hydroxy methotrexate. It is excreted mainly in the urine as unchanged drug with small amounts being excreted in the bile and feces. The percent recovered as unchanged drug in the urine is higher with short infusions than with prolonged infusions. Methotrexate has a biphasic elimination with an initial half-life of  $\approx 2$ -3 hours and a terminal half-life of 10-12 hours. Methotrexate may be “sequestered” in body fluid collections and eliminated slowly from these areas. Patients with effusions or GI obstruction should have plasma levels monitored closely for delayed excretion following high-dose methotrexate.

Formulation and stability: Methotrexate is supplied in single-dose vials containing 50mg, 100mg, 200mg, and 250 mg of methotrexate as a 25 mg/ml preservative-free solution and in vials containing 20mg, 50 mg, 100mg, 250 mg and 1000mg of lyophilized drug. It is also available in 2.5 mg tablets. Methotrexate preservative-free solution and lyophilized drug should be stored at room temperature and protected from light. Methotrexate tablets can also be stored at room temperature. The vials containing 20, 50, 100 and 250 mg of lyophilized product can be reconstituted by adding sterile water, 0.9% NaCl or D5W to a final concentration not exceeding 25 mg/ml. The 1000mg vials containing lyophilized product are reconstituted to a final concentration of 50 mg/ml.

Supplier: Commercially available

Toxicity: The dose limiting toxicities of methotrexate are generally bone marrow suppression, ulcerative stomatitis, severe diarrhea or acute nephrotoxicity. Toxicities reported frequently include nausea and vomiting, diarrhea, anorexia, hepatic toxicity and alopecia. Less common side effects include blurred vision, photosensitivity, anaphylaxis, headache, pneumonitis, skin depigmentation or hyperpigmentation, rash, vasculitis and encephalopathy. During high-dose methotrexate therapy, most patients experience a transient decrease in GFR, but renal failure can occur, particularly if the patient does not receive urinary alkalinization and aggressive hydration before, during and after receiving high dose methotrexate. Leucovorin rescue should be initiated within 48 hours of starting high-dose methotrexate and adjusted based on MTX levels to prevent bone marrow toxicity and mucositis. Leucovorin may also be necessary after IT administration, especially if IT methotrexate therapy is given to patients with renal dysfunction. Patients with Down’s Syndrome have a tendency to have delayed methotrexate clearance and a greater risk of toxicity, despite increased leucovorin rescue.

Dosage and route of administration: Methotrexate will be given at a dose of 5 g/m<sup>2</sup>/dose as an intravenous infusion over 24 hours on day 1 of each induction cycle except in patients  $\leq 31$  days of age at enrollment. These young infants will receive



methotrexate at a reduced dose of 2.5g/m<sup>2</sup>/dose. See treatment plan (section 4.2) for details.

## 5.2 LEUCOVORIN (FOLINIC ACID)

Source and pharmacology: Leucovorin is a racemic mixture of tetrahydrofolic acid, which is involved as a cofactor for 1-carbon transfer reactions in the synthesis of purine and pyrimidines. Leucovorin is a potent antidote for both the hematopoietic and reticuloendothelial toxic effects of folic acid antagonists by replenishing reduced folate pools. It is postulated that in some cancers, leucovorin enters and “rescues” normal cells from the toxic effects of folic acid antagonists, in preference to tumor cells, because of differences in membrane transport and affinity for polyglutamylation. Leucovorin is converted in the intestinal mucosa and the liver to 5-methyl-tetrahydrofolate, which is also active as a reduced folate. It is excreted primarily in the urine with minor excretion occurring in the feces.

Formulation and stability: Leucovorin is supplied in 5, 15 and 25 mg tablets and vials containing 50, 100 or 350 mg of leucovorin as a lyophilized powder. The tablets and the lyophilized powder can be stored at room temperature. The 50 mg and 100 vials can be reconstituted by adding 5 or 10 ml of sterile water or bacteriostatic water for injection respectively to yield a final concentration of 10 mg/ml. The 350 mg vials can be reconstituted with 17 ml of sterile water or bacteriostatic water for injection to yield a final concentration of 20 mg/ml. The reconstituted solution is stable for at least 7 days at room temperature. Leucovorin may be further diluted in 5% dextrose or 0.9% NaCl containing solutions.

Supplier: Commercially available

Toxicity: Leucovorin is generally well tolerated. Toxicities that have been reported uncommonly include rash, mild nausea, headache, and wheezing (possible allergic reaction). Intrathecal leucovorin is contraindicated and has caused neurotoxic deaths. There have been rare reports of leucovorin promoting seizures.

Dosage and route of administration: Leucovorin rescue will be given starting at hour 42 from the start of each methotrexate infusion. See treatment plan (section 4.2) for details.

## 5.3 VINCRISTINE (ONCOVIN®)

Source and pharmacology: Vincristine is an alkaloid obtained from the periwinkle (*Vinca rosea*) plant. It reversibly binds to microtubule and spindle proteins causing metaphase arrest. Vincristine has poor penetration into the CSF. It is approximately 75% protein bound. Extensive metabolism occurs in the liver. Excretion is primarily in the bile. A dosage decrease is recommended in patients with a bilirubin > 3 mg/dl.

Formulation and stability: Vincristine is supplied in multiple-dose 1 mg/ml vials containing 1 ml, 2 ml and 5 ml. The intact vials should be stored under refrigeration and protected from light.

Supplier: Commercially available

Toxicity: Dose limiting toxicity is neurotoxicity. This can be characterized by constipation and/or paralytic ileus, ptosis, vocal cord paralysis, weakness, jaw pain, abdominal pain, peripheral neuropathies, loss of deep tendon reflexes and “foot drop”. Peripheral neuropathy is often the first sign of neurotoxicity and is initially reversible. Other toxicities reported include alopecia, mild nausea and vomiting, SIADH, myelosuppression, orthostatic hypotension, optic atrophy, transient cortical blindness, and auditory damage. Acute shortness of breath and severe bronchospasms have been reported following the administration of vinca alkaloids. Myelosuppression is rare at usual doses. Vincristine is a vesicant and may cause severe tissue damage if extravasation occurs.

Dosage and route of administration: Vincristine 1 mg/m<sup>2</sup> will be administered by intravenous push on days 8 and 15 of each induction cycle. See treatment plan (section 4.2) for details.

#### 5.4 CISPLATIN (PLATINOL-AQ®)

Source and Pharmacology: Cisplatin is an inorganic heavy metal coordination complex that has biochemical properties similar to those of a bifunctional alkylating agent. It must undergo activation, by aquation, to form positively charged platinum complexes that react with nucleophilic sites on DNA. Cisplatin is cell-cycle, phase non-specific. Cisplatin rapidly distributes into tissues with the highest concentrations being present in the prostate, liver, and kidney and is highly protein bound (>90%). Cisplatin has an elimination half-life of 30-90 minutes. Platinum is bound to plasma constituents. Cisplatin is excreted predominantly via glomerular filtration, and dosage adjustments are necessary for patients with renal dysfunction.

Formulation and stability: Cisplatin is available in an amber multi-dose vial containing 100 mg of cisplatin at a concentration of 1 mg/ml. The unopened vial should be stored at room temperature and protected from light. The undiluted solution should not be refrigerated as a precipitate will form. Once opened, the vial should be discarded after 28 days if protected from light, or 7 days if not protected from light. Cisplatin should be further diluted in NS or 1/2 NS prior to administration.

Supplier: Commercially available

Toxicity: Nephrotoxicity is one of the major dose-limiting side effects of cisplatin. This toxicity may be irreversible and can be minimized by providing adequate hydration before, during and after cisplatin therapy. Other dose limiting toxicities

include myelosuppression, neuropathies and ototoxicity. Nausea and vomiting are common and can be severe. Delayed nausea and vomiting (occurring  $\geq 24$  hours after drug administration) can occur. Diarrhea, anorexia, electrolyte disturbances (especially hypomagnesemia), cardiac abnormalities, allergic reactions and transient elevations in liver enzymes can occur. Secondary cancers have been reported.

Dosage and route of administration: Cisplatin 75 mg/m<sup>2</sup> will be administered by intravenous infusion over 6 hours on day 8 of each induction cycle. See treatment plan (section 4.2) for details.

## 5.5 CYCLOPHOSPHAMIDE (CYTOXAN®)

Source and pharmacology: Cyclophosphamide is a nitrogen mustard derivative. It acts as an alkylating agent that causes cross-linking of DNA strands by binding with nucleic acids and other intracellular structures, thus interfering with the normal function of DNA. Cyclophosphamide is cell-cycle, phase non-specific. Cyclophosphamide is well absorbed from the GI tract with a bioavailability of  $> 75\%$ . Cyclophosphamide is a prodrug that requires activation. It is metabolized by mixed-function oxidases in the liver to 4-hydroxycyclophosphamide, which is in equilibrium with aldofosfamide. Aldofosfamide spontaneously splits into cyclophosphamide mustard, which is considered to be the major active metabolite, and acrolein. In addition, 4-hydroxycyclophosphamide may be enzymatically metabolized to 4-ketocyclophosphamide and aldofosfamide may be enzymatically metabolized to carboxyphosphamide which are generally considered to be inactive. Cyclophosphamide and its metabolites are excreted mainly in the urine. Dosage adjustments should be made in patients with a creatinine clearance of  $< 50$  ml/min.

Formulation and stability: Cyclophosphamide is available in 25 and 50 mg tablets. Cyclophosphamide is also available in vials containing 100, 200, 500, 1000 and 2000mg of lyophilized drug and 75 mg mannitol per 100 mg of cyclophosphamide. Both forms of the drug can be stored at room temperature. The vials are reconstituted with 5, 10, 25, 50 or 100 ml of sterile water for injection respectively to yield a final concentration of 20 mg/ml. Reconstituted solutions may be further diluted in either 5% dextrose or 0.9% NaCl containing solutions. Diluted solutions are physically stable for 24 hours at room temperature and 6 days if refrigerated, but contain no preservative, so it is recommended that they be used within 24 hours of preparation. Cyclophosphamide oral elixir can be compounded from simple syrup for administration to small children not able to swallow tablets. The simple syrup oral compound is stable for 8 weeks if refrigerated at 4°C and is stable for 8 days at room temperature.<sup>302, 302b</sup>

Supplier: Commercially available

Toxicity: Dose limiting toxicities of cyclophosphamide are bone marrow suppression and cardiac toxicity. Cardiac toxicity is typically manifested as congestive heart failure, cardiac necrosis or hemorrhagic myocarditis and can be

fatal. Hemorrhagic cystitis may occur and necessitates withholding therapy. The incidence of hemorrhagic cystitis is related to cyclophosphamide dose and duration of therapy. Forced fluid intake and/or the administration of mesna decrease the incidence and severity of hemorrhagic cystitis. Other toxicities reported commonly include nausea and vomiting (may be mild to severe depending on dosage), diarrhea, anorexia, alopecia, immunosuppression and sterility. Pulmonary fibrosis, SIADH, anaphylaxis and secondary neoplasms have been reported rarely.

Dosage and route of administration: Cyclophosphamide will be administered during four distinct phases of this treatment plan. See the appropriate sections of the treatment plan for details:

- *Induction (all patients):* 1500 mg/m<sup>2</sup> given by intravenous infusion over 1 hour on day 9 of each cycle. See section 4.2
- *Consolidation (low-risk patients only):* 1500 mg/m<sup>2</sup> given by intravenous infusion over 1 hour on day 1 of each cycle. See section 4.3
- *Consolidation (high-risk patients not electing CSI only):* 600 mg/m<sup>2</sup> intravenous infusion over 1 hour on day 4 and 5 of each cycle. See section 4.3
- *Maintenance (all patients):* 30 mg/m<sup>2</sup> by mouth on days 1 to 21 of each A cycle. See section 4.4.

## 5.6 MESNA (MESNEX®)

Source and pharmacology: Mesna is a synthetic sulfhydryl (thiol) compound. Mesna contains free sulfhydryl groups that interact chemically with urotoxic metabolites of oxazaphosphorine derivatives such as cyclophosphamide and ifosfamide. Oral bioavailability is ≈50%. Upon injection into the blood, mesna is oxidized to mesna disulfide, a totally inert compound. Following glomerular filtration, mesna disulfide is rapidly reduced in the renal tubules back to Mesna, the active form of the drug. Mesna and mesna disulfide are excreted primarily via the urine.

Formulation and stability: Mesna is available in 2 ml, 4 ml and 10 ml amps containing 100 mg/ml of mesna solution. The intact vials can be stored at room temperature. Mesna may be further diluted in 5% dextrose or 0.9% NaCl containing solutions to a final concentration of 1-20 mg/mL. Diluted solutions are physically and chemically stable for at least 24 hours under refrigeration.

Supplier: Commercially available

Toxicity: Mesna is generally well tolerated. Nausea and vomiting, headache, diarrhea, rash, transient hypotension and allergic reactions have been reported. Patients may complain of a bitter taste in their mouth during administration. Mesna may cause false positive urine dipstick readings for ketones.

Dosage and Administration: Mesna will be administered by intravenous infusion over 15 minutes in association with all intravenous cyclophosphamide doses. See sections 4.2 and 4.3 for details.

#### 5.7 G-CSF (FILGRASTIM) (NEUPOGEN®)

Source and pharmacology: G-CSF (granulocytic colony stimulating factor), is a biosynthetic hematopoietic agent that is made using recombinant DNA technology in cultures of *Escherichia coli*. G-CSF stimulates production, maturation and activation of neutrophils. In addition, endogenous G-CSF enhances certain functions of mature neutrophils, including phagocytosis, chemotaxis and antibody--dependent cellular cytotoxicity.

Formulation and stability: G-CSF is supplied in vials containing 300 mcg and 480 mcg of G-CSF at a concentration of 300 mcg/ml. The intact vials should be stored under refrigeration. The vials can be left out of refrigeration for 24 hours, but should be discarded if left at room temperature for longer periods of time. G-CSF can be drawn up into tuberculin syringes for administration and stored under refrigeration for up to 7 days prior to usage. G-CSF can be further diluted for IV infusion in 5% dextrose. Do not dilute in saline---precipitate may form. If the final concentration of this product is < 15 mcg/ml, it is recommended that albumin be added to a final concentration of 2mg/ml (0.2%) to minimize adsorption of the drug to infusion containers and equipment.

Supplier: Commercially available

Toxicity: G-CSF causes marked leukocytosis. Adverse reactions reported commonly include bone pain, thrombocytopenia, diarrhea, nausea, rash, alopecia, fever, anorexia and pain or bruising at the injection site. Allergic reactions, MI, atrial fibrillation, and splenomegaly have been reported rarely. G-CSF is contraindicated in patients with allergy to *E. Coli* derived products.

Dosage and route of administration: filgrastim 5 mcg/kg will be administered daily by subcutaneous or intravenous injection after all courses of myelosuppressive intravenous chemotherapy, and continue until the ANC > 2000/mm<sup>3</sup> post-nadir. See sections 4.2 and 4.3 for details.

#### 5.8 VINBLASTINE (VELBAN®)

Source and pharmacology: Vinblastine is an alkaloid extracted from the periwinkle plant (*Vinca Rosea*). It reversibly binds to microtubule and spindle proteins causing metaphase arrest. It may also block cellular utilization of glutamic acid, thereby inhibiting purine synthesis and urea formation via the citric acid cycle. Vinblastine is highly protein bound and poorly penetrates the CSF. Metabolism in the liver is extensive with one metabolite, deacetyl vinblastine, being more active than the

parent drug. The major route of elimination is via the bile. Dosages should be adjusted for patients with impaired liver function (bilirubin > 3 mg/dl).

Formulation and stability: Vinblastine is available in 10 ml vials containing 1mg/ml of vinblastine in solution. Intact vials of vinblastine solution and lyophilized vinblastine should be stored under refrigeration.

Supplier: Commercially available

Toxicity: The dose limiting toxicity is myelosuppression. Other toxicities reported commonly include alopecia, mild nausea and vomiting and constipation. Vinblastine is a vesicant and may cause severe tissue damage if extravasation occurs. Vinblastine rarely produces neurotoxicity characterized by peripheral neuropathy, loss of deep tendon reflexes, weakness, jaw pain and “foot drop”. This toxicity is much less common than with vincristine. Acute shortness of breath and severe bronchospasms have been reported following the administration of vinca alkaloids.

Dosage and route of administration: Vinblastine 1 mg/m<sup>2</sup> will be given intravenously on days 17, 19, 22, 24, and 26 of each induction cycle for high-risk patients only. See section 4.2 for details.

## 5.9 CARBOPLATIN (PARAPLATIN®)

Pharmacology: Carboplatin is an inorganic heavy metal coordination complex that has biochemical properties similar to those of a bifunctional alkylating agent. Carboplatin must undergo activation, by aquation, to form positively charged platinum complexes that react with nucleophilic sites on DNA, producing predominantly intrastrand DNA cross-links. Carboplatin is widely distributed in body tissues and fluids with highest concentrations in the kidney, liver, skin and tumor tissue. Carboplatin does not bind significantly to plasma proteins, but the activated form does bind to tissue and plasma proteins. In adults with normal renal function, the plasma elimination half-life is ≈2-3 hours. The elimination of carboplatin and its platinum-containing products is primarily dependent on glomerular filtration rate; therefore dosages may be adjusted for renal function.

Formulation and stability: Carboplatin is supplied in 20-ml amber vials containing 50, 150 or 450 mg of carboplatin as a white lyophilized powder. Vials should be reconstituted with sterile water for injection, D5W or 0.9% NaCl to give a concentration of 10 mg/ml and further diluted with D5W or 0.9% NaCl to concentration of 0.5 mg to 1.0 mg per ml. It is recommended that the reconstituted solution be discarded 8 hours after preparation.

Supplier: Commercially available.

Toxicity: Dose limiting toxicity is bone marrow suppression with thrombocytopenia being prominent. Nausea and vomiting of moderate severity are common. Hepatic dysfunction (after high doses), alopecia, ototoxicity, peripheral neuropathies, reversible renal toxicity, visual disturbances, and allergic reactions have all been reported less commonly. Pulmonary fibrosis is rare, but occurs most commonly in patients treated with cumulative doses > 1400 mg/m<sup>2</sup> or receiving bone marrow transplant dosages. Electrolyte abnormalities including hyponatremia, hypomagnesemia, hypocalcemia and hypokalemia can occur. Secondary cancers have been reported rarely.

Dosage and route of administration: Carboplatin targeted to an AUC of 5 (by the Calvert formula) will be administered intravenously over 1 hour on day 2 of each consolidation cycle for low-risk patients only. See treatment plan (section 4.3) for details. Carboplatin will be used as a substitute for cisplatin in patients with ≥ Grade 3 ototoxicity. See section 4.2.8 for details.

#### 5.10 ETOPOSIDE (VP-16) (VEPESID®)

Source and pharmacology: Etoposide is an epipodophyllotoxin derived from *Podophyllum pelatum*. It is thought to act mainly by inhibiting topoisomerase II, causing double and single strand DNA breaks. Etoposide is cell cycle, phase-specific, with activity in the G2 and S phases. Absorption of etoposide is approximately 30-40% and is highly variable and somewhat dose-dependent. It is extensively bound to serum proteins and is metabolized in the liver, including cytochrome P450 3A metabolism to several moieties that include a reactive oxidized species. Etoposide and its metabolites are excreted mainly in the urine with a smaller amount excreted in the feces. Dosage adjustments should be considered in patients with liver dysfunction, kidney dysfunction or hypoalbuminemia.

Formulation and stability: Etoposide is available in multi-dose vials containing 100mg, 150mg, 500mg and 1000mg of etoposide as a 20mg/ml solution and 30% alcohol. Etoposide is also available as a 50 mg capsule. The intact vials of etoposide solution should be stored at room temperature. The capsules should be stored under refrigeration. Etoposide solution should be diluted in D5W or 0.9% NaCl prior to administration. Solutions with a final concentration of 0.2 and 0.4 mg/ml are stable at room temperature for 96 hours and 24 hours respectively.

Supplier: Commercially available

Toxicity: Dose limiting toxicity is myelosuppression. Nausea and vomiting (usually of low to moderate severity), diarrhea, mucositis (particularly with high doses), alopecia and anorexia are fairly common. Hypotension can occur with rapid infusions. Other side effects reported less commonly include hepatitis, fever and chills, anaphylaxis and peripheral neuropathy. Secondary leukemia has been reported.

Dosage and route of administration: Etoposide 100 mg/m<sup>2</sup> will be administered intravenously over 1 hour on days 1 and 2 of each consolidation cycle for low-risk patients only. See section 4.3 for details. Etoposide 50 mg/m<sup>2</sup> will be given by mouth daily on days 1 through 21 of each maintenance cycle B for all patients with diagnoses other than medulloblastoma, high-grade glioma or ependymoma. See section 4.4 for details.

#### 5.11 TOPOTECAN (HYCAMTIN®)

Source and pharmacology: Topotecan is a semi-synthetic derivative of camptothecin that inhibits topoisomerase I activity. Topoisomerase I relieves torsional strain in the DNA helix during replication by inducing reversible single strand DNA breaks. Topotecan binds to the topoisomerase I-DNA complex and prevents relegation of single strand breaks. This results in double-strand DNA breaks during DNA synthesis when replication enzymes interact with the ternary complex formed by topotecan, topoisomerase I and DNA. Topotecan undergoes a rapid, pH-dependent opening of the lactone ring to yield a relatively inactive, hydroxy acid in plasma. At physiologic pH, topotecan exists mainly as the hydroxy acid. Metabolism occurs via a pH dependent hydrolysis of the lactone moiety. Metabolism to an N-demethylated metabolite represents a minor metabolic pathway. Mean elimination half-life is three hours. Approximately 30% of a dose is excreted in the urine. Dosage adjustment is recommended for patients with moderate to severe renal dysfunction).

Formulation and stability: Topotecan is available in single-dose vials containing 4 mg of topotecan as a lyophilized light yellow to greenish powder and 48 mg of mannitol. The intact vials should be stored at room temperature and protected from light. Each vial may be reconstituted with 4 ml of sterile water for injection to yield a final concentration of 1 mg/ml. Because the reconstituted solution does not contain a preservative, it is recommended that it be used immediately after reconstitution. The reconstituted solution can be further diluted with 5% dextrose or 0.9% NaCl containing solutions. Once diluted for administration, the drug is stable for at least 24 hours at room temperature and ambient lighting.

Topotecan for injection when reconstituted to 4 mg/4 mL with Bacteriostatic Water for Injection is chemically and physically stable for up to 28 days in amber colored plastic prescription bottles at both 5 degrees and 25 degrees centigrade.

Supplier: Commercially available

Toxicity: The dose-limiting toxicity is myelosuppression. Other toxicities reported commonly include nausea and vomiting, diarrhea, mucositis, abdominal pain, fever, rash, alopecia, anorexia, headache and flu-like symptoms. Toxicities reported less commonly include elevated liver function tests and paresthesias.

Dosage and route of administration: Topotecan will be administered by intravenous infusion over 4 hours on days 1-5 of each consolidation cycle for high risk patients



not receiving craniospinal irradiation. The initial dose of Topotecan will be based on patient's age as outlined in section 4.3.3.1.1 with subsequent doses adjusted, if necessary, to achieve a topotecan lactone AUC of  $140 \pm 20$  ng/mL\*hr. See section 4.3 for details. Topotecan will be also be administered orally at a dose of 0.8 mg/m<sup>2</sup> daily for the first 10 days of each cycle A of maintenance therapy for all patients. See section 4.4 for details.

#### 5.12 ERLOTINIB HYDROCHLORIDE (TARCEVA™)

Source and pharmacology: Erlotinib hydrochloride (erlotinib, Tarceva™) is an orally active anti-tumor agent being developed for the treatment of a variety of solid tumors. Erlotinib acts through direct and reversible inhibition of the EGFR and ERBB2 tyrosine kinase.

Formulation and stability: Tablets containing 25mg, 100mg, and 150mg of erlotinib are available. In addition to the active ingredient, tablets also contain lactose monohydrate, microcrystalline cellulose, sodium starch glycolate, sodium lauryl sulfate, and magnesium stearate. The oral tablets are conventional, immediate-release tablets containing erlotinib as the hydrochloride salt.

Supplier: Commercially available

The dose limiting toxicity after intermittent or continuous dosing of erlotinib in adults with cancer has been diarrhea. The dose limiting toxicities in a pediatric phase I trial were rash, diarrhea and hyperbilirubinemia (COG Spring 2006 Meeting, ADVL0214 Progress Report).

Table 14: Erlotinib - Known Toxicities and DLT

Common (21-100% Frequency)	Occasional (5-20% Frequency)	Rare (< 5% Frequency)
Skin rash/erythema Grade 1/2 (48-80%)	Dry skin Dry mouth	Grade 3/4 transaminase elevation
Diarrhea Grade 1/2 (20-60%)	Anorexia Headache	Grade 3/4 skin rash Grade 3/4 diarrhea
Nausea (2-30%)	Pruritus	Eyelash ingrowth/corneal pain or redness
Vomiting (10-22%)	Stomatitis	Corneal erosions
Fatigue (lethargy, malaise, asthenia) (20-30%)	Cough Decreased appetite	Uveitis Tearing Renal Toxicity Desquamation Dry eye Pneumonitis Pulmonary infiltrates, pulmonary fibrosis Blurry vision and hypersensitivity/allergic reaction Stevens-Johnson syndrome

Other side effects reported on erlotinib trials but with the relationship to erlotinib still undetermined include: anemia, leukopenia, neutropenia, leukocytosis, eosinophilia, thrombocytopenia, osteonecrosis, pulmonary embolism, deep vein thrombosis, rigors, pyrexia, alopecia, acne, sore throat, glossodynia, taste disturbance, cheilitis, constipation, abdominal pain or cramping, gastric ulceration and perforation, flatulence, dehydration, dysphagia, ischemic bowel, dyspepsia, pancreatitis, hematuria, epistaxis, melena, hemoptysis, GI bleeding, hyperbilirubinemia, transaminitis, increased alkaline phosphatase, pneumonia, urinary tract infection, infection with or without neutropenia, hypokalemia, hyponatremia, nasal dryness, rhinitis, depression, anxiety, insomnia, paresthesia, dizziness, cerebrovascular ischemia, uveitis, back pain, abdominal pain, dyspnea, increased creatinine, elevated BUN, proteinuria, renal failure, and endometritis.

Dosage and route of administration: Erlotinib will be administered at a dose of 90 mg/m<sup>2</sup> daily for 28 days during each cycle B of maintenance therapy for all patients with medulloblastoma, high-grade glioma or ependymoma. See section 4.4 for details.

**6.0 REQUIRED EVALUATIONS, TESTS, AND OBSERVATIONS**

**6.1 EVALUATIONS TO BE OBTAINED AT ENROLLMENT**

*Table 15 Baseline Evaluations*

<b>To Be Obtained Prior to Enrollment</b>	<b>To Be Obtained Within 2 Weeks of Enrollment</b>
<p><u>Required for All Patients:</u></p> <p>History and physical exam with height (cm), weight (kg), and BSA                      Lansky performance status (See Appendix II)                      Institutional diagnosis of medulloblastoma, medulloblastoma variant, PNET, PNET variant, ATRT, high-grade glioma, CPC, or ependymoma                      MRI of brain with and without contrast                      MRI of spine with contrast                      CSF cytology from lumbar spinal fluid*                      Operative Report                      Laboratory Studies: CBC with Diff, Chem 18                      Renal ultrasound to rule out synchronous renal rhabdoid tumor (required for ATRT patients only)</p>	<p><u>Required for All Patients:</u></p> <p>Auditory brainstem response (ABR)†                      Formalin-Fixed Paraffin-Embedded (FFPE) Tumor Block Specimen for Central Pathology Review                      Blood Sample for Genomic DNA</p>
<p><u>Required if Parent Consents to Optional Correlative Study:</u></p> <p>CSF for neurotransmitter studies</p>	<p><u>Required if Parent Consents to Optional Correlative Study:</u></p> <p>Neuropsychology Evaluation**</p> <p><u>Obtain as Clinically Indicated:</u></p> <p>Urinalysis , PT, PTT                      Ophthalmologic Evaluation                      Nutrition Evaluation                      Physical Therapy                      Occupational Therapy                      Speech Therapy</p>
<p>* Obtain CSF cytology from lumbar puncture even if the patient has a VP shunt or Ommaya reservoir; CSF from a VP shunt or Ommaya reservoir may be included only if lumbar puncture is medically contraindicated                      § Baseline neurological evaluation may be obtained within 4 weeks of enrollment                      † Every effort should be made to obtain ABR prior to first dose of cisplatin on day 8</p>	

6.2 EVALUATIONS DURING THERAPY

<i>Table 16 Evaluations During Induction and Consolidation</i>				
	Prior to Each Induction Cycle	Prior to Induction Cycle 3	End of Induction / Prior to Consolidation	Weekly During Induction and Consolidation
<i>CLINICAL EVALUATIONS</i>				
Complete history	X		X	
Physical exam	X		X	X
Height, weight, BSA	X		X	X
Auditory brainstem response (ABR)		X	X	
Tc-99 glomerular filtration rate (GFR) or creatinine clearance			Low risk	
Radiation oncology consult			High risk receiving CSI Intermediate risk	
<i>LABORATORY STUDIES</i>				
CBC with differential	X		X	X†
Serum creatinine and electrolytes (including Ca <sup>++</sup> , Mg <sup>++</sup> , Phos)	X		X	X**
ALT, AST, BUN, total bilirubin	X		X	
Urinalysis	X			
Endocrine screen and GH provocative test #(for consenting St Jude patients)			Intermediate risk	
Lumbar spinal fluid for CSF cytology*		X	X	
CSF for neurotransmitter studies (if enrolled in optional correlative study)		X	X	
<i>IMAGING STUDIES</i>				
MRI brain with and without contrast		X	X	
MRI spine		X	X	
RT simulation			High risk receiving CSI Intermediate risk	
RT CT scan			High risk receiving CSI Intermediate risk	
# per section 8.6 *CSF from VP shunt or Ommaya may be substituted only if lumbar puncture is medically contraindicated †Obtain CBC with diff. twice weekly while patient is receiving G-CSF **Weekly chemistry studies are not required during consolidation for patients receiving RT - obtain other studies as clinically indicated See Table20, Section 8.2 for PK studies				

Table 17 Evaluations During Maintenance and at End of Therapy							
Obtain studies prior to start of cycle except as noted							
	A1	B1	A2	B2	A3	B3	End of Therapy
Complete history	X	X	X	X	X	X	X
Physical exam	X	X	X	X	X	X	X
Height, weight, BSA	X	X	X	X	X	X	X
CBC with differential <sup>+</sup>	q wk	q wk	q wk	q wk	q wk	q wk	X
Serum creatinine and electrolytes (including Ca <sup>++</sup> , Mg <sup>++</sup> , Phos)	q 2wk	q 2wk	q 2wk	q 2wk	q 2wk	q 2wk	X
ALT, AST, BUN, total bilirubin	q 2wk	q 2wk	q 2wk	q 2wk	q 2wk	q 2wk	X
Urinalysis*	q 2wk	X	q 2wk	X	q 2wk	X	X
Endocrine Studies (TSH, Free T4, am cortisol, others as clinically indicated), including GH provocative test (for intermediate risk consenting St Jude patients) #							X
Trough PK level to assess compliance (all patients at all sites)	Cyclo wk 2 or 3	Erlotinib wk 2 or 3**			Cyclo wk 2 or 3	Erlotinib wk 2 or 3**	
Optional PK Studies (if enrolled in optional PK component)**	X			X			
Lumbar spinal fluid for CSF cytology†	X			X			X
CSF for neurotransmitter studies (if enrolled in optional correlative study)	X			X			X
MRI brain with and without contrast	X			X			X
MRI spine	X			X			X
Auditory brainstem response (ABR)	X						X
Neuropsychology evaluation §	X						X
<sup>+</sup> If no significant myelosuppression as defined as ANC < 250/mm <sup>3</sup> or platelets (unsupported) <50,000/mm <sup>3</sup> , occurs during first maintenance course, CBC may be checked every two weeks during subsequent courses. * Schedule for urinalysis may be modified per treating physician's discretion # per section 8.6 **See Table 20, Section 8.2, for details regarding PK studies; †CSF from VP shunt or Ommaya may be substituted only if lumbar puncture is medically contraindicated; **Medulloblastoma, high-grade glioma and ependymoma patients only. § Obtain time 2 evaluation 6 months (+/- 60 days) after baseline evaluation. Yearly assessments should be scheduled from time of enrollment (+/- 60 days). Obtain other studies as clinically indicated							

6.3 LONG-TERM FOLLOW-UP EVALUATIONS

<i>Table 18 Long-Term Follow-up Evaluations#</i>										
<i>EVALUATION</i>	Months Off Therapy #									Follow-up (to 60 months)
	3	6	9	12	16	20	24	30	36	
Complete history	X	X	X	X	X	X	X	X	X	q 6mo
Physical exam	X	X	X	X	X	X	X	X	X	q 6mo
Height, weight	X	X	X	X	X	X	X	X	X	q 6mo
CBC with differential	X	X	X	X	X	X	X	X	X	q 6mo
Serum creatinine and electrolytes (including Ca <sup>++</sup> , Mg <sup>++</sup> , Phos)	X	X	X	X	X	X	X	X	X	q 6mo
ALT, AST, BUN, total bilirubin	X	X	X	X	X	X	X	X	X	q 6mo
MRI brain with and without contrast	X	X	X	X	X	X	X	X	X	q 12mo
MRI spine	X	X	X	X	X		X		X	q 12mo
Lumbar spinal fluid for CSF cytology*	X	X	X	X	X		X		X	
CSF neurotransmitter studies (if enrolled in optional correlative study)	X	X	X	X	X		X		X	
Auditory brainstem response (ABR)				X			X		X	q 12mo
Neuropsychology evaluation §				X			X		X	q 12mo
Endocrine Studies (TSH, Free T4, am cortisol, others as clinically indicated) #		X##		X			X##		X	q 12mo
*CSF from VP shunt or Ommaya may be substituted only if lumbar puncture is medically contraindicated # All evaluations allow for ± 1 month from the stated schedule § Yearly assessments should be scheduled from time of enrollment (+/- 60 days) # per section 8.6 Please obtain additional studies for optimal patient care										

## 7.0 NEUROPATHOLOGY GUIDELINES

### 7.1 CENTRAL REVIEW OF PATHOLOGY

Central review of pathology will be performed for all patients in order to:

- Confirm trial eligibility based on the diagnosis of embryonal tumor, high-grade glioma, choroid plexus carcinoma, or ependymoma, with classification according to the WHO (2007) system
- Identify nodular / desmoplastic medulloblastomas for patient stratification
- Facilitate distribution of tissue for approved biological studies

### 7.2 METHODS

The working histological diagnosis will be provided by the local pathologist, but formalin-fixed paraffin-embedded (FFPE) tumor blocks and a copy of the pathology report should be submitted to St. Jude Children's Research Hospital for pathological review as soon as possible after finalization of the report.

The blocks chosen for central pathology review (and trial biological studies – see 8.1.2.2) should contain plenty of representative tumor. The address for submission of FFPE blocks is:



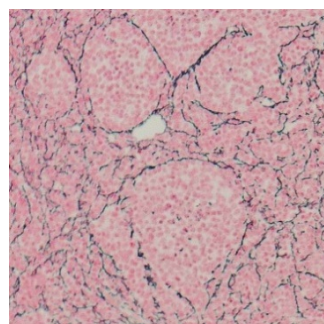
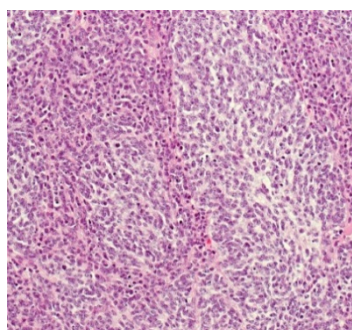
Neuropathology review will involve examination of standard histological preparations and, if required, immunohistochemistry (IHC) to look for the expression of neuroepithelial proteins (e.g. GFAP / synaptophysin / neurofilament protein / NEU-N) or non-neuroepithelial proteins (e.g. cytokeratins / smooth muscle actin / desmin / epithelial membrane antigen / Kir 7.1 protein). A Ki-67 immunolabeling index will also be determined for certain tumors, and expression of the *SMARCB1 (INI1)* gene product will be evaluated in all tumors.

#### *Subclassifying infant medulloblastomas (MBs)*

Pediatric MBs are heterogeneous. Several histopathological variants are defined in the WHO classification of CNS tumors (2007), and data from several studies suggest that some of these variants have a distinctive biology and response to treatment.<sup>303</sup> The large cell and anaplastic variants of MB have a more aggressive biological behavior than the classic MB,<sup>304,305</sup> while the nodular / desmoplastic MB,

particularly the medulloblastoma with extensive nodularity (MBEN), has a more favorable outcome.<sup>306,307</sup> The good prognosis of the nodular / desmoplastic MB has recently been shown in trial cohorts of young children,<sup>18</sup> and provides an opportunity to treat patients with this MB variant using therapies designed to maximize cure, but reduce long-term adverse events. Thus, nodular / desmoplastic medulloblastomas in patients 0 - <3 yrs of age will be treated on a separate low risk arm of this protocol. Patients diagnosed with desmoplastic medulloblastoma between the ages of 3-5 will be treated on the intermediate risk arm of the study. The diagnosis of this variant will be facilitated using reticulin preparations to highlight internodular desmoplasia.

### Figure 11 Nodular Desmoplastic Medulloblastoma



Desmoplastic/nodular MB (H&E)    Desmoplastic/nodular MB (reticulin)

## 8.0 CORRELATIVE STUDIES

### 8.1 BIOLOGICAL STUDIES ON TUMOR TISSUE

#### 8.1.1 Overview of studies on tumor tissue

Tumor from trial patients is required for diagnosis and biological studies, though the priority for utilizing tissue should be a reliable diagnosis. Tissue surplus to diagnostic requirements should be processed for biological studies. In addition to fresh / frozen tissue, this may include FFPE tissue processed initially for diagnostic purposes. However, sufficient tissue should always be reserved in pathology blocks for further diagnostic tests or medicolegal examination in the originating department of pathology.

Fresh frozen tumor samples will be analyzed using the most advanced methods available. The techniques presented in the following sections are intended to be representative of those which will be used for the biological components of the trial. As microarray technology is evolving rapidly, it is likely that specific platforms and procedural details will be different from those described below. Microarray platforms which may be used include the Human Genome U133 Plus 2.0 Array and Genome-Wide Human SNP Array 6.0. In addition, microRNAs will be studied at



the level of the genome and by expression profiling using an Illumina™ or Agilent™ platform.

Studies on FFPE material under the SJYC07 protocol are described below in 8.1.3.

#### 8.1.2 Tissue processing and storage

##### *Fresh-frozen tumor material*

Informed consent for the submission of tumor samples for all analyses will be obtained from parents. In order to simplify shipping requirements for partner sites and to facilitate tracking of submitted material, all samples from outside sites will be shipped to the principal investigator. Once sample submission has been documented by Neuro-oncology research staff, samples will be immediately transported to the SJCRH Tumor Bank, in order to ensure that the MCTC remains blinded to patient identity and clinical characteristics (including outcome). All material will then be assigned a Tumor Bank number and forwarded to the MCTC for analysis.

One or more ‘pea-sized’ aliquots of tumor should be collected from patients at the time of initial resection and any subsequent tumor surgery. Once removed from the patient, tissue elements degrade within 15-20 minutes, so liquid nitrogen for freezing the tissue should be available within close proximity to the operating room. Immediately following removal from the patient, divide tumor tissue into pea-sized aliquots (~50-100mg per aliquot, a total of 100-200mg is the preferred minimum, but please send as much tissue as available). Wrap all aliquots in foil (or place in cryopreservation tubes, if the tumor is semi-liquid). Use a waterproof marker to label foil (or cryopreservation tubes) with the PATIENT ACCESSION NUMBER. Snap-freeze the foil containing the tumor material in liquid nitrogen. For tissue obtained locally at LBCMC, A member of the NBTP will be present to transport the snap frozen samples to SJCRH. If not shipped immediately, snap-frozen samples should be transferred under liquid nitrogen (or on dry ice) to a -80°C freezer until shipped (NOTE -20°C is inadequate for storage). Samples should be shipped on dry ice with the completed ‘FRESH FROZEN TISSUE SUBMISSION FORM’ to the Principal investigator:



Frozen tumor will be processed subsequently to yield high quality DNA, RNA and protein.

*Fixed tumor material*

FFPE tumor tissue for research IHC, iFISH, DNA extraction and construction of TMAs can be obtained at the same time as tissue sections are prepared for central histopathological review. Tissue blocks for this purpose should be obtained for all patients enrolled in this study and be submitted as for central pathology review and together with a completed 'FIXED TISSUE SUBMISSION FORM', to the principal investigator:



Once sample submission has been documented by Neuro-oncology research staff, samples will be immediately transported to the SJCRH Pathology Department for processing and storage.

Tissue blocks for this component of the biological studies will be processed as follows:

- Sections (25 x 5 / 8 $\mu$ m) will be mounted on coated glass slides suitable for IHC & iFISH.
- Tissue scrolls (10 x 25 $\mu$ m) will be obtained for extraction of tumor DNA and cell nuclei (iFISH).

*Constitutional DNA whole blood sample*

Informed consent for the submission of blood samples will be obtained from parents. In order to simplify shipping requirements for partner sites and to facilitate tracking of submitted material, all samples from outside sites will be shipped to the principal investigator. Once sample submission has been documented by Neuro-oncology research staff, samples will be immediately transported to the SJCRH tumor bank, in order to ensure that the MCTC remains blinded to patient identity and clinical characteristics (including outcome). All material will then be assigned a Tumor Bank number and forwarded to the MCTC for analysis.

Five mls of whole blood should be submitted for analysis for each patient enrolled on SJYC07. Whole blood anti-coagulated with EDTA should be collected and kept at 4°C and shipped on wet ice. Using a waterproof marker, label the tubes with the patient identifying number and the date obtained, and send to:



### 8.1.3 Prioritization of tumor biology studies

#### *Frozen tissue studies*

If a limited amount of frozen tumor has been submitted, biological studies will be prioritized as follows:

1. Microarray gene expression analysis.
2. Methylation arrays
2. RT-PCR.
3. SNP array analysis.
4. Gene sequencing studies.
5. Western blot studies.
6. MicroRNA analysis and other studies.

Note: Gene expression studies will have the highest priority among biology studies. In cases where more than one block of frozen tissue has been obtained from a particular patient, extra material may be used for biology studies prior to the completion of accrual. In all such cases at least one frozen tumor block will be retained for analysis of gene expression at the end of trial accrual. In situations where only one block of frozen tumor has been obtained, no biology studies will be performed until the end of accrual, and RNA extraction for gene expression analysis and confirmatory RT-PCR studies will have the first priority when frozen tissue is analyzed.

#### *FFPE tissue studies*

FFPE tissue will be processed in Neuropathology according to a defined sequence. Submitted tissue blocks will first be processed for central pathology review, at which time standard (H&E) sections can be assessed for suitable regions that would contribute to the construction of a tissue microarray (TMA). If a tissue block appears sufficiently deep, cores will be taken for TMA construction. If not, this element of the studies will be omitted. A block will subsequently be sectioned as described below, prioritizing DNA extraction for methylation arrays over IHC and iFISH.

It is a policy of the Neuropathology department at St. Jude to return FFPE tissue blocks borrowed from other institutions with sufficient tissue for further diagnostic or medicolegal use. If at any stage tissue within a block appears thin, then sectioning will stop and the block will be returned.

#### 8.1.4 Methods for tumor biology studies

Methods for biological studies advance rapidly, especially in the area of microarray technology. The availability of specific platforms and techniques is likely to change during the accrual phase of the trial, and the most advanced techniques available at the time of sample analysis will be utilized. The methods presented below should therefore be considered to be representative of the techniques which will actually be performed in this trial.

##### *Immunohistochemistry (IHC)*

FFPE sections (5 $\mu$ m), including those for appropriate controls, will be taken to assess the following in medulloblastoma tumor samples:

- activation of the wnt signaling pathway ( $\beta$ -catenin)
- activation of the shh signaling pathway (Gli-1 / SFRP1)
- ERBB2

In addition and where appropriate antibodies are available, IHC will be used to validate novel patterns of gene expression revealed by the genome-wide approaches described below.

##### *Interphase fluorescence in situ hybridization (iFISH)*

FFPE sections (8 $\mu$ m) or cell nuclei extracted from tissue scrolls, including those for appropriate controls, will be taken to assess the following in medulloblastoma tumor samples:

- loss of chromosomes 6, 8p, 9q22
- isochromosome 17q
- amplification of *MYCC*, *MYCN*, *MYCL*

In addition, iFISH will be used to validate genetic abnormalities revealed by the genome-wide approaches described below.

##### *Tissue microarray*

Tissue microarrays will be constructed for tumor samples from patients with all diagnoses.

A maximum of four 1.0 mm diameter tissue cores will be extracted from representative tissue block(s) for the construction of tissue microarrays. H&E-stained slides from each of the paraffin donor blocks will be utilized as guides in selecting morphologically representative areas, with the Pathology section coordinator designating appropriate areas for sampling. A maximum of three 1.0 mm diameter tissue cores from each donor block will be precisely arrayed in the recipient TMA block using a tissue arrayer (Beecher Instruments, Silver Spring, MD) equipped with a thin-walled stainless steel tube (punch) with a sharpened end similar to a cork borer. A stainless steel stylet will be used to transfer tissue cores into recipient (array) blocks at defined microarray coordinates. Staggering of the first row of cores will be used to ensure reliable orientation of tissue sections and identification of each donor sample. Subsequently, 4 µm-thick sections from the microarray blocks will be mounted on positively-charged slides for future immunohistochemical and FISH analysis. Tissue cores will only be taken in cases where there is adequate tissue available and will not otherwise compromise the donor block for future diagnostic utility. All original blocks will be promptly returned to the submitting institution, along with a copy of the central laboratory pathology report.

A database will be generated identifying the microarray coordinates with individual donor cases (identifier of pathology case numbers only) with password protection for access by only the Pathology section coordinator. The database will allow for parallel entries of subsequent FISH and immunohistochemical results. Any other patient-related documents (pathology reports) will be kept in a locked file in the Pathology section coordinator's office.

#### *Microarray analysis*

We will construct gene expression-profiles for all tumor samples with 5-10µg RNA using microarray analysis.<sup>161</sup> Briefly, first and second strand cDNA will be synthesized from 5-15 µg of total tumor cell RNA using the SuperScript Double-Stranded cDNA Synthesis Kit (Gibco Life Technologies, Rockville, Maryland) and an oligo-dT<sub>24</sub>-T7 primer. This is then used to prepare cRNA that is labeled with biotinylated UTP and CTP by *in vitro* transcription using a T7 promoter coupled double stranded cDNA as template and the T7 RNA Transcript Labeling Kit (ENZO Diagnostics Inc., Farmingdale, NY). Following purification and precipitation at -20°C, 10 µg of this cRNA is fragmented by heat and ion-mediated hydrolysis at 95°C (200 mM Tris-acetate, pH 8.1, 500 mM KOAc, 150 mM MgOAc) and hybridized to the Human Genome U133 Plus 2.0 array. This contains almost 54,000 probe sets representing more than 47,000 transcripts derived from approximately 38,500 well-substantiated human genes. This set design uses sequences selected from GenBank®, dbEST, and RefSeq. Arrays are then washed at 25°C with 6 X SSPE (0.9M NaCl, 60 mM NaH<sub>2</sub>PO<sub>4</sub>, 6 mM EDTA + 0.01% Tween 20) followed by a stringent wash at 50°C with 100 mM MES, 0.1M [Na<sup>+</sup>], 0.01% Tween 20. We will then stain arrays with phycoerythrin conjugated streptavidin (Molecular Probes, Eugene, OR) and the fluorescence intensities will

be determined using a laser confocal scanner (Hewlett-Packard, Palo Alto, CA). The scanned images are analyzed using Microarray™ software (Affymetrix). We will standardize for sample loading and variations in staining by scaling the average of the fluorescent intensities of all genes on an array to a constant target intensity (2500) for all arrays used. The signal intensity for each gene was calculated as the average intensity difference, represented by  $[(PM - MM)/(number\ of\ probe\ pairs)]$ , where PM and MM denote perfect-match and mismatch probes. Microarray expression profiles will be analyzed as described in detail in the statistics section of the protocol.

#### *SNP array analysis*

SNP analysis will be performed using the Affymetrix Genome-wide human SNP array 6.0, or a similar platform, using the most appropriate technology available at the time of analysis. Such arrays are designed for high throughput parallel genotyping of almost one million SNPs that span the human genome with a median intermarker distance of 5.8 kb<sup>308,309</sup>. The sequence of each allele is determined using 40 tiled probes that span the SNP position, providing highly accurate (>99.5%) and reproducible (>99.9%) genotype calls<sup>310,311</sup>.

Samples for SNP analyses will be submitted to the microarray core laboratory in the Hartwell Center for Bioinformatics and Biotechnology and assessed for DNA purity and integrity by using UV spectrophotometry and agarose gel electrophoresis. Samples with 260/270 ratios <1.1 (indicating residual phenol) or with 260/280 ratios <1.8 (indicating residual protein) will be repurified using the Qiagen QiaAmp DNA mini-kit. Samples passing initial quality assessment will be processed as described below.

Total genomic DNA (500 ng) is digested with Nsp I and Sty I restriction enzymes and ligated to adaptors that recognize the cohesive 4bp overhangs. All fragments resulting from restriction enzyme digestion, regardless of size, are substrates for adaptor ligation. A generic primer that recognizes the adaptor sequence is used to amplify adaptor-ligated DNA fragments. PCR conditions have been optimized to preferentially amplify fragments in the 200 to 1,100 bp size range. PCR amplification products for each restriction enzyme digest are combined and purified using polystyrene beads. The amplified DNA is then fragmented, labeled and hybridized to the array. Following hybridization, arrays are washed automatically using a GeneChip Fluidics Station 450 using high stringency conditions to remove non-hybridized labeled DNA. Arrays are incubated with Streptavidin Phycoerythrin (SAPE, Molecular Probes), washed, and then incubated with biotin-conjugated anti-streptavidin antibody. After removal of the antibody solution, the arrays are retained using the SAPE, washed again, and then scanned using an Affymetrix GeneChip Scanner or similar platform. SNP array data will be analyzed as described in section 13.2.1.

#### *Gene sequence analysis*

We will use the polymerase chain reaction (PCR) and a combination of previously published and 'in-house' generated primers to amplify DNA extracted from each of the tumor samples; the products of these reactions will then be directly sequenced with the assistance of the Hartwell Center. Genes identified as potential oncogenes and tumor suppressor genes using SNP or expression microarrays will be subject to DNA sequence analysis. We will also conduct sequence analysis of constitutional DNA (extracted from white blood cells) taken from patients whose tumors contain gene mutations: this will be performed to confirm that the mutations have arisen somatically during tumor formation.

#### *Western blotting analysis*

We will analyze the expression of a number of cell signal proteins that have been implicated in the biology of medulloblastoma. Antibodies employed for western blotting will include: phospho-Y1248 ERBB2 (Upstate Biotechnology, Waltham, MA), phospho-Y204 ERK1/2 (Santa Cruz Biotechnology, Santa Cruz, CA) phospho-Ser473 of AKT1, phospho- $\beta$  catenin,  $\beta$ -catenin (New England Biolabs, Beverly, MA), ERBB2 (Novacastra Ltd, Newcastle, UK), ERK1, PDGFRB (Santa Cruz Biotechnology), AKT1 (New England Biolabs), S100A4 (Dako, Carpinteria, CA), TP53 (Oncogene Science) and  $\beta$ -actin (Sigma Chemicals, St Louis, MO) commercially available primary antibodies. We will also study the expression of additional proteins encoded by genes associated through SNP and gene expression array analysis with clinical disease behavior.

Western blot analysis will be performed using standard techniques. Briefly, 100  $\mu$ g of tumor protein lysate will be analyzed by western blotting analysis as described in detail previously (10). The first three lanes of all blots will include three separate cell line controls: (*lane 1*) Daoy.V (expresses low-levels of ERBB2, S100A4, active  $\beta$ -catenin and moderate levels of PDGFRB), (*lane 2*) Daoy.2 (express high-levels of ERBB2, S100A4, active  $\beta$ -catenin and moderate levels of PDGFRA), (*lane 3*) D341 (negative for all ERBB receptors). Following probing for the protein of interest all blots will be re-probed with an antibody for  $\beta$ -actin to control for protein loading and transfer. The expression level of each protein of interest will be determined by densitometric analysis and normalized to the levels of cell line controls (lanes 1-3) and  $\beta$ -actin. This system allows for accurate inter-tumor assessment of specific protein expression.

#### *Real-time PCR analysis*

Real-time PCR will be performed using a 7900HT Sequence Detection System (ABI) and the TaqMan One Step PCR Master Mix Reagents Kit (ABI) as described previously.<sup>312</sup> RT-PCR will be used to confirm the differential expression pattern of genes detected using microarray analysis. Real-time PCR reactions will be analyzed using SDS v2.0 software (ABI). Total RNA from human adult brain (Ambion) is

used to generate standard curves for relative quantitation. 18S rRNA is used for an endogenous control.



8.2 PHARMACOKINETIC AND PHARMACOGENETIC STUDIES

<i>TABLE 19 OVERVIEW OF PHARMACOKINETIC STUDIES</i>				
Drug	Patients	Course and Day	Time points	Sample volume (per level)
<b>Induction PK Studies</b>				
Methotrexate IV	All patients ( <i>any site</i> )	Each induction cycle - day 1	Pre-infusion and 6, 23, 42 hours from <u>start</u> of MTX, and as needed	1-2 mL
Cyclophosphamide IV	Enrolled in <u>optional</u> PK study ( <i>St. Jude only</i> )	Induction cycle 1 or cycle 2 – day 9	Pre-infusion, end of infusion, hours 3, 6, and 24 from end of infusion	1 mL
<b>Consolidation PK Studies</b>				
Topotecan IV	All high-risk receiving Cyclo/Topo consolidation ( <i>any site</i> )	Each consolidation cycle – day 1 and as needed	Pre-infusion, 5 min., 1, and 3 hours from end of infusion	2 mL
Cyclophosphamide IV  <i>Applies only to St Jude patients enrolled on optional PK study</i>	High-risk receiving Cyclo/Topo  Low-risk pts receiving Cyclo/Carbo /VP16	Each consolidation cycle – day 4,5  Each consolidation cycle – day 1	Pre-infusion, end of infusion, hours 3, 6 and 24 from end of infusion	2mL for day 4 and 24 level  1 mL for all other levels
<b>Maintenance PK Studies</b>				
Cyclophosphamide trough to assess compliance	All patients ( <i>any site</i> )	Cycle A1 and A3 – during week 2 or 3	Trough level; obtain during routine lab draw	1 mL
Cyclophosphamide PO	Enrolled in <u>optional</u> PK study ( <i>St. Jude only</i> )	Cycle A1 – day 1	Pre-dose, 0.5, 1.75, 3 and 6 hours post-dose	1 mL
Topotecan PO	Enrolled in <u>optional</u> PK study ( <i>St. Jude only</i> )	Cycle A1 – day 1	Pre-dose, 0.25, 1.5 and 6 hours post-dose	2 mL
Erlotinib trough to assess compliance	All patients receiving erlotinib ( <i>any site</i> )	Cycle B1 and B3 – during week 2 or 3	Trough level; obtain during routine lab draw	2 mL
Erlotinib PO	Enrolled in <u>optional</u> PK study ( <i>St. Jude only</i> )	Cycle B2 – day 1	Pre-dose, 1, 2, 4, 8, and 24 (±2) hours post-dose	2 mL
Alpha-1-Acid Glycoprotein (AAGP) Studies	Enrolled in <u>optional</u> PK study ( <i>St. Jude only</i> )	Cycle B2 – day 1	One sample only (record time on PK data collection form)	3 mL

### 8.2.1 Shipping information all PK studies:

All PK samples obtained at sites other than St. Jude should be shipped to the principal investigator at this address:



### 8.2.2 Methotrexate studies

*All Collaborating Sites:* Pharmacokinetic studies will be conducted in all patients enrolled on this protocol. Pharmacokinetic samples will be drawn prior to the start of the infusion and at 6, 23 and 42 hours after the start of each methotrexate infusion. Additional levels may be drawn in the event of delayed clearance or unexpected toxicity.

#### *Methotrexate plasma sampling strategy*

The plasma sampling strategy has been designed to take advantage of the routine clinical monitoring of methotrexate therapy, but also to utilize optimal sampling time points. The total blood volume collected for the pharmacokinetic studies for methotrexate (1-2 ml per sample) is no more than 6 ml per cycle.

Serial blood samples for pharmacokinetic studies will be collected prior to the start of the infusion and 6, 23 and 42 hours after the start of the methotrexate infusion.

#### *Sample collection and processing instructions*

Draw 1-2 ml of venous blood for pharmacokinetic studies. Record the exact time of sample collection and methotrexate administration on the Pharmacokinetic Data Collection Form. Centrifuge the sample at room temperature (7000X G for 10 min) within 30 to 60 minutes of blood draw. Transfer the plasma sample to individually labeled tubes and store at -20°C.

#### *CSF pharmacokinetic studies*

Rarely, patients will require placement of an externalized ventricular drain or Ommaya reservoir during treatment. In these cases, parents will be asked to consent to an optional CSF pharmacokinetic study. For patients enrolled in this component of the trial, CSF samples will be collected prior to the start of the

infusion and at 6, 23, and 42 hours from the start of the methotrexate infusion during the relevant cycle.

#### *Description of methotrexate assay*

Plasma and CSF samples will be assayed for methotrexate using fluorescence polarization immunoassay (FPIA) technology in the Clinical PK Lab, Department of Pharmaceutical Sciences, St. Jude Children's Research Hospital. This assay will be run on the TDx-FLx clinical instrument using the Abbott Laboratories drug monitoring system specifically for methotrexate in patient serum or plasma.

#### *Description of pharmacokinetic data analysis*

Methotrexate plasma concentration-time data will be fit to a compartmental pharmacokinetic model using maximum likelihood estimation as implemented in ADAPT II (ADAPT II User's Guide, 1997). Individual pharmacokinetic parameters estimated will include apparent volume of the central compartment ( $V_c/F$ ), elimination rate constant ( $K_e$ ), and half-life ( $t_{1/2}$ ). The methotrexate clearance will be calculated using the log-linear trapezoidal method.

In addition to estimating individual pharmacokinetic parameters, we will also estimate the population parameters using nonlinear mixed effects modeling methods (NONMEM). This method estimates the population parameters and both the inter- and intra-subject variability. Once the population parameters and corresponding covariance matrix are estimated, individual estimates can be obtained using post hoc analysis. Thereafter the influence of patient specific covariates such as age, body-surface area, and sex on methotrexate pharmacokinetic parameters will be tested to obtain an optimal dosing schedule for children with cancer.

### 8.2.3 Cyclophosphamide studies

#### *8.2.3.1 Sample collection*

##### *Intravenous cyclophosphamide PK Studies (St. Jude only):*

Cyclophosphamide pharmacokinetic studies will be conducted after intravenous administration in all consenting patients.

Induction: For all cyclophosphamide pharmacokinetic studies after a single intravenous dose, a 1 mL blood sample will be drawn immediately prior to beginning the cyclophosphamide infusion, at the end of the 1 hour infusion, and at 3, 6, and 24 hours after the end of cyclophosphamide infusion.

Consolidation: For cyclophosphamide pharmacokinetic studies after cyclophosphamide administration on day 1 for low risk patients and days 4 and 5 for high risk patients, a 1 mL blood sample will be drawn immediately prior to

beginning the cyclophosphamide infusion, at the end of the 1 hour infusion, and at 3 and 6 hours after the end of cyclophosphamide infusion. At 24-hours after the end of the cyclophosphamide infusion, a 2 mL blood sample will be obtained.

*Oral cyclophosphamide PK studies during maintenance:*

*All patients:* A trough cyclophosphamide level will be drawn at the time of routine laboratory studies during week 2 or 3 of cycle A1 and week 2 or 3 of cycle A3.

*St. Jude patients only:* Cyclophosphamide pharmacokinetic studies will be conducted after oral drug administration in consenting patients. For cyclophosphamide pharmacokinetic studies after oral administration, a 1 ml blood sample will be drawn immediately prior to cyclophosphamide administration and at 0.5, 1.75, 3, and 6 hours after cyclophosphamide administration on day 1 of the cycle A1. On the day of PK testing, cyclophosphamide must be administered prior to topotecan administration. As administering oral medications to very young children can be challenging, flexibility in the timing of drug administration and PK sampling is necessary; every effort should be made to obtain samples within 15 minutes before or after the schedule time point. The PK nurse administering the drug should estimate the proportion of the drug swallowed. If < 75% of the dose was swallowed, PK testing should be rescheduled for another day during week 1 of cycle A1.

*LC MS/MS assay methodology:*

Cyclophosphamide and its metabolites will be analyzed using a liquid chromatography mass spectroscopy method developed by Kalhorn and colleagues.<sup>313</sup>

*PK Studies for cyclophosphamide and metabolites (St. Jude patients enrolled in optional PK studies only):*

Blood samples will be removed from a non-CY infusion port of a central venous access catheter and aliquots of each sample will be placed into tubes containing either phenylhydrazine for analysis of 4-HCY or EDTA (ethylenediaminetetraacetic acid) for other analytes. The blood - phenylhydrazine mixture will be mixed, refrigerated or kept on wet ice for up to 16-hours; then centrifuged. Plasma will be immediately removed, frozen, and stored at -80°C until analysis.

*Trough cyclophosphamide level to assess compliance (all patients at all sites):*

Draw 1 mL whole blood and place into a green top (sodium heparin) tube. Record the exact date and time the sample was obtained. Take blood from green top tube and place in a microcentrifuge tube using a transfer pipette. Centrifuge for 2 minutes at 10,000 RPM, then transfer plasma from the microcentrifuge tube into 1

screw top tube using a transfer pipette. Put on dry ice. If shipping sample store at -80C until sample can be shipped. Ship samples on dry ice.

#### 8.2.4 Topotecan studies

##### *Pharmacokinetic studies for intravenous topotecan therapy*

Blood samples for topotecan pharmacokinetic testing will be obtained with dose 1 in all patients receiving consolidation therapy with topotecan/cyclophosphamide. Day 1 plasma topotecan concentrations will be used to adjust dosage, if needed, to attain a target topotecan lactone AUC of  $140 \pm 20$  ng•hr/mL. For St. Jude patients, topotecan PK analysis will ordinarily be performed immediately, so that the day 2 topotecan dose may be adjusted as necessary. For patients at other sites, topotecan PK samples will be shipped to St. Jude by overnight delivery for processing and analysis. All day 1 samples should be shipped together immediately after the 3-hour post infusion level is obtained. Analysis will be performed in the Stewart laboratory, and results communicated to the treating physician as soon as possible, but no later than the start of the day 4 topotecan dose. Modification of the plasma-sampling plan may be required periodically to optimize topotecan pharmacokinetic parameter estimation. After the first course of therapy, the first dose of the subsequent topotecan course will be based on the dosage required in the preceding course to attain the target topotecan AUC. If plasma samples are unable to be obtained on the scheduled day for technical reasons, then the patient will be restudied on the next possible day.

A limited sampling model will be used, with blood samples obtained prior to the infusion, and at 5 minutes, 1, and 3 hours after the end of the topotecan infusion. When possible, samples should be obtained from a different lumen than that used for drug administration.

*St. Jude patients:* At least 45 minutes before drawing blood sample or starting chemotherapy page the PK technician on call (pager 2252) in the Stewart Laboratory to have personnel available to process samples. Blood samples of 2 mL each will be collected in green-top, sodium heparin tubes. The exact date and time the sample was collected must be recorded. Immediately after the sample is collected, pipette 1 mL whole blood from the green top tube into a screw top tube (2 tubes per time point), place the tubes on dry ice, and transport to the Stewart laboratory.

*Patients at all other North American sites:* Blood samples of 2 mL each will be collected in green-top, sodium heparin tubes. The exact date and time the sample was collected must be recorded. Immediately after the sample is collected, pipette 1mL whole blood from the green top tube into a screw top tube (2 tubes per time point) and place the tubes on dry ice. Store at -80C until the sample can be shipped. Ship on dry ice.

### *Pharmacokinetic studies for oral topotecan phase*

*St. Jude patients only.* In all consenting patients, oral topotecan pharmacokinetic studies will be conducted on day 1 of the first course of oral topotecan. On the day of PK testing, cyclophosphamide must be administered prior to topotecan administration. Serial blood samples (2 mL each) for topotecan will be drawn prior to the dose and at 0.25, 1.5, and 6 hours after topotecan administration on day 1 of cycle A1. Modification of the plasma-sampling plan may be required periodically to optimize topotecan pharmacokinetic parameter estimation. As administering oral medications to very young children can be challenging, flexibility in the timing of drug administration and PK sampling is necessary; every effort should be made to obtain samples within 15 minutes before or after the schedule time point. The PK nurse administering the drug should estimate the proportion of the drug swallowed. If < 75% of the dose was swallowed, PK testing should be rescheduled for another day during week 1 of cycle A1.

At least 45 minutes before drawing blood sample or starting chemotherapy page the PK technician on call (pager 2252) in the Stewart Laboratory to have personnel available to process samples. Blood samples of 2 mL each will be collected in green-top, sodium heparin tubes. The exact date and time the sample was collected must be recorded. Immediately after the sample is collected, pipette 1 mL whole blood from the green top tube into a screw top tube (2 tubes per time point), place the tubes on dry ice, and transport to the Stewart laboratory.

### *HPLC fluorescence Assay for Topotecan Lactone*

Plasma samples will be assayed for topotecan lactone by an isocratic high performance liquid chromatography assay with fluorescence detection as implemented in the Stewart Laboratory.

#### 8.2.5 Erlotinib studies

*All patients:* A trough erlotinib level will be drawn at the time of routine laboratory studies during week 2 or 3 of cycle B1 and week 2 or 3 of cycle B3.

*St. Jude patients only:* Pharmacokinetic studies will be conducted in all consenting patients enrolled on this protocol. Pharmacokinetic samples will be drawn on day 1 of the cycle B2. If unable to collect samples on day 1, then pharmacokinetic sampling can be collected on one of the next 2 consecutive weekdays.

### *Plasma sampling strategy*

The plasma sampling strategy has been designed to produce pediatric erlotinib data that will give estimates for the single dose and steady-state pharmacokinetic parameters of erlotinib.

Serial blood samples for pharmacokinetic studies will be collected before and 1, 2, 4, 8 and 24 ( $\pm 2$ ) hours after the first dose of erlotinib in the second erlotinib cycle. As administering oral medications to very young children can be challenging, flexibility in the timing of drug administration and PK sampling is necessary; every effort should be made to obtain samples within 15 minutes before or after the schedule time point. The PK nurse administering the drug should estimate the proportion of the drug swallowed. If  $< 75\%$  of the dose was swallowed, PK testing should be rescheduled for another day during week 1 of cycle B2.

#### *Sample collection and processing instructions*

Draw 2 ml of venous blood and place in purple top tubes for pharmacokinetic studies. Record the exact time of sample collection and erlotinib administration on the Pharmacokinetic Data Collection Form. Centrifuge the sample at room temperature (7000X G for 10 min) within 30 to 60 minutes of blood drawn. Transfer the plasma sample to individually labeled tubes and store at  $-20^{\circ}\text{C}$ . Samples from sites other than St. Jude should be shipped on ice to the principal investigator at the address in section 8.2.1.

#### *Description of erlotinib assay*

Plasma and tumor samples will be analyzed for the determination of erlotinib and its metabolites OSI-420/413 by an isocratic high-performance liquid chromatography assay with tandem mass spectrometry implemented and validated in the Stewart Laboratory.

#### *Description of pharmacokinetic data analysis*

Erlotinib plasma concentration-time data will be fit to a compartmental pharmacokinetic model using maximum likelihood estimation as implemented in ADAPT II (ADAPT II User's Guide, 1997). Individual pharmacokinetic parameters estimated will include apparent volume of the central compartment ( $V_c/F$ ), elimination rate constant ( $K_e$ ), and half-life ( $t_{1/2}$ ). The apparent oral erlotinib clearance will be calculated as erlotinib dosage/AUC, and  $\text{AUC}_{0-\infty}$  will be calculated using the log-linear trapezoidal method.

In addition to estimating individual pharmacokinetic parameters, we will also estimate the population parameters using nonlinear mixed effects modeling methods (NONMEM). This method estimates the population parameters and both the inter- and intra-subject variability. Once the population parameters and corresponding covariance matrix are estimated, individual estimates can be obtained using post hoc analysis. Thereafter the influence of patient specific covariates such as age, body-surface area, and sex on erlotinib pharmacokinetic parameters will be tested to obtain an optimal dosing schedule for children with cancer.

### *Alpha-1-Acid Glycoprotein (AAGP) studies*

#### *AAGP levels*

Since erlotinib is bound by AAGP and this may influence toxicity, AAGP will also be measured at the time of pharmacokinetic analysis on day 1 of the second erlotinib cycle.

#### *AAGP samples*

Collection of AAGP Specimens (It is important that plasma be collected):  
The appropriate volume of blood to yield about 1.0 ml of plasma (approximately 3 ml of whole blood) will be collected in tubes containing heparin. Actual sampling times will be recorded on the Pharmacokinetics Data Collection Form. Isolate plasma, freeze, and store samples as indicated above for pharmacokinetic samples. Once frozen, care should be taken **NOT** to allow samples to thaw. Sample labels should bear the following information: patient ID initials/number, sample time and date.

#### *Description of assay*

AAG concentrations in plasma will be determined by a validated immunoturbidometric assay (Tina-quant, Roche).

### 8.2.6 Pharmacogenetic studies – St Jude patients only

All St. Jude patients will be asked to enroll on the institutional protocol PGEN5 for the collection of blood to process for DNA. Genotyping for genetic polymorphisms in cytochrome P450s and drug transporters will be assessed using standard molecular techniques used in the Department of Pharmaceutical Sciences, St. Jude Children's Research Hospital. We will correlate patient pharmacogenetic data with the pharmacokinetics of methotrexate, cyclophosphamide, topotecan, and their metabolites using the methodology described in sections 8.2.1-8.2.4. Patients at collaborating sites will not participate in these studies.

## 8.3 CSF NEUROTRANSMITTER STUDIES

### 8.3.1 CSF neurotransmitter and neurochemical assessment – *St Jude Patients Only*

To verify our hypothesis that neurocognitive deficits after medulloblastoma therapy result from either iatrogenic or inherent (i.e., genetic) disturbances in central dopamine transmission, we propose to study CSF neurotransmitter concentrations in patients enrolled in SJYC07. Focus will be upon dopamine and dopamine metabolite CSF concentrations since these neurochemicals are germane to our hypothesis. However, concentrations of CSF serotonin and its major metabolite 5-



HIAA will also be evaluated to estimate the contribution of lumbar puncture-associated stress on CSF neurotransmitter concentrations. In a study by Hill et al., the immediate post lumbar puncture CSF concentrations of dopamine and serotonin metabolites were roughly half the concentrations detected hours later<sup>314</sup>. However, the ratio of metabolites remained constant, particularly the HVA:5-HIAA ratio. Since our proposed analytical technique is capable of evaluating a host of neurotransmitters from one CSF sample, we will also monitor other transmitter concentrations in a descriptive manner. Such data will aid in our ability to evaluate our hypothesis.

In consenting St. Jude patients, all CSF neurotransmitter/neurochemical samples will be collected during routine sampling for other clinical tests (e.g., CSF cytology). No additional CSF tap procedures will be performed for the study. A 2 to 3 mL volume of CSF will be drawn for neurotransmitter/neurochemical evaluation during routine CSF samplings scheduled at the time of each disease evaluation (pre-treatment, week 8, 16, 24, 36, and 48). Remaining CSF will be stored for future analyses. Patients and mothers of nursing infant patients should follow a low tyramine diet for at least three days prior to CSF sampling and avoid caffeine for 24 hours prior to sampling. (See Appendix V for a list of tyramine containing foods to be avoided).

CSF processing and shipment information needed.

### 8.3.2 CSF Handling and Storage

For each consenting St. Jude patient, CSF will be collected for neurotransmitter studies at the same time the LP is done for clinical assessment of cytology. The biological study specimens are taken when the patient is scheduled for their regular LP's (see section 6.0).

1. Collect 2 - 3 mL of CSF into the 10 mL clear tube labeled "NT-CSF" that contains a pre-measured volume of antioxidant cocktail. These tubes are supplied by the Stewart Laboratory at St Jude.
2. After mixing by gentle inversion, immediately aliquot the specimen equally between two 1-2 mL amber screw top vials and freeze at -80°C. If a freezer is not immediately available, freeze vials on dry ice. Please note that dividing each neurotransmitter CSF sample into 2 separate screw top tubes will reduce error in the analysis of neurotransmitter levels. Samples will be transported to the Stewart Laboratory immediately after collection.

### 8.3.3 Genotyping for dopamine-related gene polymorphisms

All patients will be asked to enroll on the institutional protocol PGEN5 for the collection of blood to process for DNA, which will be extracted by standard techniques. Genotyping for genetic polymorphisms (e.g. DAT1, DRD4,

COMT) will be assessed using standard molecular techniques used in the Department of Pharmaceutical Sciences, St. Jude Children’s Research Hospital.

### 8.3.4 Evaluation of neurocognitive deficits and assessment of the neurocognitive phenotype for correlative analyses

Although numerous neurocognitive outcomes are available from routine evaluations of patients enrolled on SJYC07, we have chosen to limit ourselves to those most closely associated with deficient dopaminergic functioning in ADHD, the model of inattention which most closely mimics symptoms among survivors of medulloblastoma. One recent review<sup>315</sup> described the neurocognitive phenotype as characterized by specific deficits in attentional functioning, including slowed processing speed, decreased perceptual sensitivity, and decreased “hit rate” on continuous performance tasks. Our primary outcome measures will therefore be the Test of Variables of Attention (TOVA),<sup>316</sup> WJ-III Auditory Attention, WJ-III Auditory Working Memory,<sup>317</sup> the SB-V Block Span<sup>318</sup> and the BRIEF.<sup>319</sup> Testing will be routinely conducted at baseline, 6 and 12 months post-diagnosis, and yearly after completion of therapy. Additional, exploratory analyses will also be conducted with other neurocognitive outcomes.

All neurocognitive outcomes used for primary, secondary, and exploratory analyses will result in scores that are normalized for age using a standardization sample from the general population. These scores will comprise a continuous distribution at the interval level of measurement.

### 8.3.5 CSF Neurochemical Assay Methodologies

The proposed neurochemical assay is based on the method of Vaarman, Kask, and Maeorg<sup>320</sup>, and utilizes an isocratic reverse-phase HPLC with electrochemical detection. This method has been used to quantitate a number of neurotransmitters in supernatants of acid precipitated brain homogenates (Table 20). A modification of this method has been developed and validated within the Stewart laboratory at St. Jude Children’s Research Hospital.

*Table 20 Neurotransmitters and metabolites of interest measured by HPLC-EC detection*

DOPAMINE	DOPAMINE, HOMOVANILLIC ACID (HVA), DIHYDROXYPHENYLACETIC ACID (DOPAC)
Serotonin	Serotonin, hydroxyindoleacetic acid (5-HIAA)

In addition to the HPLC with electrochemical detection method that will be used to quantitate CSF neurotransmitters, samples will also be analyzed by NMR spectroscopy.

## 8.4 NEUROCOGNITIVE STUDIES

### 8.4.1 Neuropsychological Studies

#### *8.4.1.1 Overview of neuropsychological studies*

Children will be assessed at baseline, six months and yearly following treatment until 5 years from the end of therapy. Therefore, the assessment battery needs to span the years from infancy to 8 years of age. An effort was made to select measures that could assess the widest age range possible. Nonetheless, as no single measure exists that covers this entire age spectrum, separate test batteries are needed for children less than three years of age and another for children 3 years of age and older. Children less than three years of age will be administered a single comprehensive battery, the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III). All children 3 and older will be administered a separate battery, listed in Table 21. These batteries are comparable in the domains assessed such that some longitudinal analyses will be possible across batteries.

Another primary goal was to select a battery that could be administered in a circumscribed period of time to reduce the time burden for children, their parents and the research team. The administration time for the test battery is 1-3 hours, dependent on the age of the child and the speed with which they can complete tasks. It is anticipated that the average administration time will be 1.5 hours. The maximum administration time of 3 hours is only expected for older children (i.e., 7-8 years of age) or children that are notably slow (e.g., unusually slow speed of cognitive and/or physical processing). Therefore, the administration length of the current battery readily lies within the standard length of typical clinical and research batteries. It is anticipated that parents will spend on average .5 hours completing questionnaires while their child is being assessed in a separate room.

#### *8.4.1.2 Neuropsychological assessment schedule*

Baseline neuropsychological assessments should be conducted at the time of enrollment (+ up to 60 days as needed). Time 2 evaluations should be six months from the time of enrollment (+/- 60 days). The time 2 evaluation does not need to be prior to the start of maintenance therapy; however, this may be the most feasible time point logistically based on other assessments and travel to the medical center providing care. There should be approximately six months (+/- 60 days) between baseline and time 2 evaluations. Yearly assessments should be scheduled from the time of enrollment (+/- 60 days). It is likely that medical management and health status of the patient will in part guide the timing of assessments as it is important that children be well enough to participate in an evaluation to ascertain valid neuropsychological data.

Table 21 Neurocognitive Measures for children > 3 years of age

COGNITIVE DOMAIN	MEASURE	BROAD SKILL	TEST CHARACTERISTICS	AGE RANGE	TIME
<i>IQ Estimate</i>	SB-V Routing subtests	Global Cognitive Function	Object Series/Matrices and Descriptive Vocabulary Subtests	2+	15 min.
<i>Language</i>	Comprehension of Instructions, NEPSY	Receptive Language	Follow verbally presented directions of increasing length (with visual stimuli present)	3-12	5-10 min.
<i>Memory</i>	Narrative Memory, NEPSY	Verbal Memory	Children are read a short story and asked to recall as much of the story as possible (free and cued recall conditions)	3-12	10 min.
	Sentence Repetition, NEPSY	Verbal Memory	Verbatim repetition of sentences read by the examiner	3-12	5 min.
	SB-IV Bead Memory	Visual Memory	Children are shown a series of bead formations and then asked to recall the beads in the same formation	2-24	10 min.
<i>Attention &amp; Executive Function</i>	SB-V Block Span subtest	Nonverbal Attention Span	The examiner taps a series of blocks and children then recall the same sequence of blocks	2+	10 min.
	TOVA	Sustained Attention	Computerized task of attention. Records number of omission and commission errors, as well as reaction time (RT) and variability of RT	4-80	20 min.
	Auditory Attention, WJIII	Selective Auditory Attention	Children selectively listen for certain words on an audio recording that contains distracting background noise	2+	10 min.
	Auditory Working Memory, WJIII	Auditory Working Memory	Repeat words and numbers after organizing based on a pre-established rule	3.6+	5 min.
	Retrieval Fluency, WJIII	Verbal Fluency	Name foods, people and names & animals, each in 1 min. trial	3+	5 min.

Table 21 Neurocognitive Measures for children > 3 years of age (continued)

COGNITIVE DOMAIN	MEASURE	BROAD SKILL	TEST CHARACTERISTICS	AGE RANGE	TIME
<i>Achievement</i>	Bracken, School Readiness Composite (SRC)	Pre-academic skills	Tests knowledge of colors, letter/sounds, numbers/counting, as well as sizes and shapes.	3-6:11	15 min.
	Letter-Word Identification, WJIII	Basic Reading	Identify letters & single word reading	2+	5 min.
	Word Attack, WJIII	Phonological Processing Skills	Children read a series of nonwords	3.8+	5 min.
	Applied Problems, WJIII	Quantitative Reasoning and Math Calculation	Children are presented a series of math problems to solve, which are orally presented and include visual diagrams	2+	10 min.
<i>Visual Spatial &amp; Visual Motor</i>	Beery VMI	Visual Perception & Visual-Motor Integration	Green – Match shapes of decreasing size Blue – Draw shapes of increasing complexity	2-19	10 min.
	Purdue Pegboard	Fine Motor Speed & Dexterity	Place pegs using dominant, non-dominant & both hands, timed	2.6+	5 min.
<i>Processing Speed</i>	Visual Matching, WJIII	Processing Speed	Find matching pairs; timed	2.6+	3 min.
	Decision Speed, WJIII	Processing Speed	Find conceptually similar pairs; timed	2.6+	3 min.
<i>Parent Scales</i>					
Executive Function	BRIEF	Executive Function	Parent rating scales assessing the behavioral manifestation of executive functions	2-5:11/5-18	10 min.
Social-Emotional	BASC-II	Psychosocial Adjustment & Adaptive Function	Questionnaire assessing internalizing, externalizing & attention problems	2-22	15 min.

#### 8.4.1.3 Laboratory measures

##### *Intelligence/global measures of functioning*

Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III).<sup>281</sup> The Bayley-III will be used to assess children 0-3:0 years of age. This test assesses a wide range of function, including cognition, language, motor control, social-emotional and adaptive behavior development. Cognition, language and motor function are assessed through tasks administered by an examiner, in which the child is presented with a variety of stimuli, asked to participate in various activities and asked to follow various commands. Social-emotional and adaptive behavior are measured with a parent questionnaire. This test is a well standardized instrument (normative sample size of 1,700 stratified by age), which is commonly used to assess development and cognitive function in infants. Reliability is adequate, as evidenced by the internal consistency of the Cognitive (.91), Expressive Communication (.91), Receptive Communication (.87), Gross Motor (.91) and Fine Motor (.86) scales. The validity of this measure can be found through its correlations with other well validated measures of infant development (e.g., the Bayley-II, the Preschool Language Scale-4, Peabody Developmental Motor Scale-2, and Adaptive Behavioral Assessment System-II).<sup>281</sup> Additionally, scores on this measure have been shown to reliably differentiate among groups of children with clinical and medical conditions including Down Syndrome, Cerebral Palsy, prematurity and pervasive developmental disorders.<sup>281</sup> Total administration time ranges from 30 to 90 minutes depending on age, and normative data exists for children ages 0-3.

Stanford-Binet Intelligence Scales – Fifth Edition (SB:V, Abbreviated IQ).<sup>318</sup> An abbreviated form of this test, consisting of two subtests, will be used to assess intellectual function in all children greater than 3 years of age. Normative data exists for individuals spanning 2-85 years of age (standardization sample size is 4,800). Abbreviated IQ (AIQ) scores are derived from the Vocabulary and Object Series/Matrices subtests. The AIQ correlates highly with Full Scale IQ (.87 for individuals > 6 years of age and .81 for ages 2-5). This is a well standardized measure, with adequate internal consistency (.85-.96) and test-retest reliability (.84) for Abbreviated IQ. The SB:V abbreviated IQ score also correlates highly with other well validated measures of intelligence like the Wechsler Preschool and Primary Scale of Intelligence – Revised<sup>321</sup> (WPPSI-R; .83) and the Wechsler Intelligence Scale for Children-Third Edition<sup>322</sup> (WISC-III; .84). Total administration time for the AIQ is 15-20 minutes.

##### *Language measures*

NEPSY, A Developmental Neuropsychological Assessment (Comprehension of Instructions).<sup>323</sup> Receptive language is assessed by determining the child's ability to accurately follow a series of verbal instructions (commands) of increasing

complexity. This measure is well standardized with a normative sample of 1,000 children stratified by age and gender. This subtest has adequate internal reliability (.88 for ages 3-4 and .73 for ages 5-12). Furthermore, this task correlates highly with other indices of verbal reasoning such as the Verbal IQ (.62) and Verbal Comprehension Index (.58) of the WISC-III. This subtest takes approximately 5-10 minutes to administer and is normed for children 3-12 years of age.

#### *Memory measures*

NEPSY, A Developmental Neuropsychological Assessment (Narrative Memory).<sup>323</sup> For this task, the examiner reads a story and then asks the child to recall as much of the story as possible. This subtest assesses verbal memory (ability to retell the story) both under free and cued recall conditions (i.e., with true/false or multiple choice questions). This subtest has adequate internal consistency (.77-.85) and test-retest reliability (.81). This subtest also correlates with the verbal memory indices of the Children's Memory Scale<sup>324</sup> (.43-.47). Administration time is approximately 10 minutes and can be given to children 3-12 years of age.

NEPSY, A Developmental Neuropsychological Assessment (Sentence Repetition).<sup>323</sup> For this task, the examiner reads a series of sentences that become increasingly lengthy and complex. The child is required to repeat each sentence verbatim. The reliability of this subtest is adequate for internal consistency (.81-.91) and test-retest reliability (.89). This task also correlates with indices of other well validated memory measures such as the Children's Memory Scale<sup>324</sup> (.53-.59). Administration time is approximately 5-10 minutes and norms are available for children 3-12 years of age.

#### Stanford-Binet Intelligence Scales – Fourth Edition (SB:IV) Bead Memory subtest.<sup>325</sup>

This is a task of nonverbal memory. Children are shown a series of beads formations and afterwards are asked to recall the beads in the same formation as previously shown. This test has adequate test-retest reliability (.87) and internal consistency (.93-.97). Administration time is approximately 10 minutes and normative data exists for ages 2-24.

#### *Attention and executive function measures*

Stanford-Binet Intelligence Scales – Fifth Edition (SB:V) Block Span subtest.<sup>318</sup> This is a test of nonverbal attention span (i.e., spatial span). The examiner taps a number of blocks and children must recall the same sequence of blocks originally touched by the examiner. As the test progresses, the number of blocks the child has to recall increases. The subtest has high reliability (.90) and also correlates with nonverbal intelligence composites, such as performance IQ, of the WPPSI-R and WISC-III (.66-.72). Administration time is approximately 10 minutes and norms exist for ages 2 and older.

Test of Variables of Attention (TOVA):<sup>316</sup> The TOVA is a computerized continuous performance test (CPT) of attention. This task presents one of either two stimuli, a target stimulus which requires a button to be pressed when shown, and a non-target stimulus requiring the button *not* be pressed when shown. Over a period of approximately twenty minutes, these stimuli are flashed (one at a time) on the computer monitor in succession at varying rates of speed. The number of omission and commission errors, as well as reaction time and variability of reaction time are recorded. Performance across these variables during the first, second, third and fourth time quartiles are also collected. Omission errors (failure to press the button when target stimuli appear) are indicative of not paying attention (inattention). In contrast, commission errors (pressing the button for non-target stimuli) are indicative of an impulsive response style. This task is similar to the Conner's CPT but uses geometric shapes so as to exclude the use of lexical stimuli, it also includes a more reliable response method and assesses a larger age range (4-80). Research with children has found test-retest reliability coefficients to be adequate for omissions (.70), commissions (.78), reaction time (.84) and variability of reaction time (.87). The TOVA also has high correlations with other well validated computerized continuous performance tests such as the Conner's CPT (.85).<sup>316</sup>

The Woodcock Johnson Tests of Cognitive Abilities-Third Edition (WJIII: Auditory Attention):<sup>326</sup> For this test, children point to a picture (among 4 choices) indicated by a word they hear on tape, while background noise increases over time making it more difficult to selectively attend to the stimulus word. This is a task of selective attention and speech discrimination. Reliability for this measure is adequate (i.e., internal consistency ranges from .83 -.95) and it has been shown to correlate with other validated measures of auditory attention. This subtest takes approximately 10 minutes to administer and norms are available for ages 2 years and older.

The Woodcock Johnson Tests of Cognitive Abilities-Third Edition (WJIII: Auditory Working Memory):<sup>326</sup> This task presents a series of mixed word and number items to children that progressively increase in length. Children are required to repeat back the words and number using rules, in which they are required to recall items/things first followed by numbers. As such, this task assesses auditory working memory as well as auditory attention span. Reliability is adequate with internal consistency ranging from .80-.96. This measure also correlates with teacher ratings on the School Problems scale (-.37) from the Behavior Assessment System for Children (BASC),<sup>327</sup> thus providing support linking the role of working memory to academic achievement. Norms are available for ages 3.6+ years of age and administration time is approximately 10 minutes.

The Woodcock Johnson Tests of Cognitive Abilities-Third Edition (WJIII: Retrieval Fluency):<sup>326</sup> The Retrieval Fluency task requires that the child name as many items as they can that belong to three semantic categories (foods/drinks, names, and animals) within one minute per category. This task assesses speed of verbal fluency and verbal retrieval. This task has been shown to be reliable both in



terms of its internal consistency (.70-.93) and test-retest reliability (.81-.85). Cluster scores on the WJIII, which contain this subtest, also correlate with composite scores from the Stanford Binet Intelligence Scale – Fourth Edition (SB-IV; .46-.69) Administration time is approximately 5 minutes and norms exist for ages 3 and older.

*Academic achievement measures*

The Bracken Basic Concepts Scale – Third Edition: Receptive (BBCS-3:R), School Readiness Composite (SRC).<sup>328</sup> This composite assesses children’s knowledge of fundamental pre-academic skills, which include colors, letters, numbers and counting, sizes and comparisons, and shapes. Reliability data has found the SRC to have a high internal consistency (.95) as well as test-retest reliability (.84). This measure has been shown to differentiate between groups with lower or poor academic skills (e.g., mental retardation) from typically developing children. Administration time is approximately 10-15 minutes and norms exist for children ages 3-6:11 years old, although concept age equivalent values can be obtained for the entire study age spectrum of 3 and older. The Bracken will be administered first, and only if children demonstrate proficiency with pre-academic skills on this task will they be administered the achievement subtests from the Woodcock Johnson-III listed below.

The Woodcock Johnson Tests of Academic Achievement-Third Edition (WJIII: Letter-Word identification).<sup>326</sup> This task assesses single letter identification for younger children and word reading for older children, thereby testing basic letter recognition and word reading skills. This subtest has a high test-retest reliability (.88-.95). Additionally, the reading cluster composite score from the WJII correlates highly (.82) with the Reading Composite score of the Wechsler Individual Achievement Test (WIAT).<sup>329</sup> Administration time is approximately 5 minutes and norms are available for ages 2 and older.

The Woodcock Johnson Tests of Academic Achievement-Third Edition (WJIII: Word-Attack).<sup>326</sup> Word-Attack requires that children read a list of nonwords to assess their phonological processing skills. This task has adequate test-retest reliability (.83) and the Basic Reading Skills cluster score on the WJIII, which is contains this subtest, correlates highly (.82) with the Reading Composite score of the WIAT. Administration time is approximately 5 minutes and norms exist for ages 3.8 and older.

The Woodcock Johnson Tests of Academic Achievement-Third Edition (WJIII: Applied Problems).<sup>326</sup> This subtest presents a series of problems, which gradually increase in difficulty, that assess basic quantitative reasoning skills and math specific achievement skills (i.e., for more difficult problems, the child needs to both identify the correct math calculation involved and correctly execute the operation). This subtest has a robust test-retest reliability (.86) and correlates with other validated math achievement measures, such the Math Composite score of the WIAT

(.60). Administration time is approximately 10 minutes and norms exist for ages 2 and older.

*Measures of visual spatial reasoning and visual motor processing*

The Beery-Buktenica Developmental Test of Visual-Motor Integration (VMI):<sup>330</sup>

The VMI assesses visual-motor skills by requiring children to copy a series of increasingly complex shapes and figures as accurately as possible. A supplemental test of visual perception will also be administered, which requires children to match identical shapes that also become increasingly complex. As such, these two tests assess both visual-motor integration skills and visual perception skills alone, which allows one to better parse out the relative contribution of these two factors in a child's visual motor integration skills. This test is reliable with demonstrated internal consistency for both the VMI (.88) and the supplemental visual perceptual test (.85). These tasks have been shown to correlate with other validated measures of fine motor skills (e.g., Movement Assessment Battery in Children and Clinical Observations of Motor and Postural Skills) and successfully differentiate children with motor skills deficits from those without.<sup>331</sup> Administration time for these two tasks is approximately 10 minutes and norms are available for ages 2-19.

The Purdue Pegboard Test:<sup>332-334</sup> This test entails placing small pegs into a pegboard as quickly as possible with each hand separately and then using both hands together. The Purdue Pegboard is a test of fine motor dexterity and speed that allows for comparison between performance with the dominant versus nondominant hand. This measure has adequate test-retest reliability (.63-.82) and performance on this measure is sensitive in discriminating normal individuals from those with known motor impairment (e.g. cerebellar disease) as well as normal individuals with MRI white matter hyperintensities.<sup>335</sup> Administration time is 5 minutes and norms are available for ages 2.6 and older.

*Processing speed measures*

The Woodcock Johnson Tests of Cognitive Abilities-Third Edition (WJIII; Visual Matching):<sup>326</sup>

The Visual Matching subtest requires that children find as many matching pairs as possible on a form in a 3-minute time period. This task assesses visual processing speed. Reliability is adequate for both internal consistency (.84-.96) and test-retest coefficients (.78-86). The processing speed composite of the WJ-III correlates significantly with other similar measures such as the Processing Speed Index of the WISC-III (.59). Administration time is 3 minutes and norms exist for ages 2.6 and older.

The Woodcock Johnson Tests of Cognitive Abilities-Third Edition (WJIII; Decision Speed):<sup>326</sup>

This task requires that children find as many conceptually similar pairs (e.g., fruits) as possible in a 3-minute time period in order to assess semantic processing speed.

Reliability is adequate for internal consistency (.78-.92) and test-retest (.78-.92) coefficients. Together, the Visual Matching and Decision Speed subtests comprise the Processing Speed Index of the WJIII. The Processing Speed Index of the WJ-III correlates significantly with other similar measures such as the Processing Speed Index of the WISC-III (.59). Administration time is 3 minutes and norms are available for ages 2.6 and older.

#### *8.4.1.3 Parent and child report measures*

The Behavior Rating Inventory of Executive Function (BRIEF).<sup>319</sup> The BRIEF is a parent questionnaire designed to assess behavioral manifestations of executive functioning. Executive functions include goal-directed behaviors, such as the ability to plan, organize, sustain performance and change performance in response to feedback. The BRIEF questionnaire consists of 86 items from which eight clinical scales (Inhibit, Shift, Emotional Control, Initiate, Working Memory, Plan/Organize, Organization of Materials and Monitor), two indices (Metacognition and Behavioral Regulation) and one composite score (Global Executive Composite) are derived. Scores are age and gender standardized with a mean of 50 and a standard deviation of 10. The measure requires 10-15 minutes of the parent's time.

Behavior Assessment System for Children, Second Edition (BASC-2).<sup>336</sup> The BASC-2 is a questionnaire assessing behavioral, emotional and adaptive functioning. The parent form contains 10 clinical scales and 6 adaptive indices. The clinical scales include those of the externalizing problems composite (e.g., Hyperactivity, Aggression, Conduct Problems), the internalizing problems composite (e.g., Anxiety, Depression, Somatization), and the behavioral symptoms indices (Atypicality, Withdrawal, and Attention Problems). Adaptive scales include Overall Adaptive Skills, Functional Communication, Activities of Daily Living, Leadership, Social Skills and Adaptability. The reliability of the BASC-2 is strong with internal consistency averaging above .80 for all the age specific versions of this questionnaire (preschool, child, and adolescent), and average test-retest reliability is .89. This measure correlates strongly with other well-validated broad band child behavioral checklists such as the Child Behavior Checklist (CBCL). This measure will be used to assess both psychosocial function and adaptive functioning.

## 8.5 DIAGNOSTIC IMAGING STUDIES

### *8.5.1 Quantitative MR measures of white matter volume and integrity*

MRI of the brain will occur at six time points: presentation, midpoint (week 8) and end of induction phase (week 16), end of consolidation phase (week 24), and midpoint (week 36) and end of maintenance phase (week 48). These studies were designed to yield longitudinal measures of white matter structure and integrity during treatment and to determine their relationship between therapy intensity and impact on normal brain development. These clinical MR scans consist of T1 sagittal for localization followed by 4 mm thick contiguous transverse T1, T2/PD, and

FLAIR images covering the entire cerebrum, cerebellum and brain stem. Contrast is then administered with acquisition of T2\* perfusion imaging data during contrast bolus passage. Subsequently, 3D and coronal T1-weighted images are acquired, followed by a diffusion tensor imaging sequence, which acquires all the data necessary to fully evaluate the diffusion tensor. The full examination can be performed in approximately one hour and will require sedation. Scans will be evaluated by neuroradiologists for clinically relevant findings that will be captured in a data reporting form. The T1, T2/PD, and FLAIR images will be analyzed with a hybrid neural network segmentation algorithm that has been developed and validated specifically for quantification white matter changes.<sup>337-339</sup>

Quantitative volumes (cc) of abnormal and normal appearing white matter, gray matter and CSF will be determined from the segmented maps. This method has been previously applied to investigate the impact of radiation dose, age at irradiation and time since irradiation on normal appearing white matter volumes in children surviving medulloblastoma. In addition, the diffusion images will fully define the diffusion tensor for each point in the images. The tensor will then be used to evaluate the apparent diffusion coefficient (ADC) and fractional anisotropy (FA) for each point in the image.<sup>340,341</sup> The ADC measures the average diffusion distance in a region which reflects the extracellular/intracellular volume ratio and is extremely sensitive to acute ischemia. Furthermore, the FA measures the directional organization of a region and reflects the myelin integrity. The T2\* weighted imaging sets will be analyzed with an automated method which determines the global arterial input function then uses a truncated single value deconvolution combined with a standard form Tikhonov regularization with generalized cross validation to yield parametric maps of cerebral blood volume (CBV), flow (CBF) and mean transit times (MTT). The volumetric data set, diffusion parameter maps and perfusion parameter maps will be analyzed for a stack of transverse sections covering the full corpus collusum on the mid-sagittal view. To investigate regional changes in white matter structure and integrity, this volume of interest will then be divided into anatomical regions corresponding to frontal, temporal, parietal and occipital lobes. Other brain structures of interest such as the caudate, putamen, cingulate, thalamus, insula, amygdala and hippocampus may also be segmented and assessed in relationship to treatment and neurocognitive performance.

#### *8.5.2 Positron emission tomography for dose verification after Proton Beam Therapy (for participants enrolled at St Jude only)*

Each consenting patient will receive 54 CGE at 1.8 CGE per fraction PBT for 30 fractions as per the SJYC07 protocol. The number of PET activation studies will equal the number of proton treatment beams, which is estimated to be 3 for the majority of cases. On the day of the PET evaluation, the patient will be treated with a single beam corresponding to the activation beam under study. A separate plan will be developed to ensure the prescription dose to the target using a single beam on that day and that a critical normal tissue volume will not be compromised by the end of range uncertainty in RBE. The beam on time and duration for each of the single field treatment fractions will be recorded. The duration of the single beam

fraction is estimated to be 10 to 15 minutes shorter than the conventional fraction. At the end of the single beam fraction, the patient will be transported to the PET-CT; estimated time required is 10 minutes. For PET attenuation correction and anatomic localization purposes a CT of the cranium will be obtained, estimated time required is 5 minutes. The PET activation study will then be acquired for 30 minutes, the start time will be recorded.

The treatment plan and PET-CT images will be imported into a Monte Carlo program in which the UFPTI proton therapy beam and patient specific apertures and collimators have been simulated. Based on the treatment plan, a Monte Carlo simulation of the positron activation locations and intensities will be created. The predicted image and acquired image will be compared and analyzed.

#### 8.6 ENDOCRINE STUDIES FOR PATIENTS RECEIVING PROTON BEAM RADIATION RT

*(participants enrolled at St Jude only)*

Prior to radiation therapy, consenting patients will be evaluated for potential endocrine dysfunction. GH provocative testing will be performed using arginine and carbidopa-levodopa. ACTH secretion will be tested using the 1 µg ACTH test. Hypothalamic-pituitary axis function will be assessed with static assessments of fasting serum: thyrotropin (TSH), free and total thyroxine (T<sub>4</sub>), total T<sub>3</sub>, cortisol (obtained by 0800 hours), prolactin, insulin-like growth factor binding protein-3 (IGFBP-3), IGF-I, IGFBP-1 (as a marker of insulin resistance), and complete blood count. Similar evaluations will be repeated at the end of therapy and at 6 and 24 months after completion of therapy. These screening tests resemble those drawn for routine evaluation prior to radiation therapy. Understanding the pan-endocrine status is critical to interpretation of the GH data. Exceptions to the endocrine testing procedures may be made at the discretion the treating physicians. Testing procedures may be omitted or modified: 1) because the patient is receiving replacement therapy; 2) when the provocative agent is unavailable; or 3) when the procedure is deemed inappropriate based on patient's clinical condition, the intraday timing of the drug administration, or phlebotomy is constrained by logistic problems.

Comparison of GH secretion and hypothalamic radiation dosing data will follow procedures and criteria previously outlined and summarized in the statistical section. A statistical model will be used to assess the cumulative effect of the dose distribution and mean dose to the hypothalamus on GH secretion. Accurate assessment of the effect of a particular dose requires clinical and treatment information including: pre-existing medical conditions and medications; age of the patient at the time of diagnosis and irradiation; symptomatic interval and progression events prior to irradiation; tumor location and volume; hydrocephalus and its management; extent and number of surgical procedures and related morbidity; prior chemotherapy and related morbidity; neurological effects of tumor including seizures; technical factors of irradiation; neuro-imaging changes; the use of corticosteroids; auxology such as height and weight; common toxicity coding;

and the type and extent of adverse events. This information is included in the medical record of study patients and is often considered vital to routine medical care. Within the constraints of the available sample size, these data will be used in the model to estimate the incidence and time to onset of GH deficiency defined at various levels including peak serum GH values  $\leq 10$  ng/mL and  $\leq 7$  ng/mL after provocation.

## 9.0 EVALUATION CRITERIA

### 9.1 RESPONSE CRITERIA

- 9.1.1 Complete Response: Disappearance of all radiologically discernable lesions and negative CSF cytologic examination.
- 9.1.2 Partial Response:  $\geq 50\%$  reduction in tumor size as measured by the sum of the products of the maximum perpendicular diameters of all measurable lesions; or two consecutively negative CSF cytologies (if the initial cytology was positive) plus a  $< 50\%$  reduction in tumor size. No progression in any lesion, and no new lesion.
- 9.1.3 Stable Disease:  $< 50\%$  reduction in the sum of the products of the maximum perpendicular diameters of all measurable lesions and persistently negative or positive CSF cytology. No clinical progression or radiographic progression in any lesion, and no new lesion.
- 9.1.4 Progressive Disease:  $> 25\%$  increase in the size of any measurable lesion; the appearance of a new radiographically demonstrable lesion; or the conversion of negative CSF cytology to positive, confirmed by two consecutive positive cytologic evaluations following two consecutive negative CSF evaluations.

Patients with progressive disease will continue on treatment, as described in section 10.1, and will continue to be followed until off study criteria are met (see section 10.2). Recommended treatment for patients with progressive disease is discussed in Appendix VI.

### 9.2 TOXICITY EVALUATION CRITERIA

Toxicity will be monitored and graded according to the Cancer Therapy Evaluation Program Common Terminology Criteria for Adverse Events, version 3.0 (CTCAEv3.0). Adverse events not included in the CTCAEv3.0 should be reported and graded under the other adverse event within the appropriate category. A copy of the CTCAEv3.0 can be accessed from the CTEP home page: <http://ctep.info.nih.gov>

All adverse events grades 3, 4, and 5 (with the exception of those listed below) which occur during treatment and for 30 days after the end of treatment, and events which occur later than 30 days after the end of treatment which are felt to be at least possibly related to protocol treatment, are to be recorded on the case report forms. The date of resolution of the toxicity recorded on the case report forms should reflect the date the toxicity resolved to less than a grade 3. The following adverse events will not be captured on the case report forms or in the study database:

- Grade 3 and 4 hematologic toxicities which occur from the beginning of induction chemotherapy through the end of consolidation therapy with the exception of grade 3+ hematologic toxicities occurring in intermediate risk patients undergoing consolidation (i.e. during radiation therapy).
- Grade 3 elevation in serum ALT (SGPT) or AST (SGOT) which occurs within 7 days after the start of any high-dose methotrexate infusion.
- Grade 3 and 4 electrolyte abnormalities which occur from the beginning of induction chemotherapy to the end of maintenance chemotherapy. However, if the event results in a hospitalization or prolongation of an existing hospitalization, the event should be recorded on the case report form. Electrolyte abnormalities which occur after recovery from the last course of maintenance chemotherapy should be recorded on the case report forms.
- TPN or IV fluids administered to prevent significant weight loss / malnutrition from the beginning of induction chemotherapy through the end of consolidation therapy. If the patient experiences significant weight loss / malnutrition and receives TPN or IV fluids for this reason, this event should be recorded on the case report form and graded appropriately using the CTCAE v3.0. Events that occur after the recovery from the last course of maintenance chemotherapy should be recorded on the case report forms.

Ototoxicity will be graded according to the Chang Ototoxicity Grading Criteria found in Appendix IV. All ototoxicities grades 0-4 are to be recorded on the case report forms.

With amendment 5.0, the CCG ototoxicity grading scale is being replaced by the Chang scale. A recent review of the Chang scale shows it to be more sensitive and specific in identifying clinically significant hearing loss and the need for amplification and/or assistive listening devices than the CCG and other older criteria.<sup>341b</sup> [REDACTED] Lead Audiologist at St. Jude and her staff will reevaluate the previously obtained audiograms so that the protocol database will be consistent in using the Chang scale for all ototoxicity data.

## 10.0 OFF THERAPY AND OFF STUDY CRITERIA

### 10.1 OFF THERAPY CRITERIA

Patients can be taken off treatment under the following circumstances (survival, treatment, and disease status data will be collected annually until patient meets off study criteria—see section 10.2)

- Disease progression in M0 patients at diagnosis are permitted to stay on therapy (see section 9.1.4). Please refer to \* and \*\* (below)
- First disease progression in patients who are M+ at diagnosis
- Second disease progression on any patients on therapy
- Unacceptable toxicity
- Physician request
- Family request
- Completed protocol treatment

\* Patients who are M0 at diagnosis and experience local disease progression during induction are permitted to stay on therapy. These patients should have surgical resection of the residual tumor (if feasible) and continue consolidation therapy consisting of focal irradiation and consolidation chemotherapy with IV topotecan and cyclophosphamide followed by oral maintenance chemotherapy per their original tumor histology.

\*\* Patients who are M0 at diagnosis and experience metastatic or local and metastatic disease progression are permitted to stay on therapy. These patients should continue consolidation therapy on the high risk arm of the protocol followed by oral maintenance chemotherapy per original tumor histology.

Please refer to Appendix VI for examples of potential patient scenarios.

For patients who have changes consistent with progressive disease on imaging studies following completion of all therapy, it is recommended that in patients who do not require immediate surgical intervention, imaging studies be repeated in 4 weeks' time to rule out any imaging artifact due to therapy. For patients who have conversion of CSF cytology from negative to positive as the only sign of apparent progressive disease that the CSF examination be repeated in 2-4 weeks prior to calling progressive disease.

For patients who are treated at collaborating institutions it is recommended that each patient to be taken OFF TREATMENT be discussed with the PI of the study and if needed, pertinent studies sent for review to St. Jude.



## 10.2 OFF STUDY CRITERIA

Patients can be taken off study under the following circumstances:

- Family request (withdrawal of consent for further follow-up)
- Death

According to institutional procedures, patients will be deemed off study for the CPDMO upon written authorization by the principal investigator (PI). Collaborating institutions should submit the Off Study case report form accompanied by a copy of the clinic/progress note documenting that the patient was taken off study.

## 11.0 SAFETY AND ADVERSE EVENT REPORTING REQUIREMENTS

### 11.1 REPORTING ADVERSE EVENTS AND DEATHS ON TREATMENT

Only “unanticipated problems involving risks to participants or others” referred to hereafter as “unanticipated problems” are required to be reported to the St. Jude IRB promptly, but in no event later than 10 working days after the investigator first learns of the unanticipated problem. Regardless of whether the event is internal or external (for example, an IND safety report by the sponsor pursuant to 21 CFR 312.32), only adverse events that constitute unanticipated problems are reportable to the St. Jude IRB. As further described in the definition of unanticipated problem, this includes any event that in the PI’s opinion was:

- Unexpected (in terms of nature, severity, or frequency) given (1) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document, as well as other relevant information available about the research; (2) the observed rate of occurrence (compared to a credible baseline for comparison); and (3) the characteristics of the subject population being studied; and
- Related or possibly related to participation in the research; and
- Serious; or if not serious suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Unrelated, expected deaths do not require reporting to the IRB. Though death is “serious”, the event must meet the other two requirements of “related or possibly related” and “unexpected/unanticipated” to be considered reportable.

Deaths meeting reporting requirements are to be reported immediately to the St. Jude IRB, but in no event later than 48 hours after the investigator first learns of the death.

The following definitions apply with respect to reporting adverse experiences:

**Serious Adverse Event:** Any adverse event temporally associated with the subject's participation in research that meets any of the following criteria:

- results in death;
- is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- requires inpatient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant disability/incapacity;
- results in a congenital anomaly/birth defect; or
- any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition (examples of such events include: any substantial disruption of the ability to conduct normal life functions, allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse), a congenital anomaly/birth defect, secondary or concurrent cancer, medication overdose, or is any medical event which requires treatment to prevent any of the medical outcomes previously listed.

**Unexpected Adverse Event:**

- Any adverse event for which the specificity or severity is not consistent with the protocol-related documents, including the applicable investigator brochure, IRB approved consent form, Investigational New Drug (IND) or Investigational Device Exemption (IDE) application, or other relevant sources of information, such as product labeling and package inserts; or if it does appear in such documents, an event in which the specificity, severity or duration is not consistent with the risk information included therein; or
- The observed rate of occurrence is a clinically significant increase in the expected rate (based on a credible baseline rate for comparison); or
- The occurrence is not consistent with the expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the adverse event and the subject's predisposing risk factor profile for the adverse event.

Internal events: Events experienced by a research participant enrolled at a site under the jurisdiction of St. Jude IRB for either multicenter or single-center research projects.

External events: Events experienced by participants enrolled at a site external to the jurisdiction of the St. Jude Institutional Review Board (IRB) or in a study for which St. Jude is not the coordinating center or the IRB of record.

**Unanticipated Problem Involving Risks to Subjects or Others:** An unanticipated problem involving risks to subjects or others is an event which was not expected to occur and which increases the degree of risk posed to research participants. Such events, in general, meet all of the following criteria:

- unexpected;
- related or possibly related to participation in the research; and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. An unanticipated problem involving risk to subjects or others may exist even when actual harm does not occur to any participant.

Consistent with FDA and OHRP guidance on reporting unanticipated problems and adverse events to IRBs, the St. Jude IRB does not require the submission of external events, for example IND safety reports, nor is a summary of such events/reports required; however, if an event giving rise to an IND safety or other external event report constitutes an “unanticipated problem involving risks to subjects or others” it must be reported in accordance with this policy. In general, to be reportable external events need to have implications for the conduct of the study (for example, requiring a significant and usually safety-related change in the protocol and/or informed consent form).

Although some adverse events will qualify as unanticipated problems involving risks to subjects or others, some will not; and there may be other unanticipated problems that go beyond the definitions of serious and/or unexpected adverse events. Examples of unanticipated problems involving risks to subjects or others include:

- Improperly staging a participant’s tumor resulting in the participant being assigned to an incorrect arm of the research study;
- The theft of a research computer containing confidential subject information (breach of confidentiality); and
- The contamination of a study drug. Unanticipated problems generally will warrant consideration of substantive changes in the research protocol or informed consent process/document or other corrective actions in order to protect the safety, welfare, or rights of subjects or others.

*For St. Jude:* Serious and unexpected events are to be reported within five (5) working days. Unexpected deaths are to be reported to the St Jude PI within 24 hours of knowledge of the event.

*For Collaborating Sites:* Serious and unexpected events related to therapy are to be reported to the St. Jude PI (Dr. Amar Gajjar) within five (5) working days via fax or email. Unexpected deaths must be reported to the St. Jude PI via phone call or email within 24 hours of knowledge of the event. A written report must follow.

In the event that the PI is unavailable, the study coordinator [REDACTED] should be contacted regarding the event.

[REDACTED]

[REDACTED]

For the purposes of this protocol, the following events will NOT be considered serious or unexpected adverse events:

- Hospitalization for treatment related febrile neutropenia
- Hospitalization for expected complications of treatment or expected toxicities of the commercially available agents used in this study (except for grade 4 non-hematologic toxicities).
- Hospitalization for treatment of expected signs or symptoms of disease complications (line infections; shunt placement and revisions) or progression of disease
- Death unequivocally related to disease progression.

## 11.2 REPORTING TO IRB

In addition to the continuing review reports to the IRB, the Principal Investigator is responsible for reporting all serious and unexpected adverse events that impact the safety of or risk to study patients. Unexpected deaths which occur while a patient is receiving therapy, and for 30 days after protocol therapy is discontinued, or any death more than 30 days after protocol treatment which is felt to be related to protocol treatment, are to be reported by Dr. Gajjar to the St. Jude IRB office within 48 hours of knowledge of the event. Serious and unexpected events related to therapy are to be reported to the St. Jude IRB within 10 working days. All other events will be reported in the continuing review report.

All participating investigators are responsible for submitting annual continuing review reports, and serious and unexpected adverse event information to their Institutional Review Board (IRB)/Ethics Committee according to local requirements.

## 12.0 DATA COLLECTION, STUDY MONITORING, AND CONFIDENTIALITY

### 12.1 DATA COLLECTION AT ST. JUDE AND COLLABORATING SITES

Case report forms (CRFs) will be completed by each participating site and submitted to SJCRH according to the data submission table distributed with the CRFs (see Appendix VII). CRFs can be either mailed or faxed to:



The SJCRH CRA will review CRFs for accuracy and completeness. Data will then be entered into a secure database.

### 12.2 STUDY MONITORING

The study team will hold monthly meetings and review case histories or quality summaries on participants.

Source document verification of eligibility and informed consent for 100% of St. Jude participants will be performed by the Eligibility Coordinators within 10 working days of completion of enrollment.

The Clinical Research Monitor will perform monitoring of applicable essential regulatory documentation. Also, reviewing for the timeliness of serious adverse event reporting (type, grade, attribution, duration, timeliness and appropriateness) for selected study participants *semi-annually* and track accrual continuously. The monitor will verify those data points relating to the primary study objective for a certain number of study enrollees as specified in the Moderate Risk monitoring plan checklist for this study. Protocol compliance monitoring will include participant status, safety assessments, eligibility, the informed consent process, participant protocol status, off-study, and off-therapy criteria. The Monitor will generate a formal report which is shared with the Principal Investigator (PI), study team and the Internal Monitoring Committee (IMC).

Monitoring may be conducted more frequently if deemed necessary by the CPDMO or the IMC.

Continuing reviews by the IRB and CT-SRC will occur at least annually. In addition, SAE reports in TRACKS (Total Research and Knowledge System) are reviewed in a timely manner by the IRB/ OHSP.

### 12.3 MONITORING FEASIBILITY OBJECTIVES

This exploratory trial includes several feasibility objectives. Feasibility criteria and monitoring intervals have been established for each of these objectives as described in sections 13.1 and 13.2 and summarized in Table 23. The principal investigator will report the results of each feasibility assessment to the IRB and CPSRMC, along with a proposed response to such assessments, if criteria are not being met. Responses may include changes in methodology, revision of study objectives, or possibly deletion of secondary objectives.

*Table 23 Feasibility Monitoring Plans*

Objective	Data to be Reviewed	Feasibility Criteria	Potential Responses to Failure to Meet Criteria	Initial Assessment	Subsequent Assessments
1.1.1 and 1.2.1.3	Proportion of Enrolled Patients with Frozen Tissue	> 40% of patients have frozen tissue	Modify tissue acquisition procedures  Re-evaluate feasibility of primary objective	After 30 patients enrolled (any diagnosis)	Every 30 patients
1.2.1.3	Proportion of Enrolled Patients with FFPE Tissue Available for Research Studies	>60% of patients have FFPE tissue	Modify tissue acquisition procedures  Re-evaluate feasibility of objective	After 30 patients enrolled (any diagnosis)	Every 30 patients
1.2.2.4	Proportion of induction courses with delay >7 days due to toxicity	<25% of courses delayed >7 days	Reduce vinblastine dose  Eliminate vinblastine	After 10 high-risk patients have completed induction	When every 10 high-risk patients complete induction
1.2.2.5	Proportion of topotecan/cyclo courses in which subsequent chemo is delayed > 7 days	< 25% of courses delaye > 7 days	Reduce cyclophosphamide dose  Re-evaluate feasibility of objective	After 10 high-risk patients complete both consolidation courses	When every 10 high-risk patients complete both consolidation courses
1.2.2.6	Proportion of oral chemotherapy doses received	>75% of doses received	Modify drug administration techniques  Re-evaluate feasibility of objective	After 20 patients complete maintenance	After every 30 patients complete maintenance
1.2.2.9	Number of medulloblastoma patients between 3-5 yrs of age who have experienced an event among the first 21 patients enrolled and followed for 1 year	>14 patients are event free at 1 year	Stop enrollment of patients on this arm of the study	After 21 patients are followed for 1 year post enrollment	After 35 patients are followed for 1 year post enrollment
1.2.3.3	Proportion of courses in which target AUC is achieved	Target achieved in > 60% of courses	Modify targeting strategy	After 10 St. Jude high-risk patients complete both consolidation courses	When every 10 St. Jude high-risk patients complete both consolidation courses (on amendment 3.0 or later)
1.2.4.1, 1.2.4.2	Ability to formulate specific hypotheses for subsequent testing		Test specific hypotheses in remaining subjects  Eliminate CSF neurotransmitter studies	After 30 patients enrolled (any diagnosis) and followed for 1 year	

#### 12.4 MONITORING FOR EXCESSIVE TOXICITY IN THE PRESENCE OF PROTON BEAM RADIATION THERAPY (PBRT)

Due to the concerns regarding the potential for excessive toxicity in patients receiving PBRT, amendment 9 of this protocol will implement a monitoring rule to alert the study team if excessive toxicities are observed in patients receiving PBRT. The following toxicities are of potential concern and will be incorporated into the monitoring rule. The participating sites were informed via a memorandum sent on June 12, 2014 that these toxicities must be entered into the CRIS database and will be monitored by the study team.

- Neurology: CNS necrosis/cystic progression grades 3+ that begin after start of radiation. Patients would require HBO and necrosis would be noted in the MRI reports.
- Neurology: CNS cerebrovascular ischemia grades 3+ that begin after start of radiation. These would be noted in the MRI reports. Patients would have TIAs, CVAs or strokes.
- Vascular: Vessel injury-artery grades 3+ that begin after start of radiation. This would require re-vascular surgical repair.
- Neurology: Ataxia (incoordination): grades 3+ that begin after start of radiation.
- Neurology: Encephalopathy: grades 3+ that begin after start of radiation.
- Neurology: Leukoencephalopathy: grades 3+ that begin after start of radiation.
- Neurology: Neuropathy (cranial, motor or sensory): grades 3+ that begin after start of radiation.
- Neurology: Seizure: grades 3+ that begin after start of radiation.

In SJYC07 PBRT is an optional modality for patients enrolled on the intermediate risk arm. Patients who choose not to pursue PBRT are treated with photon-based RT approaches (conformal, IMRT, etc.). There are no suitable historical data available from a young patient cohort on which we can base the toxicity comparisons, however the cohort of patients who are being treated with photons on this protocol can serve as an informal ‘control group’ to aid our interpretations in the context of toxicity comparisons. It should be noted however that since the selection of patients for PBRT is not random and is based on patient/family/physician preferences, this comparison is not statistically optimal.

As part of the effort to develop monitoring rules for excessive toxicity during or after PBRT, we analyzed the adverse event data for the intermediate risk patients using the data which were current as of 8/21/2014. At that time there were 117 patients who were enrolled as intermediate risk and 1 whose risk group was changed from high to intermediate. For the purpose of toxicity monitoring, patients who were on the intermediate risk arm of SJYC07 at any time and who received focal radiation (i.e., not craniospinal radiation) will be included in the monitoring of excess toxicity related to proton beam radiation therapy. Furthermore intermediate risk patients enrolled prior to amendment 8 who were 3-5 years of age, had

anaplasia or MYCN gain or amplification and who received focal RT will also be included in these toxicity analyses. Per protocol, these patients will be excluded only from biology and therapeutic primary objectives.

Of the 118 intermediate risk patients as defined above, 35 patients were excluded because they had no RT data available (23 came off treatment before receiving RT, 3 were reassigned risk group, and 9 are pending RT) at the time of this analysis. The remaining 83 patients were included in this analysis; 45 of these patients received proton beam RT and 38 received photon-based RT. Among the patients who are still alive, the median follow-up times (range) for those treated with photons vs. protons were 44.7 months (6.9-77.1 months) and 32.0 months (1.0-59.5 months), respectively. We compared these two groups with respect to age at diagnosis, gender, tumor diagnosis and tumor location and noted no statistical differences with respect to these four characteristics.

Four of the 45 patients who received proton beam had one of the adverse events of interest, compared to 0/38 patients who received photon-based RT. Please see the table below for details. Considering adverse events with attributions at least possibly related, 3/45 patients who received proton beam RT had one of the AE's of interest (6.7%).

List of intermediate risk patients with adverse events of interest

Patient #	Adverse Event	Timeframe	Grade	AE relation
1	Vessel injury-artery, Carotid	36 Months Off Therapy	3	3=Possible
1	CNS cerebrovascular ischemia	36 Months Off Therapy	3	3=Possible
2	Ataxia (incoordination)	Maintenance - Cycle B2	3	2=Remote
3	CNS necrosis/cystic progression	3 Months Off Therapy	3	4=Probable
4	Seizure	Maintenance – Cycle A1	3	3=Possible

**Safety monitoring for clinically significant necrosis or vasculopathy**

The occurrence of clinically significant necrosis or vasculopathy that is at least possibly related to PBRT will constitute a primary endpoint for this safety monitoring effort. More specifically, the events listed in the first 3 bullets above (grade 3+ CNS necrosis/cystic progression, grade 3+ CNS cerebrovascular ischemia, and grade 3+ vessel injury-artery) will be used for the primary safety monitoring.

As noted above at the time of the analysis in preparation for this amendment, 38 SJYC07 intermediate risk patients were treated with conformal photon radiation therapy and none of them experienced clinically significant necrosis or



vasculopathy. Though follow-up is not the same in all of these patients, using a binomial approach and based on the observed data, the exact 90% upper bound estimate of the proportion of patients who may experience these toxicities when treated with photon-based RT is 5.9%. These data are evolving however and thus are subject to change.

Based on discussions with the study team, if adequate evidence accumulates to suggest that the percentage of patients who experience clinically significant necrosis or vasculopathy at least possibly related to RT exceeds 5%, this would be considered unacceptable. A Bayesian monitoring rule with prior density of Beta(1,15) on the toxicity rate will be used as a trigger for careful assessment of the toxicity data. This prior has mean  $p=0.0625$  and median  $p=0.0435$ , with approximately 90% of support for  $p<0.15$ . The monitoring criterion is met when the posterior probability  $P(p>0.05 \mid \text{data})>0.85$  which indicates 85% certainty that the toxicity rate exceeds 5% given the data and the assumed prior density. Using this approach, the following table summarizes the monitoring thresholds:

Number of Intermediate Risk patients who complete PBRT	Initiate data review if the number of patients who experience clinically significant necrosis and/or vasculopathy associated with PBRT satisfies the following:
40	$\geq 4$
50	$\geq 5$
60	$\geq 6$
70	$\geq 6$
80	$\geq 7$
90	$\geq 7$
100	$\geq 8$

Note that the monitoring thresholds listed above are based on number of patients and not number of events and thus a patient with two of these events will be counted only once. Based on the table above, the toxicity monitoring rule was not triggered at the time of this amendment since there were 2 patients in 45 treated with PBRT who experienced one of these events.

**Safety monitoring for permanent neurological conditions or deficits excluding the effects of necrosis and vasculopathy**

No formal monitoring rule is being proposed for the rest of the toxicities listed above since their attribution to PBRT can be less clear. We will however utilize a similar approach to monitor for permanent neurological conditions or deficits at least possibly associated with PBRT (excluding the effects of necrosis and vasculopathy) at the same time an assessment is conducted for necrosis and vasculopathy. Patients who have experienced necrosis or vasculopathy will be excluded from this cohort to allow a separate assessment, since those patients would have already counted towards the toxicity monitoring rule above. We will use the

same thresholds as above for these toxicities as well to trigger a more extensive review of the data.

This safety monitoring for PBRT related toxicities will be incorporated into our monthly monitoring report and thus will be assessed monthly. If any of the monitoring thresholds above are crossed, a prompt and extensive review of the toxicity data will be undertaken. If a boundary is crossed, we will also compare the toxicities in the photon vs. proton intermediate risk cohorts from SJYC07 using cumulative incidence functions to better inform the decision making process. At that time a decision will also be made regarding whether protocol directed choice of allowing participants to receive photon radiation therapy would be temporarily suspended during this review.

*As of October 29, 2015 the option to receive proton beam therapy was withheld.*

The pre-planned stopping rule for toxicity for clinically significant necrosis or vasculopathy in the proton beam radiation cohort was met with the occurrence of a 6th event in 55 evaluable patients (11%) who received proton beam radiation.

Six participants treated with the proton beam therapy have experienced grade 3 necrosis or grade 3 vasculopathy.

- Three participants experienced grade 3 radiation necrosis within 4 – 6 months after starting radiation. Two of these participants are alive without disease but with sequelae of radiation necrosis. The third participant died of progressive disease within 8 months of the onset of necrosis.
- Three participants experienced grade 3 CNS vascular injury requiring re-vascular surgery within 45-60 months after starting radiation. One participant required a second re-vascular surgery and is alive without disease with minimal sequelae. One participant was unable to be re-vascularized and is alive on daily aspirin. The third participant had re-vascular surgery on [REDACTED] and is alive without disease.

## 12.5 CONFIDENTIALITY

Study numbers will be used in place of an identifier such as the name or medical record number. No research participant names will be recorded on the data collection forms. The list containing the study number and the medical record number will be maintained in a locked file and will be destroyed after all data have been analyzed.

## 13.0 STATISTICAL CONSIDERATIONS

### 13.1 PRIMARY OBJECTIVES

1.1.1 **Biology:** *To identify patterns of tumor methylation profiling that are associated with progression-free survival among young medulloblastoma patients treated with risk-adapted therapy.*

*Responsible Investigators: Amar Gajjar, Giles. Robinson*

*Responsible Biostatistician: Arzu Onar-Thomas, Tong Lin*

1.1.2 **Therapeutic:** *To estimate the event free survival distribution of young medulloblastoma patients treated with risk-adapted therapy*

*Responsible Investigator: Amar Gajjar*

*Responsible Biostatistician: Arzu Onar-Thomas*

All patients with medulloblastoma who were diagnosed prior to their 3<sup>rd</sup> birthday will contribute to both the biology and therapeutic primary objectives of this protocol. Furthermore patients who were  $\geq 3$  and  $< 5$  years old at the time of diagnosis will also be included in the cohort for these primary objectives as long as they meet the eligibility criteria listed in section 3.1.2 of version 8 of this protocol. Patients in the 3-5 year old age cohort who enrolled on previous versions of this protocol and who do not meet the criteria in section 3.1.2 listed in version 8 will be excluded from these analyses.

The outcomes of interest for the biology and therapeutic primary objectives are progression-free survival and event free survival, respectively. PFS will be measured from date on treatment until the date of first progression, medulloblastoma-related death or date of last contact for patients who have not experienced an event (failed). EFS will be measured from date on treatment until the date of first progression, second malignancy or death due to any cause; or date of last contact for patients who have not experienced an event (failed). For both objectives the primary analyses will be consistent with 'intent to treat' and thus all eligible patients who receive any methotrexate will be included in these analyses. Patients who go off treatment due to toxicity or for any reason other than disease progression or medulloblastoma-specific death, will still be followed on study until disease progression, second malignancy or death. Patients who experience a second malignancy or die from other causes will be considered as having had a competing event in the analysis for the biology objective since the intent there is to look for associations between gene expression and tumor progression. Patients who remain progression free or are lost to follow-up will be treated as censored at the date of last contact.

With respect to EFS, a subset analysis will also be done by removing patients who have died from unrelated causes.

Protocol therapy will be risk directed based on the following three strata:

Low risk:

- Patients with gross-totally resected M0 medulloblastoma with nodular desmoplastic histology.
- Patients with M0 high-grade glioma, regardless of extent of resection.

Intermediate risk:

- Patients with M0 medulloblastoma with histology other than nodular desmoplastic; and/or patients with less than gross-totally resected tumors.
- Patients with M0 PNET/pineoblastoma, ATRT and other diagnoses (ependymoma, CPC, etc.)  
Patients who are  $\geq 3$  and  $< 5$  yrs of age with non-metastatic medulloblastoma and less than 1 cm<sup>2</sup> of residual tumor. Patients with anaplastic histology, large cell histology, melanotic differentiation, or myogenic differentiation or tumors with MYC or MYCN gain or amplification are excluded.

High risk:

- Patients with M+ medulloblastoma, PNET/pineoblastoma, ATRT or other diagnosis (high-grade glioma, ependymoma, CPC, etc.)

Note that the intermediate and high-risk groups will also include patients with ATRT, PNET/pineoblastoma, PNET variants (ependymoblastoma, CNS neuroblastoma), high-grade glioma, ependymoma and choroid plexus carcinoma. Only medulloblastoma patients will contribute to the primary objectives. Although patients with other eligible tumors will not contribute to answering the primary study objectives, they will contribute to the toxicity monitoring rule as well as to most of the secondary objectives. Further, tumor tissues from these patients will be subjected to similar exploratory genome-wide analysis of DNA abnormalities and analysis of gene expression profiles as indicated in the secondary objectives.

As part of the risk directed therapy, patients in the high-risk stratum who are 3 years or older at the end of induction therapy will be offered optional craniospinal irradiation (CSI). CSI is expected to improve EFS for these patients and thus reduce the expected numbers of events on which the power of the proposed analyses is based. Nevertheless patients who elect to receive CSI will be included in the primary analyses. Provided the number of events is adequate, we will also perform a secondary subset analysis by considering these patients as censored on the date of their irradiation.

It is also possible that there will be some very young patients enrolled on the intermediate-risk stratum for whom focal irradiation (which is part of the risk-directed therapy) will not be appropriate. In this case, these patients will receive therapy consistent with the low-risk consolidation and maintenance phases until they reach one year of age, at which time they will undergo focal irradiation, followed by further maintenance therapy to complete six total maintenance courses.

### 13.1.1 Overview of historical survival data:

The Kaplan-Meier estimates of distributions of EFS from infant medulloblastoma studies in the literature reveal a ‘cure model’ pattern where early failures are followed by a long stabilization (cure) period in the EFS rates. Though somewhat less pronounced, this pattern also holds for EFS curves of PNET/pineoblastoma and

ATRT patients. Thus a model which captures this “cure pattern” is needed in order to estimate the expected number of failures and hence approximate the duration of the trial.

There is considerable uncertainty in the historical data regarding when the above-mentioned stabilization may be expected to occur. The historical St Jude data indicates that a vast majority of failures will be observed within the first year, whereas the data in the literature indicates that this period may be around 2 years. The following are the EFS curves from some of the relevant studies in the literature. Several of these plots also illustrate the effect of the factors being utilized in defining the risk strata on EFS:

*Figure 12: EFS by Regimen in Medulloblastoma Patients Treated on CCG-9921<sup>16</sup>*

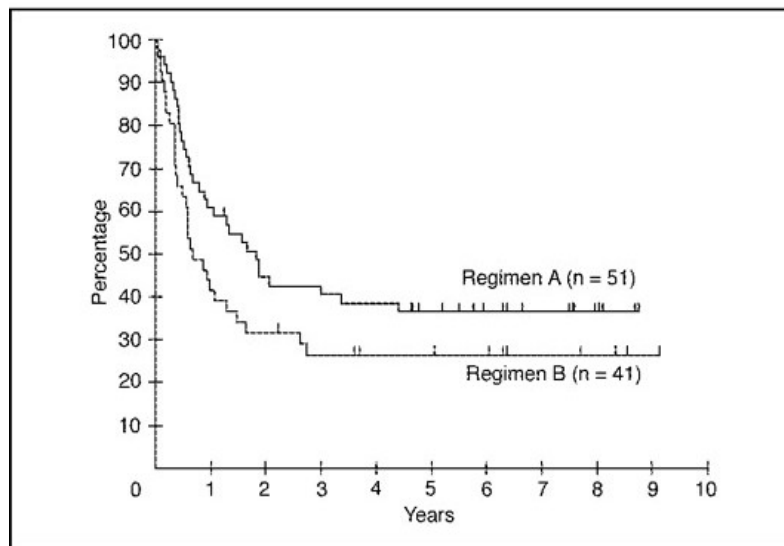


Figure 13: EFS by Pathology for Patients Treated on CCG-9921<sup>16</sup>

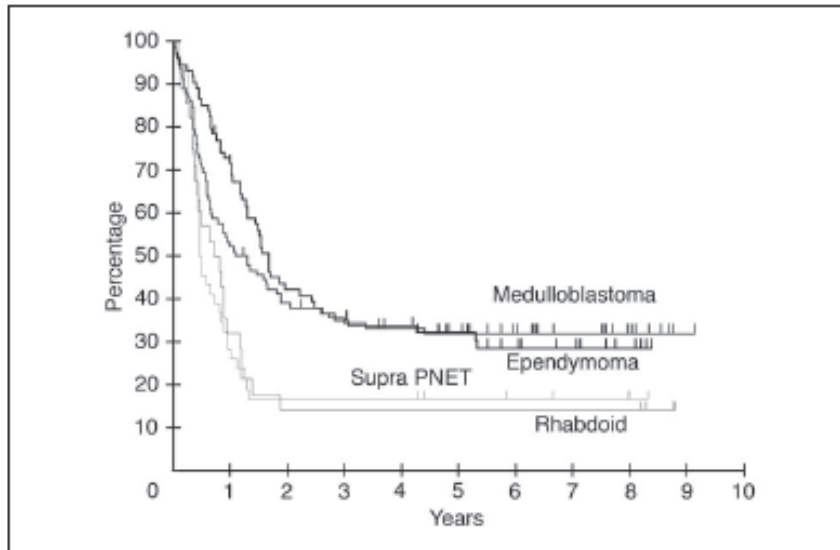


Figure 14: PFS in R0M0 Patients by Extent of Resection in Medulloblastoma Patients Treated on the French BBSFOP Trial<sup>17</sup>

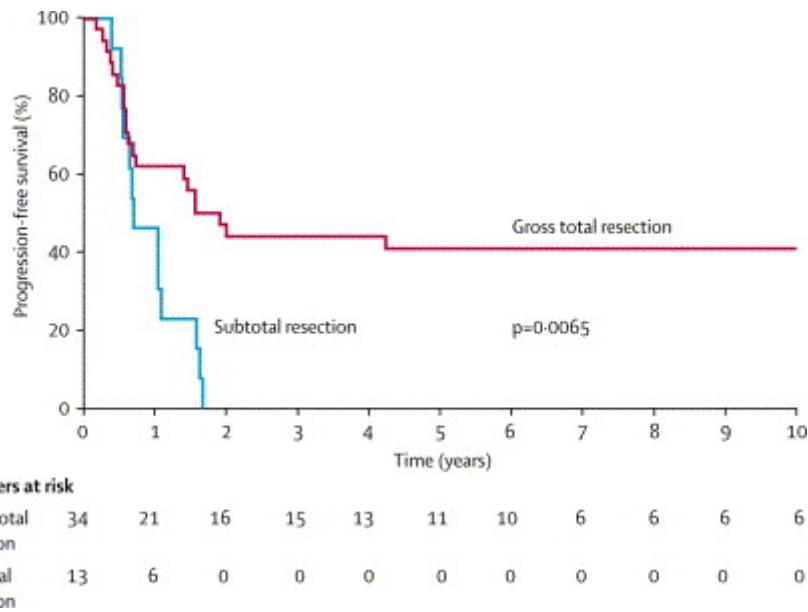


Figure 15: PFS and OS Curves for PNET Patients Treated on the German HITSKK '87 and HITSKK '92 Trials<sup>22</sup>

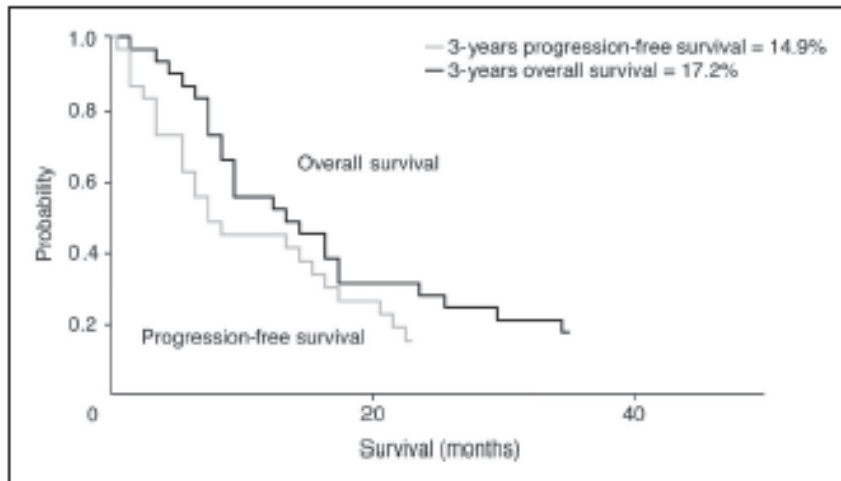


Fig 4. Kaplan-Meier plots of the estimated overall survival and progression-free survival rates of all 29 children.

Figure 16: EFS for Children < 3 Years of Age with Metastatic Medulloblastoma Treated on Headstart II<sup>19</sup>

There were 9 children under 3 years of age at the time of diagnosis who were treated on this protocol (5 were M3 and 2 each were M2 and M1). The plot below was produced from the data presented in the paper.

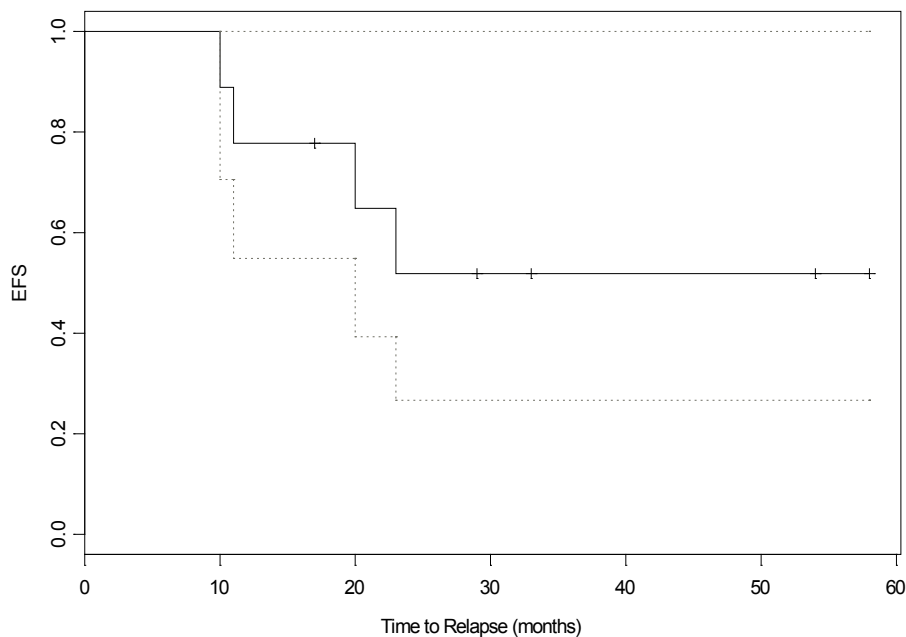


Figure 17: St Jude Historical Infant Medulloblastoma Data:

This cohort includes 56 patients (33 M0, 23 M+) treated during 1984-2006. Follow-up time was truncated at 10 years in order to enhance visualization. A few censoring times are available beyond this cutoff but no events were observed.

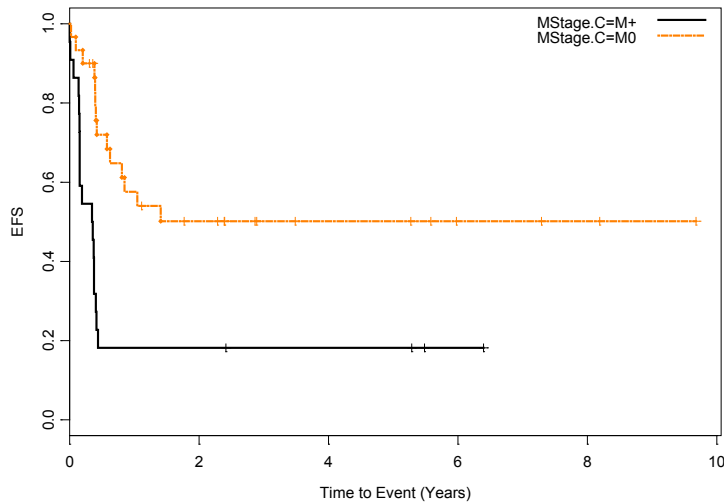
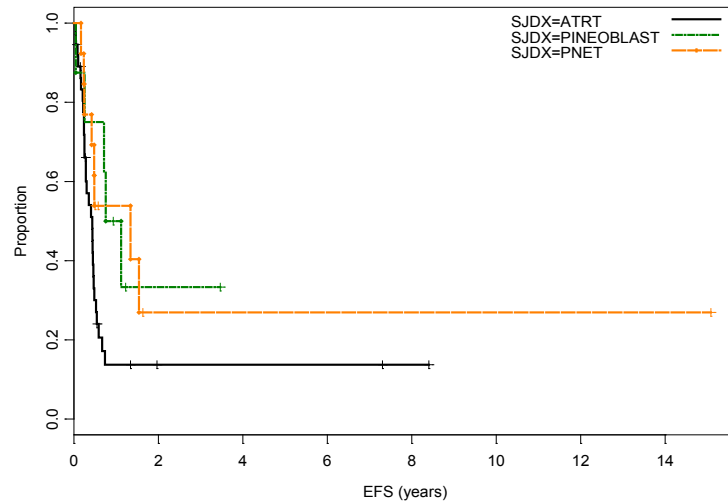


Figure 18: St Jude Historical Infant PNET/Pineoblastoma and ATRT Data:

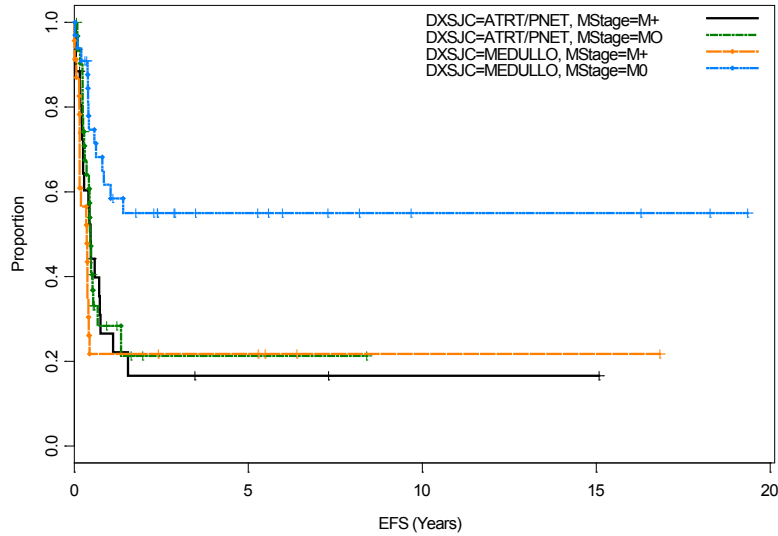
This cohort includes 58 patients (37 ATRT, 8 Pineoblastoma and 13 PNET) treated during 1984-2006.





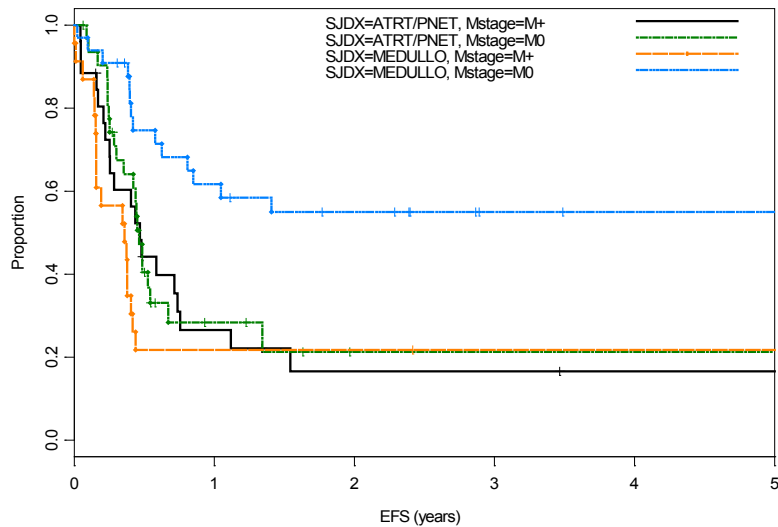
Putting together the St Jude historical data for Medulloblastomas, PNET/pineoblastomas and ATRTs and incorporating M-status in light of the stratification proposed for this trial, leads to the following Kaplan-Meier Plot:

Figure 19: St Jude Historical Infant Medulloblastoma, PNET/Pineoblastoma and ATRT Data:



To enhance visualization, the x-axis of the plot below was truncated at 5 years to produce the following:

Figure 20: St Jude Historical Infant Medulloblastoma, PNET/Pineoblastoma and ATRT Data (Truncated):



The following table was produced based on the St. Jude Historical Infant Data and displays the EFS as well as accrual information available:

*Table 24: Number (Percentage) of Patients by Histology and M-Status in SJ Historical Cohort*

	<b>Number (Percentage) of Patients</b>	<b>1-Year EFS</b>
M0 Medulloblastoma	33 (28.9%)	61.7% (46.8-81.4)
M0 PNET/Pineoblastoma	10 (8.8%)	60.0% (36.2-99.5)
M0 ATRT	22 (19.3%)	13.6% (4.2-44.4)
M+ Medulloblastoma	23 (20.2%)	21.7% (10.0-47.2)
M+ PNET/Pineoblastoma	11 (9.6%)	21.2% (6.3-71.6)
M+ ATRT	15 (13.2%)	14.4% (4.0-52.0)
<b>Total</b>	<b>114 (100%)</b>	

Recall that the low-risk stratum includes M0 Medulloblastoma patients whose tumors are desmoplastic and are gross-totally resected. The following information was compiled from the historical St Jude medulloblastoma data in order to obtain some idea regarding the proportion of patients who can be expected to be treated in this stratum:

- There were 40 medulloblastoma patients in the St Jude cohort whose tumors were tested for desmoplasia. The following table summarizes this information:

*Table 25: Medulloblastoma Patients in the St Jude Cohort whose Tumors were Tested for Desmoplasia*

	<b>M0</b>	<b>M+</b>	<b>Total</b>
<b>Desmoplastic</b>	6 (15%)	3 (7.5%)	9 (22.5%)
<b>Non-desmoplastic</b>	16 (40%)	15 (37.5%)	31 (77.5%)
<b>All</b>	22 (55%)	28 (45%)	40

All 6 M0 desmoplastic tumors were gross-totally resected.

- The effect of desmoplasia on EFS was also investigated using the specimens collected during UKCCSG CNS 9204 trial from 31 infants. Based on personal communication with Dr. Richard Grundy, 17 (60.7%) of the 28 tumor specimens which had sufficient material for the necessary pathology studies were desmoplastic. The 1-year EFS rate for the 17 patients with desmoplastic tumors was 88.2% (72.9-100%) compared to 18.2% (0.0-41.0%) for the 11 non-desmoplastic tumors.

The table on the next page summarizes the relevant historical information that was reviewed in compiling the background information for this trial.

		Headstart II Chi et al. (2004 JCO)	Headstart I Mason et al. (1998 JCO)	“The French Study” BBSFOP (Lancet 2005)	“German Study” HITSKK’92 (NEJM 2005)	CCG-9921 (JCO 2005)	Historical St Jude Data
Patients	Medullo	9 M+ patients	N=13 “most with limited disease” 46% w/ complete resection	75 patients 15 of whom were M+	43 patients 31 of whom were M0/M1 and 12 were M2/M3.	92 patients 38 M0 w/ min residual 23 M0 other, 31 M+	56 patients (33 M0, 23 M+) treated during 1984-2006
	PNET	NA	14 patients	NA	13 treated on HIT-SKK87 16 treated on HIT-SKK92	20 M0 w/ min residual 17 M0 other, 9 M+	21 PNET/Pineoblastoma 11 M0, 10 M+
	ATRT	N=6 (2004 ISPNO abstract)	6 patients (from Reddy(2005) Neuro Oncol 75:309-313	NA	NA	13 M0 w/ min residual 5 M0 other, 10 M+	37 ATRT 22 M0, 15 M+
Tx		400mg/kg of Mtx + Other chemo	Intensive Chemo and Bone Marrow Rescue	Postoperative Chemo alone	Chemo that included IT MTX. RT was an option for patients who did not achieve complete remission	Multiagent Chemo with RT for patients with residual disease or M+ status	Various
1-year PFS/EFS (95% CI)	Medullo	PFS: 77.8% (54-100)	EFS: 69% (43-95)	Not provided. 5-year PFS: R0M0 29%(18-44); R1M0 6% (1-27); RXM+ 13% (4-38)	1 yr EFS (read from plots) - M2/M3: 40% (n=17) - M0/M1 no residual: 100% (n=14) - M0/M1 with residual: 85% (n=12)	1-year EFS - M0 min resid 66% - M0 other: 43% - M+: 42%	1-year EFS - M0: 61.7% (47-81.4) - M+: 21.7% (10.7-47.2)
	PNET	NA	1 yr EFS: 79% (57-100); 2-year EFS: 43% (17-69)	NA	1 yr EFS (read from plots) (not available separately for HIT-SKK87 and HIT-SKK92): 45%	1-year EFS - M0 min resid 25% - M0 other: 29% - M+: 22%	1-year EFS - M0: 60% (36-99.5) - M+: 42.4% (21-87)
	ATRT	PFS: 50% at 1-, 2-, 3- years (2004 ISPNO abstract)	PFS, OS: 0% (though no time is given) (2004 ISPNO abstract)	NA	NA	1-year EFS - M0 min resid 31% - M0 other: 20% - M+: 40%	1-year EFS - M0: 13.6% (4.2-44.4) - M+: 14.4% (4.0-52.0)
Stable	Medullo	~2 years	Not enough information	All except 1 had PD w/n 2 yrs of dx.	~ 2 years	~2-3 years	~1 Year
	PNET	NA	~ 2 years	NA	~ 2 years (but not much)	~2 years	~2 years
	ATRT	~1 year	NA	NA	NA	~2 years	~2 years
Desmoplasia	Medullo	NA	NA	25 desmo cases. Not signif. wrt EFS	20 desmo cases. 1-year EFS desmo vs. not: 100% vs. 60%. Effect significant.	NA	1-year EFS: Desmo(n=9): 77.8% (39-100) Non(n=31):29% (11-50.3)

Table 26: Historical Medulloblastoma, PNET/Pineoblastoma and ATRT Data

### 13.1.2 Background Information on Gene Expression for Medulloblastoma Patients:

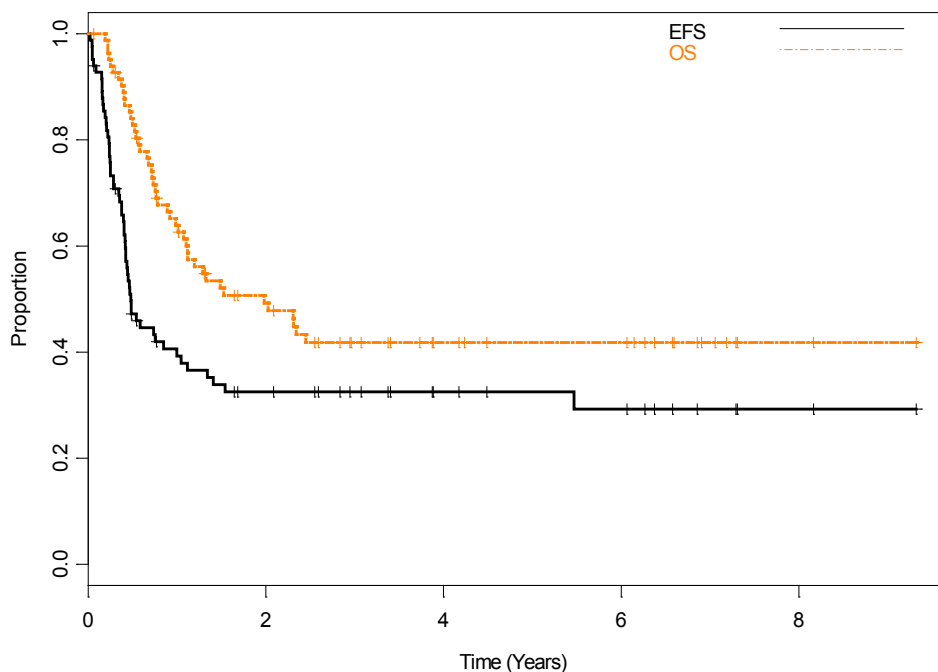
As stated previously, the primary biology objective of the study is perform molecular studies with the intent of assessing various biologic findings for their association with progression-free survival for very young medulloblastoma patients in the context of the risk-directed therapy proposed for this protocol. As the historical data summarized above indicate, progression-free survival is affected by M-stage, extent of resection and histologic subtype, and therefore these factors will have to be included in the same model before the effect of molecular aberrations on PFS can be properly studied. A typical model for such studies is the Cox proportional hazards model.

Well-accepted statistical guidelines indicate that approximately 10 events are needed for each variable studied in a multi-variable Cox model. An important implication of this recommendation, i.e. 10 events per covariate, in the context of this trial is that in order to be able to study the association of a given biologic marker while simultaneously accounting for M-stage, extent of resection and histologic subtype, approximately 40 events will be needed. Note that it is possible even with 40 events that we may not be able to account for the association of all of these factors in a satisfactory fashion. For example, if we observe very few events in the low-risk stratum, it may be difficult to isolate the effect of desmoplasia from M0-status or GTR, depending on the number of events observed in the intermediate and high risk groups and the distribution of these factors among those patients. An alternative approach to accounting for these three factors in the same model as the biologic marker is to use risk-stratum as a proxy. Although this approach may limit the inference regarding surgical resection status, desmoplasia and M-stage, it may allow associations to be observed between biologic marker and PFS with fewer events, which is the primary goal. In the case where risk stratum is used, the model would include 3 covariates (2 indicator variables for risk strata and gene expression) and thus approximately 30 events should be adequate.

### 13.1.3 Background information on St Jude Historical Cohort

St. Jude historical cohort treated during 1998-2006 were used in the initial design of this study and appropriate subsets of this cohort will be used as part of the outcome evaluation. The entire St Jude historical cohort includes 83 patients, 35 of whom were medulloblastoma patients and 32 of whom were ATRT patients. The 1- and 5- year event-free survival rates of the historical cohort are 0.393 (0.0548) and 0.325 (0.053), respectively; whereas 1- and 5- year overall survival rates of the historical cohort are 0.639 (0.0537) and 0.418 (0.0571). The corresponding Kaplan-Meier plot is given below:

Figure 21: Kaplan-Meier Plot of Event-Free and Overall Survival for St. Jude Historical Cohort (all histologies)



With respect to the risk strata defined in this study, 3 patients in the historical cohort would have been classified as low risk, 37 patients would have been graded as intermediate risk and 35 patients would have been grouped as high risk. Note that 8 of the 83 patients could not be classified into one of the three risk strata as these were medulloblastoma patients with M0 disease and gross total resection but the desmoplasia status of their tumor was unknown. Of the 35 patients classified into the high risk stratum 14 were medulloblastoma patients; and of the 37 patients in the intermediate risk stratum 10 were medulloblastoma patients. The following tables and plots summarize the EFS and survival information for the St Jude historical cohort by risk stratum:

**Table 27 Summary Statistics for Event-Free Survival of St Jude Historical Cohort by Risk-Stratum**

	n.obs	events	mean	se(mean)	median	0.95LCL	0.95UCL
RiskStratum=High	35	28	1.83	0.470	0.405	0.285	0.756
RiskStratum=Int	37	23	3.39	0.727	0.485	0.422	NA
RiskStratum=Low	3	1	4.24	1.650	NA	0.203	NA

**Table 28 Summary Statistics for Overall Survival of St Jude Historical Cohort by Risk-Stratum**

	n.obs	events	mean	se(mean)	median	0.95LCL	0.95UCL
RiskStratum=High	35	25	2.61	0.496	1.12	0.718	2.31
RiskStratum=Int	37	18	4.68	0.748	1.49	1.074	NA
RiskStratum=Low	3	1	4.40	1.525	NA	0.663	NA

**Table 29 One and Five-Year EFS and OS Rates of St Jude Historical Cohort by Risk-Stratum**

	1-Year EFS	5-Year EFS	1-Year OS	5-Year OS
RiskStratum=High	0.286 (0.076)	0.222 (0.071)	0.510 (0.085)	0.262 (0.077)
RiskStratum=Int	0.365 (0.083)	0.332 (0.082)	0.680 (0.080)	0.447 (0.089)
RiskStratum=Low	0.667 (0.272)	0.667 (0.272)	0.667 (0.272)	0.667 (0.272)

**Figure 22 Kaplan-Meier Plot of Event-Free Survival for St. Jude Historical Cohort by Risk-Stratum (all histologies)**

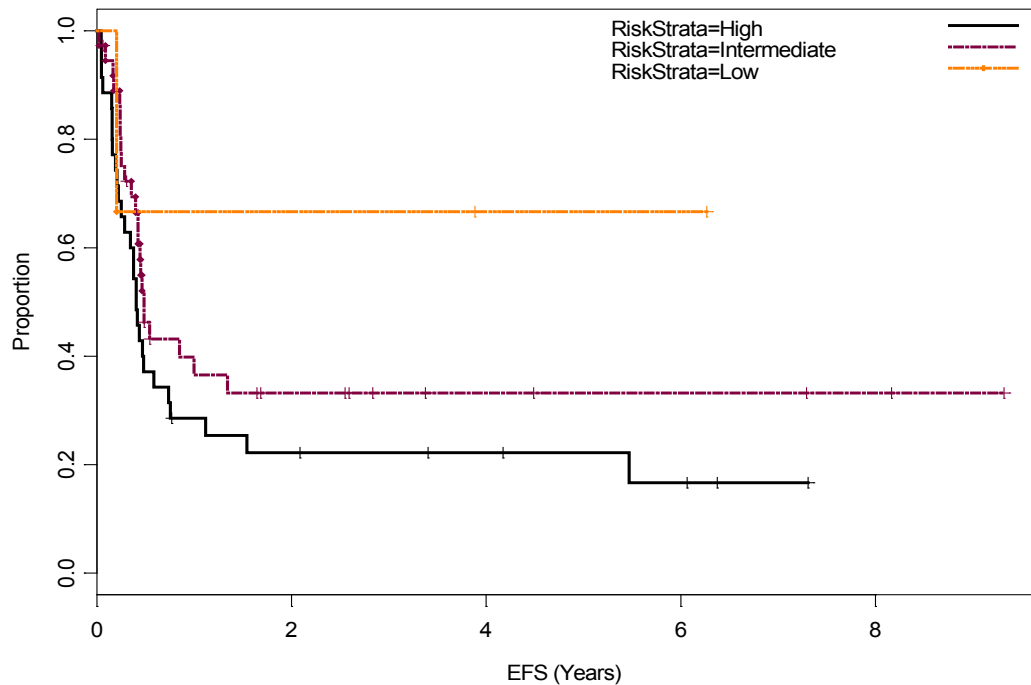
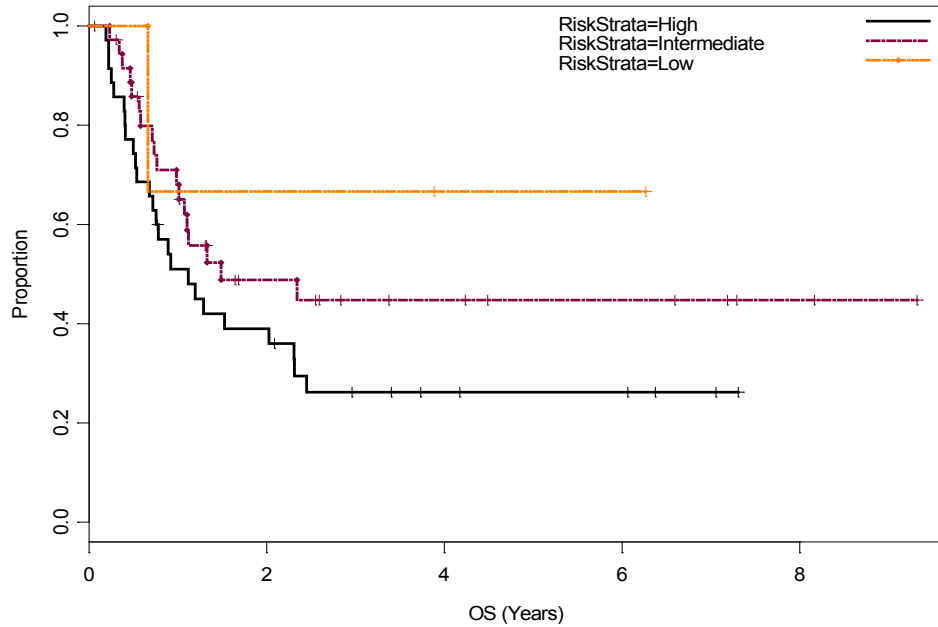


Figure 23 Kaplan-Meier Plot of Overall Survival for St. Jude Historical Cohort by Risk Stratum (all histologies)

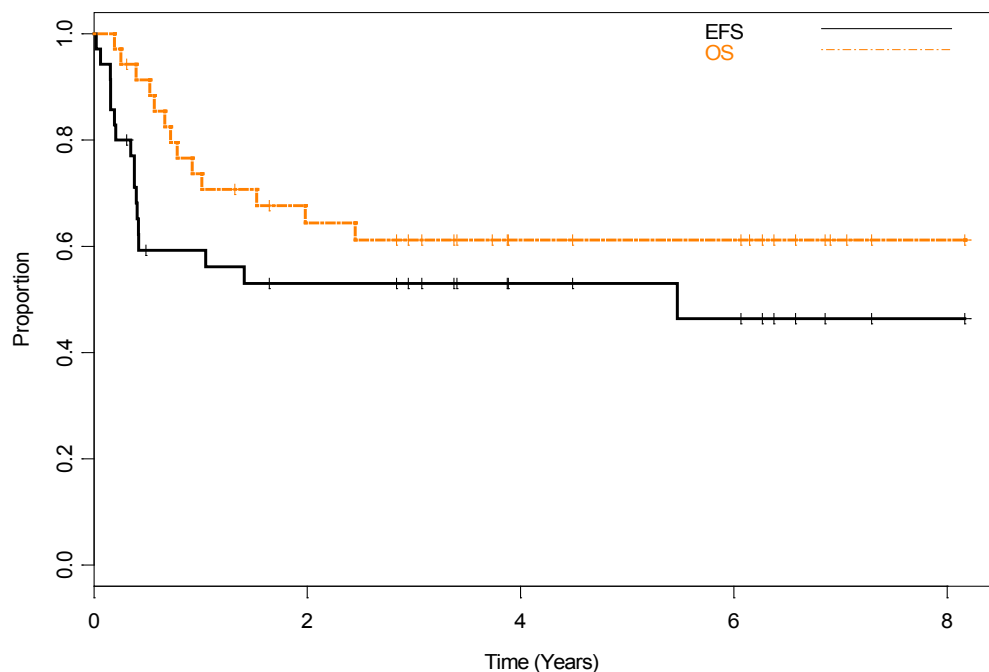


Note however that the proposed primary therapeutic analysis will not be specific to risk group. In other words the EFS distribution of medulloblastoma patients will be estimated across all three risk groups. The following table and plot provide some summary information regarding the St. Jude historical medulloblastoma cohort. The 1- and 5 year EFS rates for this cohort are estimated as 0.593 (0.084) and 0.530 (0.086), whereas the 1- and 5 year OS rates are given as 0.737 (0.075) and 0.612 (0.085).

Table 30 Summary Statistics for Event-Free and Overall Survival of St Jude Historical Medulloblastoma Cohort

	N Obs	Events	Mean	SE (Mean)	Median	0.95 LCL	.095 UCL
<b>EFS</b>	35	17	4.34	0.661	5.468	0.416	NA
<b>OS</b>	35	13	5.36	0.613	NA	2.449	NA

*Figure 24 Kaplan-Meier Plot of Event Free and Overall Survival for St. Jude Historical Medulloblastoma Cohort*



The unusual “cure model” shape of the EFS and survival curves for the medulloblastoma cohort makes it infeasible to use the typical one-parameter exponential distribution-based methods in describing this distribution. Note that the historical data that was initially used to design this trial did not come from a single study, rather the cohort consisted of patients mainly treated on BB98, P9934 and PBTC001, two of which were Phase I trials. Given the heterogeneity of these data as well as its relatively small size, a historical control based comparative design could not be employed. Further, a standard treatment does not exist for the patient population in question and thus there were no other more relevant data which could be used in planning this study at the time it was first designed. Therefore the objective of this study was not to perform a formal comparison; rather it was and is to estimate the EFS distribution for the cohort treated on this trial to provide efficacy information for the proposed regimen and to build an appropriate historical cohort for future studies.

*Update on Published Relevant Literature as of Amendment 8:*

Since the writing of this protocol, multiple studies reporting outcome of medulloblastoma occurring in young patients (<5 years at diagnosis) treated primarily with chemotherapy with the intent to avoid or delay radiation therapy have been published. The table below adapted from Leary et al. (2011)<sup>368</sup> summarizes the outcome reported in these studies:



Trial			Event-Free Survival		Overall Survival		Initial Treatment		
	N	PFS Time (yrs)	DMB	Non-DMB	DMB	Non-DMB	CSI	HDC ASCR	HD MTX
HIT-SKK2000	45	5	95%	30% <sup>a</sup>	100%	68%	No	No	Yes
HIT-SKK92	43	5	85%	34%	95%	41%	No	No	Yes
HIT-SKK87	29	10	89%	30%	89%	40%	Yes	No	Yes
UKCCSG/SIOP CNS9204	31	5	35% <sup>a</sup>	33% <sup>b</sup>	53%	33%	No	No	Yes
Meta-analysis	270	8	55%	27%	77%	42%	Yes <sup>c</sup>	Yes <sup>c</sup>	Yes <sup>c</sup>
CCG-9921	76	5	77%	17%	85%	29%	No	No	No

DMB indicates desmoplastic medulloblastoma, including desmoplastic nodular medulloblastoma and medulloblastoma with extensive nodularity; CSI, craniospinal irradiation; HDC ASCR, high-dose chemotherapy with autologous stem cell rescue; HDMTX, high-dose methotrexate.

<sup>a</sup> outcome for Non-DMB patients is based on classic medulloblastomas only. The trial only included 3 anaplastic patients.

<sup>b</sup> All differences in survival are statistically significant except for UKCCSG/SIOP CNS9204 event-free survival.

<sup>c</sup> Patient cohorts in meta-analysis treated on six different regimens, including reverences HIT-SKK 87 and 92 trials and UKCCSG/SIOP CNS9204 trials above in addition to Italian and Head-Start regimens which used HDC ASCR.

With the drastically reduced sample size of the revised trial (version 8 onwards) we will use published data to set monitoring rules for early stopping as detailed in relevant sections below.

*Enrollment of Medulloblastoma Patients 3-5 years of age*

With amendment 5.0, the eligibility for enrollment to SJYC07 was expanded to include patients  $\geq 3$  -  $<5$  years of age in order to explore the possibility of treating these patients without Craniospinal Irradiation (CSI), which is known to have devastating neurocognitive effects in the long run. It is the investigators' belief that the risk adapted therapy proposed for this trial would provide a comparable outcome to the one that can be achieved via CSI for patients with tumors that do not harbor high risk features. Starting with amendmet 6 of the current study 3-5 year old medulloblastoma patients with anaplastic or large cell histology or C-MYC or N-MYC amplification have been excluded from enrolling on this trial as these are well established adverse risk factors in these patients. With amendment 8 patients with melanotic or myogenic differentiation have also been excluded as these subtypes are also thought to have worse outcome.

While the initial study design had proposed a separate stratum and a separate assessment for these patients, due to accrual limitations, with amendment 8, we chose to combine them with patients <3 years of age as have routinely been done with contemporary early childhood medulloblastoma protocols including HIT 2000 from the German group as well as ACNS1221, the new COG study.

#### 13.1.4 Accrual estimates and expected trial duration

##### *Overview of accrual estimate and trial duration*

This study will be considered complete when 90 medulloblastoma patients have been enrolled and the last patient has been followed for 1-year. It is expected that the total enrollment period will last approximately 10 years. The planned total of 90 patients include an estimated 70 medulloblastoma patients < 3 years of age at the time of diagnosis as well as 20 medulloblastoma patients 3-5 years of age at the time of diagnosis.

As of Feb 27, 2014 198 eligible patients have been enrolled on the study, 55 of whom are medulloblastoma patients (1 without central path review at the time of the writing amendment 8). While it is difficult to estimate the total accrual for the entire study (all histologies), based on the current information and expectations for future enrollment patterns, we project that total accrual to the trial will be approximately 315 patients (70 <3 year-old medulloblastoma patients + 225 <3 year-old patients with other histologies and 20  $\geq$ 3-<5 year-old intermediate-risk medulloblastoma patients). Note that with the opening of SJATRT protocol, young ATRT patients who have been historically enrolled on this trial will enroll on that study instead. Hence we anticipate a slight drop in the number of non-medulloblastoma patients enrolled on this trial for the remainder of the accrual period.

##### *Accrual to Date (as of amendment 8)*

The initial accrual goal of this study was 140 <3-year old medulloblastoma patients to be enrolled in approximately 7 years. However as of March 2014, approximately 6.5 years after the trial opened to accrual only 44 <3-year old medulloblastoma patients have been enrolled. Thus based on a recommendation of the St Jude CT-SRC we have dramatically revised the accrual goals as well as the planned analyses in order to complete this trial in a more timely fashion. The table below provides detailed accrual information in patients <3 years. We expect to accrue the remaining 26 patients in the next 3 years:

**Table 32: SJYC07 Accrual by Year and Site, Diagnosis Age <3 Years of Age  
(as of 2/27/2014)**

<b>Medullo</b>	<b>2007</b>	<b>2008</b>	<b>2009</b>	<b>2010</b>	<b>2011</b>	<b>2012</b>	<b>2013</b>	<b>2014</b>	<b>Grand Total</b>
Collab	0	1	4	5	4	5	1	0	20
St Jude	1	3	3	8	5	2	2	0	24
<b>Grand Total</b>	<b>1</b>	<b>4</b>	<b>7</b>	<b>13</b>	<b>9</b>	<b>7</b>	<b>3</b>	<b>0</b>	<b>44<sup>^</sup></b>

<b>Non-Medullo</b>	<b>2007</b>	<b>2008</b>	<b>2009</b>	<b>2010</b>	<b>2011</b>	<b>2012</b>	<b>2013</b>	<b>2014</b>	<b>Grand Total</b>
Collab	0	0	5	8	8	7	3	0	31
St Jude	0	20	20	16	14	17	16	2	105
<b>Grand Total</b>	<b>0</b>	<b>20</b>	<b>25</b>	<b>24</b>	<b>22</b>	<b>24</b>	<b>19</b>	<b>2</b>	<b>136<sup>^</sup></b>

<b>All Patients</b>	<b>2007</b>	<b>2008</b>	<b>2009</b>	<b>2010</b>	<b>2011</b>	<b>2012</b>	<b>2013</b>	<b>2014</b>	<b>Grand Total</b>
Collab	0	1	9	13	12	12	5	2	54
St Jude	1	23	23	24	20	20	18	3	132
<b>Grand Total</b>	<b>1</b>	<b>24</b>	<b>32</b>	<b>37</b>	<b>32</b>	<b>32</b>	<b>23</b>	<b>5</b>	<b>186<sup>^</sup></b>

<sup>^</sup> 6 patients did not have central pathology results at the time of data extraction. These patients were not included in the medullo vs. non-medullo accrual numbers, but were included in the overall (All Patients) section.

The following table summarizes the accrual rates observed to date in the 3-5 year old Medulloblastoma cohort. Note that accrual to this cohort was initiated in October 2010.

**Table 33: SJYC07 Accrual by Year and Site, Diagnosis Age ≥3-<5 Years of Age**

<b>Medullo</b>	<b>2007</b>	<b>2008</b>	<b>2009</b>	<b>2010</b>	<b>2011</b>	<b>2012</b>	<b>2013</b>	<b>2014</b>	<b>Grand Total</b>
Collab	0	0	0	0	3	2	1	0	6
St Jude	0	0	0	0	1	1	2	0	4
<b>Grand Total</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>4</b>	<b>3</b>	<b>3</b>	<b>0</b>	<b>10</b>

<b>Non-Medullo</b>	<b>2007</b>	<b>2008</b>	<b>2009</b>	<b>2010</b>	<b>2011</b>	<b>2012</b>	<b>2013</b>	<b>2014</b>	<b>Grand Total</b>
Collab	0	0	0	0	0	0	0	0	0
St Jude	0	0	0	1	0	0	0	0	1
<b>Grand Total</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>1</b>

<b>All Patients</b>	<b>2007</b>	<b>2008</b>	<b>2009</b>	<b>2010</b>	<b>2011</b>	<b>2012</b>	<b>2013</b>	<b>2014</b>	<b>Grand Total</b>
Collab	0	0	0	0	3	2	1	0	6
St Jude	0	0	0	1	1	1	2	0	5
<b>Grand Total</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>4</b>	<b>3</b>	<b>3</b>	<b>0</b>	<b>11</b>

Thus to date we have enrolled 10 intermediate  $\geq 3$ - $< 5$  year-old Medulloblastoma risk patients. With the projected timeline of 3 more years of accrual we expect to enroll approximately 10 more patients in this cohort, barring early stopping. Note that with amendment 8 of the protocol, rather than accrued to a separate stratum, these patients will be combined with  $< 3$ -year old patients in the intermediate risk stratum.

*Accrual Patterns Observed to Date (Amendment 8 onwards):*

Of the 44  $< 3$ -year old medulloblastoma patients enrolled on this trial as of Feb 27, 2014, 13 are high risk, 13 are intermediate risk and 18 are low risk. Note that this enrollment pattern is very different than the St Jude historical cohort on which the original design was based. In the historical cohort of 23 medulloblastoma patients 14 were high risk, 10 were intermediate risk and 3 were low risk. Thus with this major amendment we will modify the analysis plan and monitoring approaches. Clearly with the above noted difference in accrual pattern across strata, it would not be reasonable to use a combine- cohort-based approach to monitor outcome.

Barring early stopping based on various monitoring rules noted below, the trial will remain open until 90 medulloblastoma patients are enrolled which we anticipate will take another 3 years. The rationale for this sample size is pragmatic rather than statistical. While in the initial version of the protocol the biology primary objective was based on gene expression that required fresh frozen tissue, in this version the biology objective is being modified to utilize biologic data generated from fixed tissue. To date 95% of patients enrolled on the study (regardless of histology) have provided fixed tumor tissue. In our initial sample size justification of 140  $< 3$ -year old medulloblastoma patients, we had assumed that 40%, i.e. 56 patients, would provide good quality RNA for gene expression analyses. Further based on the SJ historical cohort, we had estimated that we would observe approximately 30 events in these 56 patients at the end of the trial. Based on 90-95% acquisition rate of FFPE tissue observed to date on this trial, we estimate that if 90 patients are enrolled approximately 80 (90%) patients will have adequate FFPE tissue for the planned biology analyses. If the accrual distribution across risk strata as well as outcome are similar to the one observed in the St Jude historical cohort and taking into account the longer observation period we have acquired due to slow accrual, in 90 patients we expect to observe a median of 47 events (90% CI: 38-56). While it is not clear whether we will have similar power for the biology objective as the original design which was based on gene expression, we expect that these data will provide useful information since even with the smaller overall sample size we will have a larger number of events due to longer follow-up as well as tissue availability from a large majority of patients. Note that the current distribution of patients across risk strata is skewed towards the low risk patients and we expect to see fewer events in this stratum however the intermediate risk stratum in the SJ historical cohort (n=10) had only 1 event during the limited follow-up at the time of the original protocol design which appears to be very unusual based on published data from various large studies with much longer follow-up utilizing 'chemotherapy only' approaches to treat this

population and thus we anticipate that it is highly likely we will observe more than 30 events in the overall cohort.

***Monitoring Rules:***

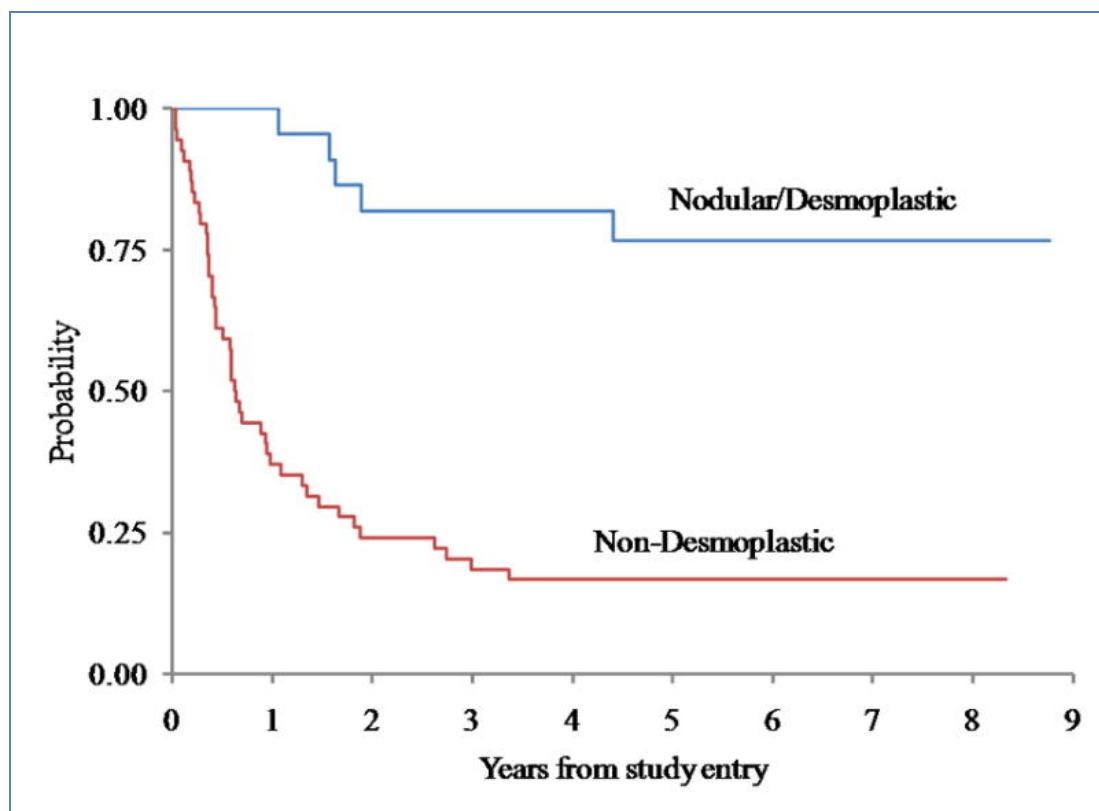
Please note that the outcome data already available on this trial was not used in the modification of the trial design and monitoring rules that have been implemented as of amendment 8 of the protocol.

***Amendment 8 onwards:***

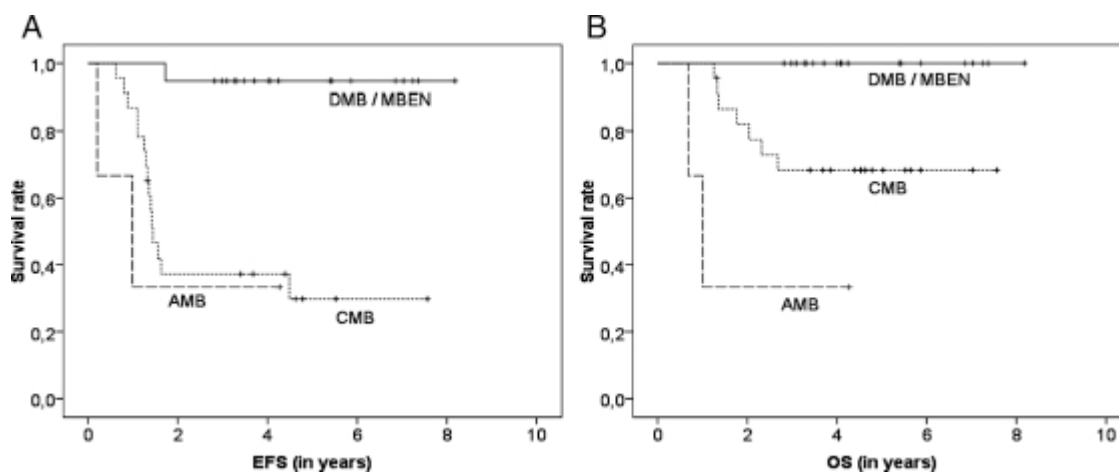
Note that with the drastically reduced target accrual the originally proposed approach to interim analysis becomes infeasible as the operating characteristics of that approach are inadequate. Furthermore, the differing proportions of patients allocated to various risk strata between the historical cohort and the current trial also make the original approach less than desirable. Thus we propose stratum specific monitoring rules based on recently published literature utilizing similar treatment approaches for young medulloblastoma patients.

***Published Data for Young Medulloblastoma Patients:***

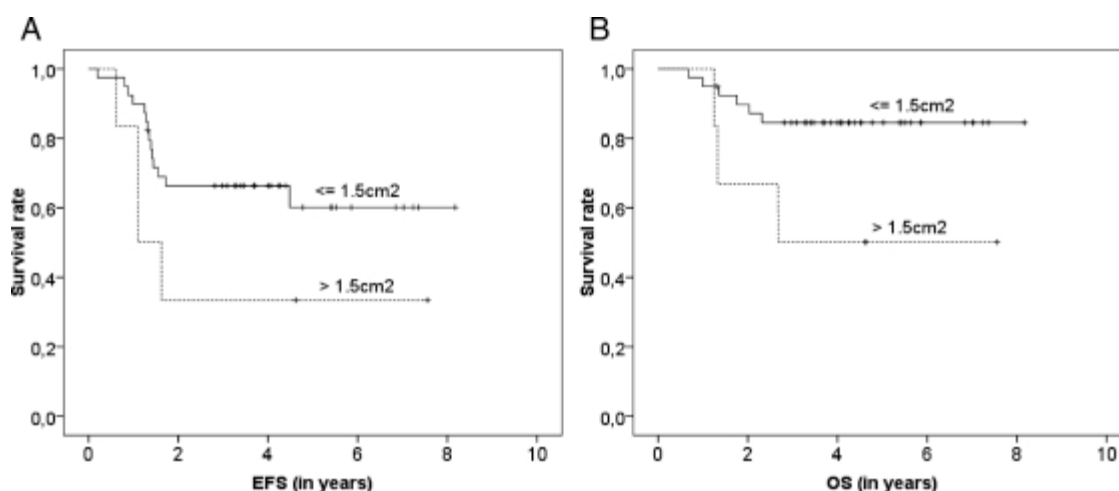
The following KM plots are from a variety of papers displaying the outcome observed in similar patient cohorts based on chemotherapy dominated regimens with the intent to delay or avoid radiation.



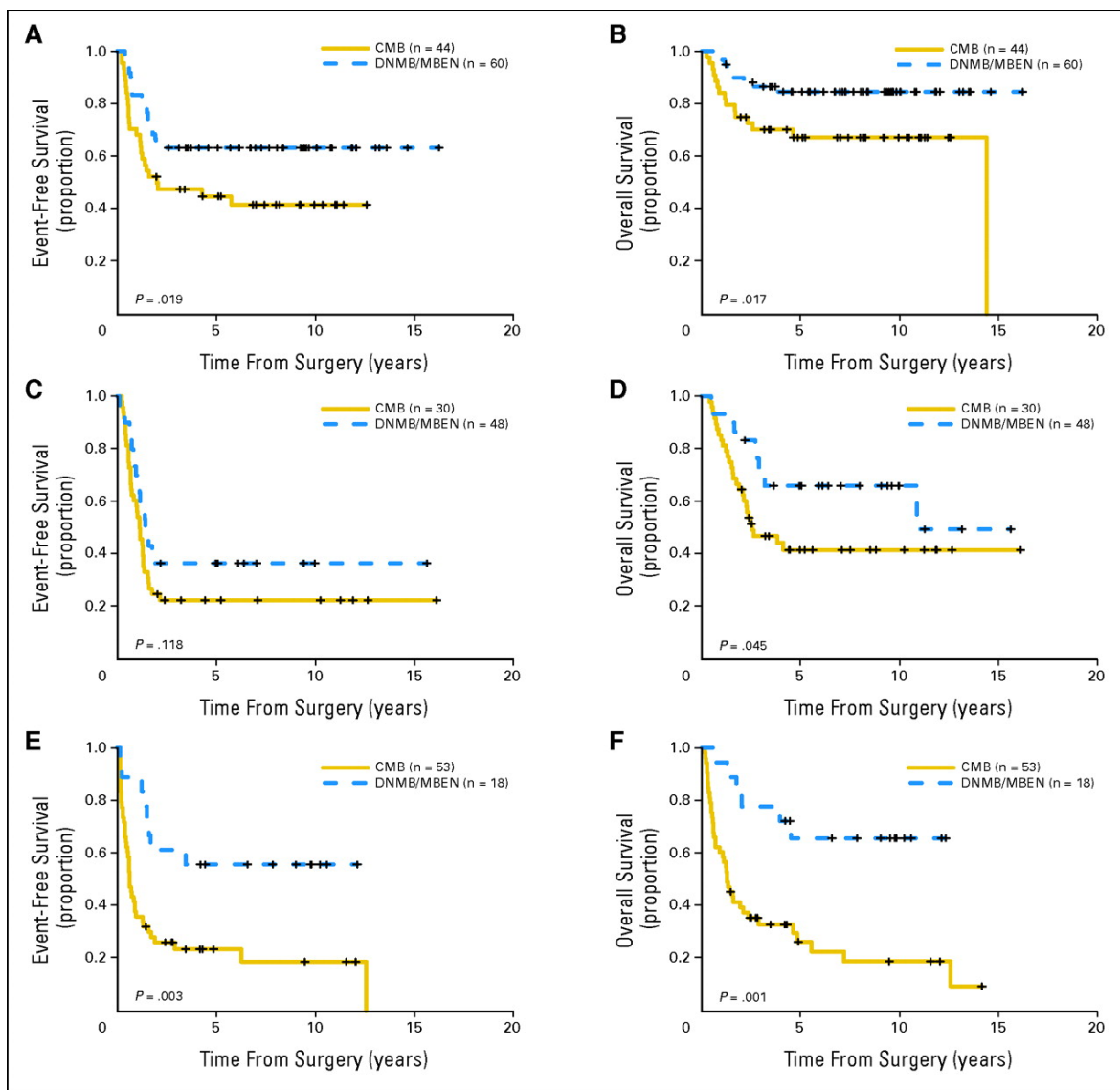
Event-Free Survival for Medulloblastoma comparing Desmoplastic Groups ( $p < 0.0001$ ) from Leary et al.<sup>368</sup>



Survival according to histology from van Beuren et al.<sup>369</sup>. Nineteen patients (13 patients with desmoplastic medulloblastoma [DMB] and 6 patients with medulloblastoma with extensive nodularity [MBEN]) had better event-free survival (EFS; panel A) and overall survival (OS; panel B) rates (5-year rates,  $95\% \pm 5\%$  and  $100\% \pm 0\%$ , respectively) than did 23 patients with classic medulloblastoma (CMB;  $30\% \pm 11\%$  [ $P < .001$ ] and  $68\% \pm 10\%$  [ $P = .008$ ], respectively).



Survival according to postoperative residual tumor from van Beuren et al.<sup>369</sup>. Thirty-nine patients without residual tumor or with small residual tumor ( $\leq 1.5 \text{ cm}^2$ ) had slightly better rates of event-free survival (EFS; 5-year rates,  $60\% \pm 9\%$  vs  $33\% \pm 19\%$ ;  $P = .110$ ) (panel A) and overall survival (OS; 5-year rates,  $84\% \pm 6\%$  vs  $50\% \pm 20\%$ ;  $P = .044$ ) (panel B), compared with 6 patients with residual tumor  $> 1.5 \text{ cm}^2$ .



Event-free survival (EFS) and overall survival (OS) of patients with desmoplastic/nodular medulloblastoma (DNMB)/medulloblastoma with extensive nodularity (MBEN) versus classic medulloblastoma (CMB) within the clinical risk groups (A and B) M0R0, (C and D) M0R+, and (E and F) M+ from Rutkowski et al.<sup>370</sup>

*Monitoring Rule for Low Risk Medulloblastoma Patients (this monitoring rule has been in the protocol since its inception):*

One of the innovative parts of the proposed treatment regimen is to remove focal RT from the treatment of low risk medulloblastoma patients (M0 gross-totally resected nodular desmoplastic tumors). In order to guard against the possibility that the proposed treatment strategy may yield inferior outcome for this patient group an ad-hoc monitoring rule will be utilized. Note that sample size constraints make it infeasible to implement a formal monitoring strategy in this case. As part of the German Study (HITSKK'92) 20 patients with desmoplastic tumors were treated with IT methotrexate as part of that study's regimen, all of whom were event free at one year. The 95% exact lower bound (Blyth-Still-Casella) of this

estimate is 87.4%. In contrast, the St. Jude historical data contains 9 patients with desmoplastic tumors whose 1-year EFS estimate is 77.8% (7 were event free at 1 year) which leads to a 95% Blyth-Still-Casella lower bound of 48.5%. For the current trial we will monitor the 1-year EFS rates of low-risk medulloblastoma patients (those with M0 gross-totally resected desmoplastic tumors) starting with the 10<sup>th</sup> patient. If at any point, this estimate falls below 80%, we will consider revising our approach. This estimate will be updated after every 10 low-risk patients reach the 1-year time point.

As of December 2, 2015 accrual to the low risk medulloblastoma cohort has been suspended based on the above stopping rule. Routine data monitoring found 8 of the 23 low risk medulloblastoma participants had events, bringing the 1-year EFS to 78.3%, which is below the 80% boundary.

*Monitoring Rule for Intermediate Risk Medulloblastoma Patients:*

The intermediate risk Medulloblastoma cohort in this protocol is composed of M0R+ patients or M0R0 non-desmoplastic patients. Rutkowski et al. panel C provides outcome information for a cohort that is similarly defined i.e. M0R+ patients. While this cohort does not include patients with LCA tumors who typically have worse outcomes and are included in our intermediate risk cohort, our cohort also includes M0R0 non-desmoplastic patients who have slightly better outcome as shown in panel A of the same plot. So on average we feel comfortable assuming for monitoring purposes that the outcome reported in panel C of Rutkowski et al. for classic medulloblastoma patients may serve as an appropriate boundary. While the short-term, i.e. 1- or 2-year EFS rates are not formally reported in the manuscripts referenced above, this information can be approximated from the plots above. Based on the information from this plot, the 2-year PFS rate is approximately 25%. So similar to the approach proposed for high risk patients we will use 20% 2-year EFS as threshold for the intermediate risk cohort. If at any point this threshold is crossed the study team will review all available data and may consider treatment revision or stopping accrual in this cohort. We will utilize this approach after 15 intermediate risk patients have been followed for 2 years and will repeat it with every 15 additional intermediate risk patients.

*Monitoring Rule for High Risk Medulloblastoma Patients:*

Based on published data summarized above, it appears that 2-year PFS/EFS rate of M+ non-Desmoplastic patients is approximately 25% (panel E of the KM plots in Rutkowski et al. above). While our high risk cohort (defined by M+ disease) includes Desmoplastic Nodular patients, it also includes large cell or anaplastic patients. While the former has been shown to have better EFS, the latter, albeit more rare, usually has worse EFS than desmoplastic patients. Thus taking all of these factors into account as well as the small number of patients which will be enrolled on our trial, for monitoring purposes we will use 20% 2-year EFS as threshold for the combined M+ cohort. If at any point this threshold is crossed the



study team will review all available data and may consider treatment revision or stopping accrual in this cohort. We will utilize this approach after 15 high risk patients have been followed for 2 years and will repeat it with every 15 additional high risk patients.

*Toxicity Monitoring Rule:*

An informal toxicity monitoring rule will be employed during the trial where the accrual will be stopped to reassess safety concerns in the event that a total of 3 patients, regardless of histology, experience Grade 3 elevation in creatinine or decrease in glomerular filtration rate across all strata at any point during the study, as defined by CTCAE criteria (version 3.0). See section 12.3 for a summary of monitoring plans.

13.1.5 Analysis plan for the primary objectives:

*Biology objective:*

As indicated above total accrual on this trial is expected to last approximately 10 years. Tumor tissue will be collected from patients during this time, however the sample and array processing will not be performed until the accrual is complete. This will eliminate various sources of variability which would otherwise be introduced into the data. Further, in light of the rapidly progressing sample and array processing methodologies, waiting until the end of the accrual period to perform the necessary analyses would allow us to utilize the most up-to-date methods at that time. Once all tumor samples have been accrued, study investigators, the Biostatistics Department, and the Hartwell Center will collaborate to develop the most informative design and analysis of the biologic data given the labor, material, and cost constraints at that time. Topics to be decided include the selection of reference samples, replication, and associated plans for statistical analysis and inferences.

The same rapid emergence of new approaches is also true for statistical methodology regarding genomic data hence we will not pre-specify a particular approach that we will use for analysis which is expected to be performed approximately 11 years after study initiation. In general terms we anticipate that we will fit a Cox-type survival model per gene while simultaneously accounting for M-stage, extent of resection and desmoplasia or alternatively the risk-group in which patients were treated on protocol. For these models patients who experience disease progression or medulloblastoma specific death will be counted as having had an event; whereas patients who remain progression free or are lost to follow-up will be treated as censored at the date of last contact. If some of the patients experience competing events such as second malignancy or death by non-tumor related causes including toxicity, we will utilize an appropriate competing risks model (for example the method of Fine and Gray (1999)) to provide an alternative look at the data.

Provided adequate numbers of events are available per risk-stratum, an alternative subset analysis may be performed where patients within each risk-directed stratum are analyzed separately via a cause-specific survival model using PFS as the endpoint. Based on this approach the analysis performed for the low-risk stratum will only have to adjust for the genomic variable as all patients in this stratum will have M0, gross totally resected nodular desmoplastic Medulloblastoma. The analysis performed for medulloblastoma patients treated in the intermediate- and high-risk strata will only have to be adjusted for desmoplasia and resection-status, as all patients within the intermediate-risk stratum will be M0 and all patients within the high-risk stratum will be M+.

Further, in all these analyses we will adjust the p-values for multiplicity using an FDR-like approach in an effort to minimize the number of false positives.

*Therapeutic objective:*

As indicated previously, due to the lack of an adequate historical control to which the results of this trial can be compared, the primary therapeutic objective is to estimate the EFS distribution of the medulloblastoma cohort treated with the proposed risk directed therapy. Kaplan-Meier estimates of event-free survival (EFS) distribution for all eligible medulloblastoma patients treated on this trial will be provided. Following the ‘intent to treat’ principle, all medulloblastoma patients who receive at least one dose of methotrexate will be included in these estimates. EFS will be measured from date on treatment until the date of first progression, second malignancy or death due to any cause; or date of last contact for patients who have not experienced an event (failed). Patients who go off treatment due to toxicity or for any reason other than an event, as defined above, will still be followed on study until disease progression, second malignancy or death. Patients who remain progression free or are lost to follow-up will be treated as censored at the date of last contact.

## 13.2 ANALYSIS PLAN FOR SECONDARY OBJECTIVES

The caveat regarding rapidly evolving statistical methodology applies to the secondary objectives also. The analysis plans given below represent the current knowledge and may be modified at the time of the analysis if better approaches become available.

### *13.2.1 Biological aims*

*Responsible Investigator(s): Amar Gajjar, Giles Robinson*

*Responsible Biostatistician: Arzu Onar-Thomas, Tong Lin*

*To perform high resolution genome-wide analyses of chromosomal abnormalities and gene expression patterns, and evaluate the relationship of these to other clinicopathological variables.*

For patients from whom fresh frozen tumor is available at the time of surgery microarray analysis will be performed on the tumor sample obtained at initial or repeat surgery prior to treatment. The association between clinicopathological variables (e.g., M-stage, desmoplasia, age etc.) and presence of mutations as well as gene expression profiles will be explored.

To compare expression between known clinicopathological subtypes, the Kruskal-Wallis test<sup>350</sup> for equal medians will be applied to the expression of each probe. For large enough group sizes, the chi-square approximation to the sampling distribution of the Kruskal-Wallis statistic will be used to obtain a  $p$ -value. For small group sizes,  $p$ -values will be obtained by comparing the observed Kruskal-Wallis statistic to the set of Kruskal-Wallis statistics obtained by pairing all possible permutations of group labels (or a large sample of the possible permutations) with the ranks.

To assess the association of expression with other uncensored continuous variables (such as age, levels of certain proteins or other biologically meaningful quantities), the Spearman rank correlation test will be used.<sup>351</sup> For large enough sample sizes, the  $t$ -distribution approximation to the sampling distribution of the statistic will be used to obtain a  $p$ -value. For smaller samples,  $p$ -values will be obtained by comparing the observed Spearman rank correlation to the set of Spearman rank correlation statistics obtained from pairing all possible permutations (or a large sample of these permutations) of the first  $n$  digits (e.g. 1, 2,  $n$ ) with the ordered first  $n$  digits, where  $n$  is the number of individuals under consideration.

For each set of  $p$ -values obtained by testing one of the hypotheses mentioned above, multiplicity issues will be examined using the concepts introduced by Pounds and Morris.<sup>342</sup> However, instead of using the beta-uniform mixture model to estimate the distribution of  $p$ -values, a cubic-spline approximation of the cumulative distribution function will be used to estimate the distribution of  $p$ -values.<sup>352</sup> The approach can be used to explore various measures of the expected occurrences of Type I and Type II errors for any selected significance threshold. Additionally, the  $q$ -value approach introduced by Storey et al. may be used to examine multiplicity issues.<sup>353</sup>

Unsupervised clustering algorithms, such as k-means and hierarchical clustering, will be used descriptively to explore the possibilities of discovering novel subgroups based on gene expression. Additional descriptive analyses may include principle component analysis and discriminant analysis.<sup>354</sup>

SNP chips are relatively new and rapidly developing technology designed to simultaneously measure the number of copies per cell of many genes at the DNA level. We will collect germline and tumor samples from each subject for DNA SNP microarray analysis. The method of Carvahlo et al.<sup>355</sup> will be used to compute summary signals for each array by removing the effects of fragment length and DNA probe sequence. Genotype calls will be generated using the

CRLMM algorithm<sup>355</sup> and regions of loss of heterozygosity will be inferred via hidden Markov modeling of the within-subject comparison of genotype calls in tumor and germline samples.<sup>356</sup> For copy number analysis, the summary signals will be reference marker normalized.<sup>357</sup> Inferences of change in copy number will be generated via comparison of reference-marker normalized tumor signals and germline signals. The copy number inferences will be obtained via median smoothing,<sup>358</sup> hidden Markov modeling,<sup>359</sup> or circular binary segmentation.<sup>360,361</sup> Biological considerations and the estimated prevalence of lesions will be used to select regions of inferred LOH or copy number change for laboratory verification. Finally, we will explore the association of germline genotypes and inferred copy number change and LOH with clinical presentation and outcome, pharmacokinetic and pharmacodynamic variables, and microarray gene expression values using previously described statistical methods<sup>362</sup> and adjust for multiple testing by using methods to control or estimate the false discovery rate.<sup>363,364</sup> As the field of statistical genomics changes rapidly, improved methods may be available and used at the time of analysis.

*To evaluate specific tumor types for molecular abnormalities with suspected prognostic or therapeutic significance*

Specific tumor types within medulloblastoma will be scrutinized for the presence of molecular abnormalities with putative prognostic or therapeutic significance such as the activation of the wnt or shh signaling pathways, loss of chromosomes 6, 8p, 9q22, isochromosome 17q and amplification of MTCC, MYCN and MYCL. Contingency table analysis will be used to examine the association of molecular abnormalities with other categorical characterizations of tumors. If appropriate, associations of continuous markers with specific tumor types will be examined using ANOVA or the Kruskal-Wallis test to compare the mean or median of the continuous variable across subgroups as defined by this technique. Significance will be determined using exact, permutation, or resampling methods.

*To evaluate the feasibility of collecting frozen and fixed tumor samples suitable for analysis using high-resolution molecular biology tools.*

As indicated in the statistical design for the primary objective we estimate that approximately 60% of patients will have frozen tissue available and approximately 70% of the available frozen tumor tissue will be suitable for high-quality RNA extraction. A similar proportion of samples are expected to yield high-quality protein, and a larger proportion to yield high-quality DNA. It is anticipated that most of the fresh-frozen tumor tissue will be available in small amounts making it infeasible to use some of it to assess RNA, DNA, and protein quality upon arrival at the biology lab. As discussed in the plan for the primary objective, we will estimate the proportion of patients with frozen tumor tissue after the first 30 patients (with any histologic diagnosis) have been enrolled on the study, and re-evaluate the feasibility of completing this objective of the trial if this proportion is below 40%. See section 12.2.

Compared to the percentage of patients with frozen tissue, we anticipate that a much higher percentage of patients will have fixed tumor tissue available. Nevertheless we will consider it unacceptable if less than 60% of patients have fixed tissue available for biological studies as well as for pathological diagnosis. Note that whereas the primary objective concerns only medulloblastoma patients, this secondary objective involves all patients enrolled on the study. As will be done for frozen tissue, after the first 30 patients (of any histologic diagnosis) have been enrolled on the study we will estimate the proportion of patients with fixed tumor tissue. If this proportion is below 60%, we will re-evaluate the feasibility of completing this objective. Beyond the first 30 patients, the estimate of the proportion of patients with fixed tumor tissue will be updated with every 30 new patients enrolled on the trial and if this percentage falls below 60% we will re-assess the feasibility of this objective. See section 12.2 for further discussion.

### *13.2.2 Therapeutic aims*

*Responsible Investigator: Amar Gajjar*

*Responsible Biostatistician: Arzu Onar-Thomas*

*To estimate the event-free and overall survival for patients treated with the proposed risk-adapted therapy regimen, and to descriptively compare these survival rates to historical controls.*

Histology specific Kaplan-Meier estimates of distributions of survival and event-free survival (EFS) for all eligible patients who received at least one dose of methotrexate will be provided separately for each stratum. EFS will be measured from the date of initial treatment to the earliest date of disease progression, second malignancy or death for patients who fail; and to the date of last contact for patients who remain at risk for failure. Note that toxicity will not be treated as an event in these estimates. The distribution estimates will be consistent with intent-to-treat.

Provided the accrual for the proposed study allows a reliable analysis to be performed, EFS and OS distributions will be estimated for each group stratified based on the proposed risk directed therapy and possibly by histology (for the intermediate and high risk groups). These will then be compared to the St. Jude historical cohort using the Mantel-Haenszel Log-rank test for the intermediate and high risk strata. Clearly, regardless of the number of patients enrolled on the low risk arm of this study, a meaningful stratum-specific comparison with the historical cohort cannot be made due to the fact that the historical cohort includes only three patients who would have been classified as low risk. Note that the comparison with SJ historical cohort will be made based on updated historical data as available at the time of analysis.

*To estimate the rates of local and distant disease progression in patients treated with focal irradiation of the post-operative tumor bed using a 5 mm clinical target volume margin.*

We will estimate the rate of local and distant disease progression in patients treated in the intermediate risk stratum (M0 non-desmoplastic medulloblastoma and other eligible tumors) after focal irradiation of the post-operative tumor bed using a 5 mm clinical target volume margin. This analysis will be done once all patients in this stratum have been followed for at least 1 year from the end of their radiation treatment. We will provide exact 95% confidence intervals for the local and distant failure rates for the overall group and in the cases where patient numbers are adequate to make such estimates meaningful, for histology-specific sub-groups. Historical control data for this objective are limited. In the St. Jude historical control group (treated since 1998), 13 M0 medulloblastoma patients received focal RT at doses of 45 to 54 Gy and subsequently remained disease-free. Six M0 medulloblastoma patients treated during this era experienced disease recurrence or progression; 4 of these events occurred before focal RT was scheduled to be given. Two patients had events after RT; one experienced local recurrence after receiving a dose of 45 Gy to the tumor bed, and the other experienced disseminated recurrence after receiving low-dose CSI (18 Gy). The St. Jude historical control group also included 27 M0 ATRT, PNET, and pineoblastoma patients. Of these, only 5 reached the time of planned focal RT without experiencing disease progression, and among those 5 patients 3 became long-term event-free survivors and 2 developed disease recurrence (1 local and 1 disseminated). Both patients who experienced recurrence after focal RT received a tumor bed dose of 45 Gy. Thus, in the St. Jude historical control group as a whole, only 3 patients experienced failure after receiving focal RT, and all 3 received a tumor bed dose (45 Gy) substantially lower than the 54 Gy which will be used on SJYC07. Unlike in SJYC07, patients in this historical control group also received a radiation dose to the entire posterior fossa ranging from 18 to 36 Gy. CTV margins were also larger in the historical control group, and ranged from 1 cm to 2 cm. By the time SJYC07 is complete, results from PBTC-001 and P9934 should be available, and may provide additional historical control data relevant to this objective.

The following variables will be considered in relation to the rate and patterns of disease progression: planning target volume margin (3mm or 4mm or 5mm), planning target volume conformity index (e.g., 80% or 95% or 100%), recurrent tumor volume dose-volume histogram, method-modality (3DCRT, IMRT, proton beam radiation therapy).

*To estimate the objective response rate (sustained for 8 weeks) to induction chemotherapy including high-dose intravenous methotrexate for patients with residual or metastatic disease.*

For patients treated in the intermediate and high risk strata with residual or metastatic disease we will estimate the stratum-specific objective response rate (CR or PR) as defined in Section 9.1. Consistent with intent to treat, all patients who receive at least 1-dose of methotrexate are evaluable for response. Objective responses **must** be sustained for **at least** eight weeks. Provided it is confirmed and sustained, the date of response will be the imaging date at which the response was

first noted, or (in the case of patients with M1 disease and no radiographically evident residual tumor), the date of the first negative CSF cytology sample. We will provide exact 95% confidence intervals for the objective response rate separately for each stratum and if patient numbers allow, for each histologic subtype within each stratum. From the definition of the risk strata, all M0 patients with residual disease are in the intermediate-risk group and all M+ patients are in the high-risk group. Very few patients are expected to be M1 (only 5 in the historical cohort, 1 medulloblastoma and 4 other tumors, and none of these had residual disease) and even fewer would have measureable residual disease which would make obtaining meaningful estimates for this sub-group quite difficult. Within the high risk stratum, we will provide estimates of objective response separately for the M1 and M2+ patients, if adequate M1 patients have been accrued. Otherwise we will provide response estimates for the M+ group with and without the M1 patients. According to the SJ historical cohort identified for this study, 13 out of the 47 M0 patients and 13 out of the 35 M+ patients had measureable residual disease.

*To evaluate the feasibility and toxicity of administering low dose intravenous vinblastine in conjunction with induction chemotherapy for patients with metastatic disease.*

In order to evaluate the feasibility of administering low dose intravenous vinblastine in conjunction with induction chemotherapy for patients with metastatic disease, after the first 10 patients with metastatic disease have completed induction therapy, we will estimate the proportion of courses during which subsequent therapy was delayed for more than 7 days due to toxicity. If this percentage is greater than 25%, we will consider it unacceptable and will consider re-evaluating our approach. This percentage will be updated when every 10 new patients with metastatic disease complete induction and will be compared against the 25% threshold.

*To evaluate the feasibility and toxicity of administering consolidation therapy including cyclophosphamide and pharmacokinetically targeted topotecan to patients with metastatic disease, and to estimate the sustained (for 8 weeks) objective response rate (complete response + partial response, as defined in section 9.1) to such therapy in patients with measurable residual disease after induction.*

In order to evaluate the feasibility of administering consolidation therapy including cyclophosphamide and pharmacokinetically targeted topotecan to patients with metastatic disease, after the first 10 patients in the high-risk stratum have completed both courses of consolidation, we will estimate the proportion of courses during which subsequent chemotherapy administration was delayed for more than 7 days due to toxicity. If this percentage is greater than 25%, we will consider it unacceptable and will consider re-evaluating our approach. This percentage will be updated with every 10 new patients who complete both high-risk consolidation courses and will be compared against the 25% threshold.

For patients on the high-risk stratum with measurable residual disease after induction treated with consolidation therapy including cyclophosphamide and pharmacokinetically targeted topotecan, we will estimate the objective response rate (CR or PR) as defined in Section 9.1. Consistent with intent to treat, all patients who receive at least 1-dose of cyclophosphamide or topotecan during consolidation are evaluable for response. Objective responses **must** be sustained for **at least** eight weeks. Provided it is confirmed and sustained, the date of response will be the imaging date at which the response was first noted. We will provide exact 95% confidence intervals for the objective response rate for each histologic subtype separately, if patient numbers allow.

*To evaluate the feasibility and toxicity of administering oral maintenance therapy in children younger than 3 years of age at diagnosis.*

The overall strategy of this protocol is to facilitate the development and implementation of novel therapies with more specific anti-tumor activity and less toxicity than standard cytotoxic agents. The large majority of new drugs in oncology are designed to be administered orally. The ability to receive oral therapy at home has tremendous appeal to adults and older children with cancer, but administration of oral medication of any kind to infants and toddlers can be quite difficult. We therefore intend to evaluate the feasibility of administering oral chemotherapy for a prolonged period of time (24 weeks) to children in this population.

After the first 20 patients complete maintenance therapy, we will assess the feasibility of this objective. Based on patient diaries, we will estimate the percentage of total scheduled doses patients received per course for each of oral topotecan, cyclophosphamide, erlotinib and etoposide. We believe that if the average number of doses received per course is less than 75% of the scheduled number of doses due to toxicity or patient refusal to take the agent, then the feasibility of administering oral maintenance therapy in very young children may be in question. The feasibility assessment will be repeated after every 30 patients complete maintenance therapy.

*To use quantitative MR measures (volumetric, diffusion, and perfusion) of brain tumor patients less than 3 years of age receiving chemotherapy including high-dose intravenous methotrexate to assess impact of treatment on developing brain.*

Quantitative MRI measures of change in neurostructure (especially white matter volume and integrity) over time will be assessed using a random effects model incorporating various covariates. Covariates to be considered include age at diagnosis, time since diagnosis and risk-arm. Differences in quantitative MRI measures of neurostructure volume and integrity between patient groups will be evaluated as a metric of structural neurotoxicity of therapy. Exploratory analyses will also investigate the association between patterns of neurostructure changes and clinical and demographic variables.



*Positron Emission Tomography for Dose Verification after Proton Beam Therapy  
(for participants enrolled at St Jude only)*

All intermediate risk patients treated at St Jude are eligible to receive proton beam therapy (PBT) and will be routinely referred to the University of Florida Proton Therapy Institute (UFPTI), though study investigators expect that family preferences (e.g. difficulties associated with relocating to Florida for approximately eight weeks, etc.) may play a role in determining the percentage of patients who will choose not to receive PBT. Reasons (clinical or family-based) for opting out of PBT will be documented and summarized. Additionally if adequate number of intermediate risk patients fall in the non-PBT category to make such a comparison meaningful, these patients will be compared to patients who received PBT based on relevant clinical and demographic characteristics such as age, tumor location, tumor type and extent of resection to investigate differences between the two groups.

Based on St Jude historical data for the cohort who would have been eligible for SJYC07, approximately 50% of patients are expected to be classified as intermediate risk. This figure is consistent with the first 79 St Jude patients who have been enrolled on the protocol to date, 41 of whom are intermediate risk. The accrual goal of the protocol is 90 medulloblastoma patients. It is estimated that approximately 225 additional non-medulloblastoma patients will also be accrued during the same period. As indicated previously 50% of all patients are expected to be classified as intermediate risk, hence the estimated number of intermediate risk patients who will enroll on the protocol is 157 and we estimate that 50-60% of these patients (78-94) will be treated at St Jude. St Jude historical data suggests that EFS at 4 months, which is the end of induction for this cohort of patients is 72.3% (s.e. 7.46%), hence approximately 56-67 patients will be available to be referred for PBT. The study investigators expect 50%-66% of these patients to receive PBT and to consent to participate in the required PET studies. Hence we estimate that images from 28-44 patients will be available for this secondary objective.

This objective aims to assess the feasibility of using post-PBT PET as an in-vivodosimetric and distal edge verification system in this patient population. To this end we will estimate the percentage of PET scans where loss of signal intensity or resolution were observed. The former may be due to activation decay of relevant isotopes and the latter due to biological washout and patient motion. In addition we will estimate the differences observed between the measured and simulated parameters such as positron activation locations and intensities.

*13.2.3 Pharmacologic aims*

*Responsible Investigator: Clinton Stewart*

*Responsible Biostatistician: Arzu Onar-Thomas*

*To develop a population pharmacokinetic model containing covariates which explain inter- and intra-patient pharmacokinetic variability for high-dose methotrexate in young children with brain tumors, and to explore the relationship*

*between clinical effect (toxicity and antitumor efficacy) and methotrexate pharmacokinetics.*

A compartmental pharmacokinetic model will be fit to the methotrexate plasma concentration-time data using maximum likelihood estimation as implemented in ADAPT II (ADAPT II User's Guide, 1997). Individual pharmacokinetic parameters estimated will include volume of the central compartment (Vd), elimination rate constant (Ke), and half-life (t1/2). The methotrexate clearance will be calculated using the log-linear trapezoidal method.

In addition to estimating individual pharmacokinetic parameters, we will also estimate the population parameters using nonlinear mixed effects modeling methods (NONMEM). This method estimates the population parameters and both the inter- and intra-subject variability. Once the population parameters and corresponding covariance matrix are estimated, individual estimates can be obtained using post hoc analysis. Thereafter the influence of patient specific covariates such as age, body-surface area, and sex on methotrexate pharmacokinetic parameters will be evaluated.

A Cox-type survival model, possibly with time dependent covariates, may be used to explore the relationships between toxicity and efficacy vs. PK parameters.

*To assess the extent of inter-patient variability in the pharmacokinetics of intravenous and oral cyclophosphamide and metabolites in young children with brain tumors, and explore possible associations between cyclophosphamide pharmacokinetic parameters and patient specific covariates (e.g., age, sex, race, weight).*

Previous studies by Yule et al. have failed to demonstrate a relationship between patient specific cofactors, such as a child's body surface area, age, gender, glomerular filtration rate, or hepatic function, and exposure to cyclophosphamide metabolites DCCY and CEP<sup>365,366</sup>. However, the effects of such cofactors on the exposure to the active metabolite HCY have not been evaluated in children. We will therefore estimate the population pharmacokinetic parameters for cyclophosphamide and metabolites (i.e. HCY) using nonlinear mixed effects modeling methods (NONMEM). This method estimates the population parameters and the inter-subject variability. Once the population parameters and corresponding covariance matrix are estimated, individual estimates can be obtained using post hoc analysis. Using such a method, the influence of patient specific covariates such as age, BSA, and sex on cyclophosphamide/4-HCY pharmacokinetic parameters will be tested to optimize dosing in children with medulloblastoma.

*To assess the ability to achieve the target systemic exposure of intravenous topotecan in children diagnosed with metastatic brain tumors at a young age.*

Based on the accrual as of amendment 5.0, we expect approximately 20-25% of the patients enrolled on this study to have metastatic brain tumors, which leads to

an estimated 18-23 patients from a total of 90 patients with medulloblastoma and another 45-57 patients from the non-medulloblastoma cohort. We anticipate that approximately 50-60% of these patients will be treated at SJ and only SJ patients will contribute to this objective. The 6-month EFS rate for the high-risk cohort is 37.1% (s.e. 8.17%), hence we expect samples from 15-20 patients will be available for this objective.

Pharmacokinetic studies will be performed on day 1 of IV topotecan therapy during the consolidation phase. If the systemic exposure (AUC) of topotecan lactone is not within the target range of  $140 \pm 20$  ng/ml\*hr on day 1, the topotecan dosage will be adjusted on subsequent days to achieve this target. If a dosage adjustment is made, another pharmacokinetic study will be performed to determine whether the target AUC has been achieved with the new dosage. If pharmacokinetic studies have been performed three times in one course and the AUC is still not within the target range, one final dosage adjustment will be made without further pharmacokinetic studies during that course. The final dosage from course 1 will be used for the initial day 1 dosage during course 2. The same pharmacokinetic studies will be performed during course 2 to achieve the target AUC.

For analysis of our targeting success rate, we will consider AUC values resulting from a pharmacokinetically-guided dosage adjustment differently than those determined solely from a predetermined dosage, such as the first dose of cycle 1. In the latter instance, we will refer to the AUC value as a “dose success” or a “dose failure,” and will reserve the terms “pharmacokinetic targeting success” and “pharmacokinetic targeting failure,” respectively, for situations in which the dosage adjustment does or does not place the patient’s topotecan lactone AUC within the target range. The initial topotecan dosage of course 1 will not count towards this feasibility objective, since this dosage is predetermined.

Based on prior experience with topotecan targeting at St. Jude, we expect to achieve the target exposure in approximately 70% of targeted PK studies. We will assess the targeting success rate (i.e., studies that are targeting successes/total number of eligible targeting studies) after the first 10 SJ patients have been treated with targeted topotecan on protocol version 3.0 or later. If the targeting success rate is less than 60%, we will modify the targeting strategy. See section 12.2 for details. As part of the analysis we will also provide simple descriptive statistics for each group (e.g., dose success and failure, and pharmacokinetic targeting success and failure).

*To assess the extent of inter-patient variability in the pharmacokinetics of intravenous and oral topotecan in young children with brain tumors, and explore possible associations between topotecan systemic exposure and patient specific covariates (e.g., age, sex, race, weight).*

We have evaluated the relationship between patient specific cofactors, such as a child’s body surface area, age, gender, glomerular filtration rate, or hepatic

function, and topotecan systemic exposure in a population analysis. However, this analysis focused on older children and only included 14 children less than 2 years old. Thus, we will estimate the population pharmacokinetic parameters for topotecan using nonlinear mixed effects modeling methods (NONMEM). This method estimates the population parameters and the inter-subject variability. Once the population parameters and corresponding covariance matrix are estimated, individual estimates can be obtained using post hoc analysis. Using such a method, the influence of patient specific covariates such as age, BSA, concomitant drugs, pharmacogenetic factors, and sex on topotecan pharmacokinetic parameters will be tested to optimize dosing in very young children with brain tumors.

*To assess the extent of inter-patient variability in the pharmacokinetics of oral erlotinib in young children with medulloblastoma, high-grade glioma and ependymoma,, and explore possible associations between erlotinib pharmacokinetic parameters and patient specific covariates (e.g., age, sex, race, weight).*

We will estimate the population pharmacokinetic parameters for erlotinib using nonlinear mixed effects modeling methods (NONMEM). This method estimates the population parameters and the inter-subject variability. Once the population parameters and corresponding covariance matrix are estimated, individual estimates can be obtained using post hoc analysis. Using such a method, the influence of patient specific covariates such as age, BSA, concomitant drugs, pharmacogenetic factors, and sex on erlotinib pharmacokinetic parameters will be tested to optimize dosing in very young children with brain tumors.

*For St. Jude patients enrolled on the PGEN5 protocol, to assess the relation between pharmacogenetic variation in drug metabolizing enzymes and drug transporters, and the pharmacokinetics of methotrexate, cyclophosphamide, topotecan, and erlotinib in young patients with brain tumors.*

All St. Jude patients will be asked to enroll on the institutional protocol PGEN5 for the collection of blood to process for DNA. PGEN5 is a non-therapeutic study that investigates the influence of genetic polymorphisms on the disposition or effects of therapeutic agents. In this analysis, we will assess the relation between appropriate metabolism (e.g., CYP3A5\*1) and transporter genotypes (e.g., ABCG2 34G>A), and pharmacokinetic metrics (e.g., AUC or clearance) for cyclophosphamide, topotecan, methotrexate, and erlotinib. We will also explore the relation between genotype, pharmacokinetic phenotype, and patient outcome.

#### *13.2.4 Cancer control aims*

##### *CSF neurotransmitter studies*

*Responsible Investigator: Clinton Stewart; Heather Conklin*

*Responsible Biostatistician: Hui Zhang*

Correlations between complex neurochemical CSF phenotypes and attentional functioning, as well as the effect of genotype on each these two outcomes have never been studied in young children with primary tumors of the central nervous system. Because of the lack of data to formulate specific hypotheses, we will analyze the following two CNS neurotransmitter objectives after 30 patients have been followed for one year (approximately 3-4 CSF samples and 2-3 neurocognitive scores). The primary investigator as well as co-investigators from pharmaceutical sciences, cancer control, and biostatistics will review the data and analysis results for either formulating specific hypotheses to be tested in the remaining patients accruing to the study or to stop investigation because of the obvious futility of this research. Results may be reported at a national or international meeting emphasizing the exploratory nature of the findings. If the plan is to continue with the investigation, we will report the intention to confirm the results in an independent set of data from the same protocol, SJYC07. No changes in clinical practice will occur due to this initial unconfirmed analysis.

*To explore possible associations between CSF neurotransmitter concentrations, with emphasis placed upon concentrations of dopamine and its metabolites, and the development of neurocognitive deficits as identified by standardized tests.*  
*Responsible Investigator: Clinton Stewart; Heather Conklin*  
*Responsible Biostatistician: Hui Zhang*

To investigate whether neurocognitive deficits after medulloblastoma therapy are associated with suspected iatrogenic disturbances in central dopamine transmission, an exploratory study of CNS neurotransmitters will be undertaken with particular focus placed upon dopamine and metabolite profiles. Short term relationships will be investigated by calculating differences in the pre-treatment and 1 year values for both the neurotransmitter and attentional outcomes. Appropriate regression analyses (parametric or nonparametric, e.g. splines) will be used to assess the relationship between the change in neurotransmitter values vs. the change in neurocognitive function. Long term effects, estimating the change in neurotransmitter values and the change in attentional function and working memory through treatment and post-treatment, will be investigated using random coefficient models or some other appropriate model for longitudinal data. Ideally all analyses must take into consideration risk group, neurocognitive intervention, and other covariates deemed important however sample size considerations may make this infeasible. The effects of serotonin and its metabolite, 5-HIAA, will also be investigated in the same manner.

*To explore the association between genetic polymorphisms affecting central dopaminergic transmission and specific phenotypes, including CNS neurotransmitter and neurocognitive performance phenotypes.*  
*Responsible Investigator: Clinton Stewart; Heather Conklin*  
*Responsible Biostatistician: Hui Zhang*

Genotyping for genetic polymorphisms (e.g. DAT1, DRD4) will be assessed using standard molecular techniques. Based on the observed allelic frequencies, we expect to analyze the genotypes on a homozygous/heterozygous categorization. If, however, low frequencies are observed, patients will be grouped by presence or absence of particular polymorphisms for analysis. We will report the distribution of homozygosity and heterozygosity, as well as the distribution based on presence vs. absence for each polymorphism.

To assess the effect of pharmacogenetic variation on CNS transmitters, we will initially consider only the dopamine and serotonin neurotransmitters and metabolites provided in Table 21. Kruskal-Wallis tests will be used to evaluate differences in neurotransmitter concentrations at study enrollment by DAT1\*10 and DRD4\*7 polymorphisms. Random coefficient models or other models appropriate for longitudinal data will be used to evaluate the association of the DAT1\*10 and DRD4\*7 polymorphism with the change in neurotransmitter levels throughout treatment and post-treatment.

To assess the correlation of pharmacogenetic variation with neurocognitive performance phenotypes, random coefficient models or other longitudinal models will be used to evaluate the association of genetic polymorphisms with changes in attentional functioning and working memory

*Neuropsychological objectives*

*Responsible Investigator: Heather Conklin*

*Responsible Biostatistician: Hui Zhang*

*To investigate changes in neuropsychological performance among patients enrolled in the study, and examine the impact of the proposed treatment regimen and other disease related factors (e.g., hydrocephalus) on neuropsychological performance.*

Hypothesis 1: There will be a decline (negative slope) in global cognitive functioning for the group as a whole.

Hypothesis 2: Hydrocephalus (correcting for CSF diversion) will be associated with a steeper decline in global cognitive functioning.

Hypothesis 3: Higher radiation doses to specified structures (e.g., the frontal lobes) will be associated with steeper declines on related functions (e.g., attention and working memory).

Patients treated on this protocol will be subjected to serial neuropsychological evaluations (baseline, 6- and 12-months from treatment initiation and yearly after the first year.) Random-coefficient models relating the longitudinal neuropsychological performance measures to demographic variables (e.g., gender, age at diagnosis) disease-related covariates (e.g., tumor type, tumor location,

number of surgeries, extent of resection, presence of hydrocephalus. duration of time between symptom onset and treatment, endocrine abnormalities [particularly growth hormone], presence of a shunt, number of shunt revisions) and treatment-related factors (e.g., chemotherapy agents and radiation dosimetry) will be studied in single or multivariable models based on data availability. If the number of longitudinal measurements is deemed too small per patient to make longitudinal random coefficient models effective then an attempt will be made to associate disease and treatment related covariates to changes in neuropsychological performance measures via parametric or non-parametric regression approaches. Such regression models will also be used to study short term changes in neuropsychological performance measures (differences in the pre-treatment vs. 6-month and pre-treatment vs. 1 year values) via their association with these covariates.

Our ability to assess structure-function relationships (Hypothesis 3) will depend on the number of patients who receive a significant radiation dose to the structures of interest, and survive long enough to undergo repeated neurocognitive testing. Anatomical structures of interest and their corresponding neurocognitive functions are presented in Table 34. Assessment of relationships between posterior fossa RT dose and motor coordination should be quite feasible, given the expected preponderance of medulloblastoma patients enrolled on the trial; medulloblastomas are located in the posterior fossa by definition and have moderate survival rates. We plan to evaluate each of the other relationships if the number of surviving patients with relevant radiation exposure permits. Additional structure-function relationships may also be evaluated, if there is evidence from the available literature to suggest such a relationship.

<i>TABLE 34 POSSIBLE STRUCTURE-FUNCTION RELATIONSHIPS TO BE ASSESSED</i>	
<b>Anatomical Structure</b>	<b>Neurocognitive Function</b>
Posterior Fossa	Motor Coordination
Frontal Areas	Attention; Working Memory
Medial Temporal Lobes	Memory
Left Temporal Lobe/Perisylvian Area	Language; Reading
Right Parietal/Occipital Lobes	Visual-spatial Reasoning

Variables for dose-volume analyses will include, for a specified normal tissue volume, representing the lobes of the brain and smaller functional elements, the mean dose and  $V_{0-5 \text{ Gy}}$ ,  $V_{5-10 \text{ Gy}}$ ,  $V_{10-15 \text{ Gy}}$ ,  $V_{15-20 \text{ Gy}}$ ,  $V_{20-25 \text{ Gy}}$ ,  $V_{25-30 \text{ Gy}}$ ,  $V_{30-35 \text{ Gy}}$ ,  $V_{35-40 \text{ Gy}}$ ,  $V_{40-45 \text{ Gy}}$ ,  $V_{45-50 \text{ Gy}}$ ,  $V_{50-55 \text{ Gy}}$ ,  $V_{55-60 \text{ Gy}}$ . The latter variables represent the percent volume receiving dose over the specified interval which will be used for correlation with the magnitude and time to onset of clinically significant CNS effects including cognitive, endocrine, audiometric, neurologic and structural abnormalities.

Hydrocephalus measurements (evans index, cella media index, ventricular angle and frontal horn diameter) corresponding to the time of diagnosis, enrollment, completion of therapy and two years after the initiation of therapy will be recorded for each patient along with clinical information about CNS diversion (presence or absence of ventriculostomy, CSF shunting and shunt revisions). Correlations between hydrocephalus measurements and biologic, neuropsychological and neurostructural outcomes have not been studied in this patient population. We hypothesize that the baseline and longitudinal changes in hydrocephalus measurements, when corrected for temporary or permanent CSF diversion will influence the baseline biologic, neuropsychological and neurostructural measurements. These data will be generated for use by study co-investigators to study the magnitude and rate of change in hydrocephalus parameters and to determine their value as covariates in the secondary objective aims for this protocol. Results may be reported at a national or international meeting emphasizing the exploratory nature of the findings.

*Interim Analysis to assess neurocognitive development of young children:*

We are interested in conducting an interim analysis to take a preliminary look at the trajectory of neurocognitive development among children diagnosed with malignancies of the CNS in infancy and treated with risk-adapted therapy using a comprehensive battery to assist with planning for dissemination of findings through conference presentations and manuscripts. Any manuscript which results from the interim look at the data will clearly state that these results are preliminary. Furthermore, the manuscript that reports the final results from these data at the end of the trial will acknowledge the interim look at the data and reference the relevant manuscripts or presentations.

Our primary domains of interest are detailed below:

- Intelligence as measured by the Cognitive Scale Composite Score, Bayley Scales of Infant and Toddler Development, Third Edition or Abbreviated IQ from the Stanford-Binet Intelligence Scales, 5th Edition.
- Attention as measured by the Test of Visual Attention, Auditory Attention from the Woodcock Johnson Tests of Achievement, 3<sup>rd</sup> Edition and Parent Report on the Behavior Assessment System for Children, Second Edition.
- Executive Function as measured by Retrieval Fluency and Auditory Working Memory from the Woodcock Johnson Tests of Achievement, 3<sup>rd</sup> Edition, and Parent Report on the Brief Inventory of Executive Function.
- Processing Speed as measured by the Visual Matching and Decision Speed subtests of the Woodcock Johnson Tests of Achievement, 3<sup>rd</sup> Edition.

By assuming intelligence (as measured by the Cognitive Scale Composite Score, Bayley Scales of Infant and Toddler Development or Abbreviated IQ from the Stanford-Binet Intelligence Scales) drops by about 1/3 of a standard deviation from baseline to 3-year assessment, 73 subjects will be needed to obtain 80% statistical power and 5% type 1 error by paired t-test. Therefore, an interim



analysis will be performed once we have at least 73 patients who have completed their 3-year neuropsychology assessment and have been measured for at least one of the domains mentioned above at 3 years (+/- 3 months) since enrollment and at least one additional assessment prior to this one. Patients who may have missed their 3-year assessment but who have been assessed at a later time point will also be counted towards the 73 patients provided they have at least 1 more measurement before the 3-year time point. Although paired t-test was used to estimate sample size, a mixed-effect model will be employed to study change over time since treatment initiation in these data. While these 73 patients will determine the timing of the proposed interim analysis, our model will incorporate all the patients who have at least two neuropsychology assessments with the constraint that they must have at least 1 measurement at or beyond 1-year. To address the association of other factors (e.g., treatment intensity, patient age and presence/absence of posterior fossa syndrome), they will be included as covariates in the mixed-effect modeling. We may also consider other potential factors for inclusion in these models as appropriate.

*Diagnostic imaging objectives*

*Responsible Investigator: Eugene Reddick, Heather Conklin*

*Responsible Biostatistician: Hui Zhang, Yimei Li*

*To assess the impact of changes in quantitative MR measures in the frontal lobe on neurocognitive performance in attention, working memory, and fluency.*

Associations between changes in quantitative MR measures in the frontal lobe and changes in neurocognitive performance in attention, working memory, and fluency will be studied via random effects models if adequate longitudinal data is available. Note that one test battery will be used to evaluate children younger than 3 years of age and a different battery will be used to assess children older than 3 years of age. Some neurocognitive measures will only be included in the test battery for older children; for these measures, analyses will be performed based on data collected after patients reach 3 years of age. Several other measures (e.g., IQ, adaptive functioning and executive function) will be included in both batteries, and will be more amenable to longitudinal analysis. If the amount of longitudinal data for a particular measure is deemed inadequate for analysis, regression (parametric or nonparametric) type approaches may be utilized to study changes (e.g. baseline vs. 6-months; baseline vs. 1-year) in these variables.

*To assess the impact of changes in quantitative MR measures in the right frontal-parietal regions on neurocognitive performance on visual-spatial reasoning and processing speed.*

Quantitative MRI measures of change in neurostructure may provide *in vivo* measures of the impact of therapy on the normal brain. Models incorporating measures of therapy (risk arm, radiation dose) and quantitative MRI of neurostructure (especially white matter volume and integrity) will be evaluated first to ensure conditions necessary to test for statistical inference by computing partial correlation coefficients, controlling for age at diagnosis and time since diagnosis in all analyses. Random effects models may also be utilized if the longitudinal nature of the data is deemed adequate. As discussed above, however, some neurocognitive measures will only be obtained in children older than 3 years of age, and the amount of longitudinal data for these measures may therefore be limited. With sufficient sample size, it would be preferable to use path analysis or structural equation modeling (SEM) to examine each conceptual model.

*To evaluate endocrine function before and after radiation therapy (for participants enrolled at St Jude only)*

*Responsible Investigator: Thomas Merchant*

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It is estimated that 66-80 St Jude patients will be eligible for referral for proton beam therapy (PBT) and the study investigators expect that 45-60 patients will receive PBT. Serial GH testing (at baseline, the end of therapy, and at 6 and 24

months after completion of therapy) will be performed on consenting patients in order to estimate longitudinal change in GH secretion as measured by mean peak GH values, with the intent to explore associations with radiation dose to the hypothalamus. Since determination of proton- or photon-based radiotherapy is not based on randomization, it will not be possible to compare the endocrine outcome between the patients with and without PBT. However, the differences between these two clinical cohorts with respect to clinical and demographic variables of interest will be summarized via descriptive statistics. Due to the same constraints, the results of the modeling exercises described below are exploratory and should be interpreted as hypothesis generating.

The intent of this objective is to estimate the longitudinal change in abnormal GH secretion as measured by mean peak GH values via a mixed effects model for the patients who receive PBT. If sample size is sufficient ( $n > 10$ ), a separate model will be developed for patients receiving photon-based radiation therapy, though no direct comparison between the two cohorts will be made.

Within the constraints of the available sample size, it is also of interest to incorporate various clinical and treatment-related variables into the model. These other variables include pre-existing medical conditions and medications, age of the patient at the time of diagnosis and irradiation, symptomatic interval and progression events prior to irradiation, tumor location and volume, hydrocephalus and its management, extent and number of surgical procedures and related morbidity, prior chemotherapy and related morbidity, neurologic effects of tumor including seizures, technical factors of irradiation, neuro-imaging changes, the use of corticosteroids, measures such as patient height and weight, common toxicity coding and the type and extent of adverse events. Since sample size is expected to be a notable constraint in these analyses, it will not be possible to build a model which will include several of these variables simultaneously. A well-established convention in the statistical literature uses 10 patients per covariate included in the model<sup>6</sup>. We will also use this approach to determine the number of covariates that can be simultaneously modeled. Also, please note that the 6-month and 1-year PFS estimates for the St Jude historical cohort for the intermediate risk patients are 46.3% and 36.5%, respectively. Hence, significant missingness may be expected in the later time-points for the GH tests. If this is observed, the effect of these missing values on the overall conclusions will be investigated via various imputation methods. In the event that the percentage of patients with missing data at the later time points exceeds 50%, the longitudinal models will be solely based on the earlier time points. Additional exploratory models will be used to investigate the effect of the above-mentioned clinical and treatment-related factors on the incidence and time to onset of GHD defined at various levels (including peak serum GH  $\leq 10$  ng/mL and  $\leq 7$  ng/mL).

## 14.0 OBTAINING INFORMED CONSENT

The process of obtaining informed consent will follow institutional guidelines. Informed consent will be obtained by the attending physician or his/her designee in the presence of at least one witness. After the eligibility criteria have been confirmed and the research participant is deemed eligible, we will seek consent from the parents or guardians. As all research participants enrolled on this trial will be less than 5 years old at the time of diagnosis, assent from the research participant will not be obtained.

### 14.1 INFORMED CONSENT PRIOR TO RESEARCH INTERVENTIONS

Written informed consent must be obtained prior to performing any research interventions, evaluations or tests done as part of eligibility determination. This can be accomplished by institutional screening consent or with SJYC07 IRB-approved informed consent.

### 14.2 CONSENT WHEN ENGLISH IS NOT THE PRIMARY LANGUAGE

When English is not the patient, parent, or legally authorized representative's primary language, the Social Work department will determine the need for an interpreter. This information documented in the participant's medical record. Either a certified interpreter or the telephone interpreter's service will be used to translate the consent information. The process for obtaining an interpreter and for the appropriate use of an interpreter is outlined on the Interpreter Services, OHSP, and CPDMO websites.

### 14.3 COLLECTION OF COLLABORATING INSTITUTION CONSENT FORMS

St. Jude research staff will fax signed collaborating institution's consent forms to the CPDMO Eligibility Office at [REDACTED]

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**APPENDIX I: DRUGS THAT MAY AFFECT CYCLOPHOSPHAMIDE/4-HCY AND  
ERLOTINIB/OSI-420 METABOLISM.**

CYP 3A4/5 Moderate and Potent Inhibitors	
Cannabinoids	Itraconazole**
Cimetidine	Ketoconazole**
Clarithromycin**	Nefazodone
Cyclosporine	Nelfinavir <sup>§</sup>
Delavirdine	Nevirapine
Diltiazem	Ranitidine
Erythromycin**	Ritonavir** <sup>§</sup>
Fluoxetine**	Saquinavir <sup>§</sup>
Fluvoxamine**	Sertraline
Grapefruit juice**	Zafirlukast
Indinavir** <sup>§</sup>	

\*\*Strong inhibitors

<sup>§</sup>Protease inhibitors

CYP3A4 Inducers
Phenytoin
Carbamazepine
Rifampicin
Phenobarbital
St. John's Wart
Glucocorticoids (i.e. dexamethasone)

**APPENDIX II: LANSKY PERFORMANCE SCALE**

PERFORMANCE STATUS CRITERIA	
Lansky performance scores are intended to be multiples of 10	
<i>Score</i>	<i>Description</i>
100	Fully active, normal
90	Minor restrictions in physically strenuous activity
80	Active, but tires more quickly
70	Both greater restriction of and less time spent in play activity
60	Up and around, but minimal active play; keeps busy with quieter activities
50	Gets dressed, but lies around much of the day; no active play, able to participate in all quiet play and activities
40	Mostly in bed; participates in quiet activities
30	In bed; needs assistance even for quiet play
20	Often sleeping; play entirely limited to very passive activities
10	No play; does not get out of bed.

### **APPENDIX III: TREATMENT OF SYNCHRONOUS EXTRANEURAL ATRT**

Occurrence of a synchronous extraneural tumor in patients with ATRT is an uncommon event associated with a dismal prognosis. Patients with such tumors are eligible for treatment on this protocol. In general, treatment will be based on CNS staging, so that patients with otherwise M0 disease are treated according to the intermediate risk arm of the study, and those with M1-3 disease are treated according to the high risk arm. Local control of extraneural disease sites should also be performed in a manner appropriate for the disease site, including complete resection if possible and local RT. RT dose should be chosen by disease site in coordination with the principal investigator and Radiation Oncology Section Head. Examples are provided below:

Example 1 – Patient with M0 CNS disease (no intracranial or spinal metastasis on MRI, negative CSF cytology) and synchronous renal rhabdoid tumor. After initial complete resection of CNS tumor and nephrectomy, chemotherapy should be administered according to the intermediate risk arm. Consolidation should consist of conformal RT to the intracranial tumor bed to 54.0 Gy as detailed in this protocol, plus appropriate flank or abdominal radiation. Abdominal RT dose and field should be based on the current standard therapy for patients with high risk renal tumors (e.g., 19.8 Gy as per COG study AREN0321).

Example 2 – Patient with M3 CNS disease and synchronous renal rhabdoid tumor. After initial maximal safe resection of CNS tumor and nephrectomy, chemotherapy should be administered according to the high risk arm. Consolidation should consist of craniospinal irradiation or topotecan/cyclophosphamide as detailed in this protocol. Decisions regarding flank/abdominal RT will be made on a case by case basis.

**APPENDIX IV: CHANG OTOTOXICITY GRADING CRITERIA**

**Chang Ototoxicity Grading Scale\***

<b>Chang Grade (New Scale)</b>	<b>Sensorineural Hearing Threshold (dB HL) Bone conduction or air conduction with normal tympanogram</b>	<b>CCG Grade (Old Scale)</b>
0	≤ 20 dB at 1, 2, and 4 kHz	No change from baseline is grade 0
1a	≥ 40 dB at any freq 6 to 12 kHz	1
1b	> 20 and < 40 dB at 4kHz	2
2a	≥ 40 dB at 4 kHz and above	--
2b	> 20 and < 40 dB at and freq below 4kHz	3
3	≥ 40 dB at 2 or 3 kHz and above	4
4	≥ 40 dB at 1 kHz and above	Not defined



**APPENDIX V: HIGH-TYRAMINE FOODS TO AVOID 72 HR PRIOR TO CSF  
NEUROTRANSMITTER SAMPLING**

Smoked, aged, or pickled meat or fish

Sauerkraut

Aged cheeses (e.g., swiss, cheddar, blue, boursault, camembert, emmenthaler, stilton)

Yeast extracts

Fava beans

Beef or chicken liver

Aged sausages (e.g., bologna, pepperoni, salami, summer sausage);

Game meats (e.g., venison, rabbit)

## **APPENDIX VI – RECOMMENDED TREATMENT FOR PATIENTS WITH PROGRESSIVE DISEASE**

Treatment of patients with progressive disease will vary based on the timing and site of disease progression. In general, it is recommended that treatment include components of SJYC07 therapy to which the patient has not yet been exposed. In particular, the intravenous topotecan/cyclophosphamide regimen described in section 4.3.3 should be administered as consolidation therapy for patients with progressive disease who have not previously been treated with this regimen. Treatment of St. Jude patients with progressive disease will be discussed at the weekly St. Jude multidisciplinary brain tumor conference. For patients from other sites, it is strongly recommended that management of disease progression be discussed with the principal investigator. Specific scenarios are discussed below.

### *Local Recurrence or Progression:*

Patients with M0 disease at initial diagnosis who experience local disease recurrence or progression should undergo maximal safe resection, followed by focal RT (if not already given) and consolidation with intravenous topotecan/cyclophosphamide, as detailed in the high-risk consolidation regimen (see section 4.3.3).

RT for patients with all diagnoses other than high-grade glioma should be given according to the RT guidelines in section 4.6. Radiation therapy for patients with high-grade glioma will include different targeting and dose guidelines. The gross tumor volume will be based on the combined MRI post-contrast T1- and T2-weighted imaging sequences with an anatomically constrained clinical target volume margin of 2cm. The planning target volume margin will include a geometric expansion of 0.3-0.5cm. The minimum dose prescribed to the planning target volume should be 54Gy. A total dose of 59.4Gy may be prescribed to the same volume or a subvolume at the discretion of the treating physician. The normal tissue dose constraints will be similar to those specified for the embryonal tumor group.

Maintenance therapy according to the initial treatment plan (see section 4.4) may subsequently be administered, if it has not already been given.

Example 1: Patient with M0 classical medulloblastoma (initially intermediate risk) develops local recurrence after 2 induction cycles. Recommended treatment would include resection followed by focal RT, then consolidation with two cycles of intravenous topotecan/cyclophosphamide as detailed in section 4.3.3, then maintenance with alternating oral cyclophosphamide/topotecan and erlotinib (see section 4.4).

Example 2: Patient with M0 ATRT (initially intermediate risk) develops local recurrence after maintenance cycle A2. Recommended treatment would include resection followed by consolidation with two cycles of intravenous topotecan/cyclophosphamide (see section 4.3.3).

*Distant Recurrence or Progression:*

Patients with distant (M1+) recurrence or progression have a dismal prognosis. The decision to pursue further treatment should be made only after careful discussion with the family which considers the patient's age, functional status, tumor histology, and previous treatment. For patients younger than 3 years of age at the time of disease progression, chemotherapy with intravenous topotecan/cyclophosphamide is recommended, if it has not been administered previously. For patients older than 3 years at the time of disease progression, CSI may also be considered.

The older cohort of medulloblastoma patients enrolled on the study ( $\geq 3$ - $< 5$  yrs of age) who have a distant recurrence should be treated with CSI as outlined in the HR arm of the study and continue with the oral chemotherapy portion of the study.

**APPENDIX VII – REQUIRED DATA AND TIME TABLE FOR SUBMISSION**

<b><i>DATA</i></b>	<b><i>SUBMISSION GUIDELINE</i></b>
On Study (All Pre-treatment Assessments)	Within 14 days of enrollment
Chemotherapy	Within 14 days of completion of a course of treatment
Disease Assessment	Within 14 days of completion of disease evaluation
Serious and Unexpected Adverse Events	Within 5 working days of the event
Death	Within 24 hours of knowledge of the event
Follow Up Evaluations	Within 14 days of follow-up evaluation
Off-Treatment	Within 14 days of off-treatment date
Off-Study	Within 14 days of off-study date

## APPENDIX VIII – RSV TREATMENT GUIDELINES

### **PROPHYLAXIS**

#### *Principles*

- Monoclonal anti-RSV antibody (palivizumab) has been proven effective in reducing severe RSV infection in premature infants and young children with chronic lung disease. While it has not been evaluated in randomized trials in immunocompromised children, judicious use of this compound for prevention of RSV disease in patients at high risk of RSV related morbidity and mortality is indicated.

#### *Candidates*

- Patients  $\leq 2$  years of age on SJYC07, during the RSV season (November – March), may be considered for prophylaxis on a case by case basis in consultation with the Department of Infectious Diseases.

#### *Recommended Procedure*

1. Palivizumab is administered intramuscularly at a dose of 15 mg/kg, monthly from the beginning of November to the beginning of March. While not FDA-approved, the intravenous route may be safe and well-tolerated. Contact the local pharmacy or review the online St Jude Formulary Handbook for more information on IV administration.
2. If a patient who is receiving immunoprophylaxis has a breakthrough RSV infection, prophylaxis should continue through the RSV season because the patient can get re-infected. RSV subtypes may show variable susceptibility to palvizumab and failure of immunoprophylaxis once is not necessarily a predictor of its success (or lack of) in the future.
3. Palivizumab is packed in 100- and 50-mg vials that must be used within six hours after they have been opened. Coordination with the pharmacy is encouraged to cohort patients requiring prophylaxis and to minimize wastage of this expensive compound. For patients weighing less than 9 kg, the pharmacy will round the dose to the nearest 25 mg.
4. For patients weighing equal to or more than 9 kg, the pharmacy will round the dose to the nearest 50 mg.

### **TREATMENT**

#### *Principles*

- RSV infected patients, especially those who are severely immunocompromised, frequently have co-infections. RSV upper respiratory tract infection (URTI) does not rule out lower respiratory tract infection (LRTI) by another co-pathogen. In severely immunocompromised patients, further tests including bronchoalveolar lavage in patients with LRTI should be done as clinically indicated.
- High morbidity and mortality result from LRTI (RSV pneumonia).
- Risk factors for the progression of URTI to LRTI and LRTI to death include:

- Lymphopenia ( $<100/\text{mm}^3$ )
- Age  $\leq 2$  years
- In patients who meet the criteria for treatment, early treatment is beneficial.
  - Survival is better in patients treated  $> 24$  hours prior to respiratory failure requiring mechanical ventilation versus those treated late.
  - Survival is better with preemptive treatment at the RSV URTI stage as compared to historical controls treated late.
- There are no randomized clinical trials that examine the efficacy of palivizumab or ribavirin in immunocompromised pediatric patients. The recommendations shown in this guideline are based on experience with RSV infection at this institution, existing literature and expert opinion. These guidelines do not obviate the role of patient specific infectious disease consults and case by case decision making.
- For purposes of these treatment guidelines:
  - LRTI is defined as hypoxia, tachypnea or apnea and/or radiographic evidence of pneumonia (infiltrates, consolidation). Clinical signs such as wheezing, cough or radiological signs such as parenchymal thickening, atelectasis and effusion alone do not fulfill criteria of LRTI.
  - Immunocompromised is defined as  $< 3$  months post completion of cancer therapy or  $< 2$  years post HSCT.

### ***Candidates***

- Immunocompromised patients with **LRTI**.
- Patients with **URTI** who are  $\leq 2$  years of age and/or lymphopenic (absolute lymphocyte count  $< 100/\text{mm}^3$ ) and/or HSCT recipients (pre engraftment or  $< 2$  months post engraftment or with grade 3 or 4 GvHD) and/or have SCIDS

### ***Recommended Procedures***

1. All cancer patients with respiratory symptoms should get a fresh, well-collected nasal wash sample sent for viral DFA and culture. A nasal wash is recommended over a nasal swab. Procedures for collecting a nasal wash or a nasal culture are available in the Nursing Policy and Procedure Manual (Volume II).
2. Ribavirin: Ribavirin, 20 mg/ml (6 grams diluted in 300 ml distilled water) over 12 to 18 hours OR administered as three 2 hour pulses aerosolized through a SPAG unit into a respiratory tent. Ribavirin therapy is generally given for 5-10 days but duration of therapy has to be determined on a case by case basis (see below).

Palivizumab: 25-30 mg/kg IV single dose. Further treatment doses must be approved in consultation with the Department of Infectious Diseases for patients at SCRH.

3. For patients weighing less than 9 kg, the pharmacy will round the dose to the nearest 25 mg.
4. For patients weighing equal to or more than 9 kg, the pharmacy will round the dose to the nearest 50 mg.
5. For patients meeting the criterion for anti-RSV treatment, discontinuation of treatment is based on clinical evidence of respiratory improvement in the context of documented evidence of improvement of the immune system.
6. Discontinuation of therapy should not be based solely on presence or absence of RSV in the upper respiratory tract. A negative RSV DFA from an upper airway specimen does not rule out RSV lower airway disease and conversely a prolonged period of positive
7. RSV DFA in a recovering, stable patient is not uncommon. Additionally, ribavirin use can reduce the sensitivity of RSV culture from respiratory secretions.