NB2012
Initial version, dated: 06-27-2012. Resubmitted to CT-SRC. Resubmitted to IRB 11-27-2012 and 12-14-2012
Amendment 1.0, dated 07-02-2013. Resubmitted to IRB 10-17-2013 (IRB approved: 10-23-13).
Activation date: January 15, 2014
Amendment 2.0, dated: 08-27-2014 (IRB approved: 10-28-14). Activated: November 18, 2014
Amendment 3.0 (consent form changes only), dated: 03-16-2015. (IRB approved: 3-24-15). Activated: April 13, 2015
Amendment 4.0, dated July 22, 2015. (IRB approved: 10-13-15) Activated: November 11, 2015
Amendment 5.0, dated February 2, 2016. Resubmitted to CT-SRC May 17, 2016. Resubmitted to IRB June 23, 2016
(IRB approved: 06-28-2016). Activation date: August 10, 2016
Amendment 6.0, dated April 20, 2017. Resubmitted to IRB 05-25-2017 (IRB approved: 05-25-2017) Activated: July 10, 2017
Amendment 7.0, dated October 12, 2017 (IRB approved: 12-08-2017)
Amendment 8.0, dated January 23, 2018 Resubmitted to IRB 02-06-2018 (IRB approved: 02-07-2018)
Activated: 02-16-2018
Revision 8.1, dated July 20, 2018 (IRB approved: 08-01-2018) Activated: 08-09-2018

NEUROBLASTOMA PROTOCOL 2012: THERAPY FOR CHILDREN WITH ADVANCED STAGE HIGH-RISK NEUROBLASTOMA

(IND#100,617; IDE#11,533)
NCT01857934

Principal Investigator
Wayne L. Furman, MD
Department of Oncology

Co-Investigators - Department of Oncology

Michael W. Bishop, MD
Rachel C. Brennan, MD
Sara M. Federico, MD
Mark E. Hatley, MD, PhD
Sara Helmig, MD
Alberto Pappo, MD
Hong Ha Rosa Nguyen, MD
Victor M. Santana, MD
Elizabeth A. Stewart, MD

Department of Surgery
Andrew M. Davidoff, MD
Andrew J. Murphy, MD

Department of Radiation Oncology
Matthew Krasin, MD
John Lucas, MD

Department of Diagnostic Imaging

Department of Bone Marrow Transplant & Cellular Therapy
Stephen Gottschalk, MD
William E. Janssen, PhD
Renee Madden, MD
Ewelina Mamcarz, MD
Amr Qudeimat, MD
Ashok Srinivasan, MD
Aimee Talleur, MD

Mary E. McCarville, MD
Barry L. Shulkin, MD

Brandon M. Triplett, MD

Department of Pharmaceutical Sciences
Allison Bragg, PharmD

Department of Biostatistics
Deo Kumar Srivastava, PhD
April Sykes, MPH

Department of Pathology
John Choi, MD, PhD
Armita Bahrami, MD
Paul E. Mead, PhD
Michael R. Clay, MD
Teresa Santiago, MD

Department of Oncology
Nancy Miles Bailey, FNP-BC

Comprehensive Cancer Center
Dana Hawkins, BSN, CCRC
Gwendolyn Anthony, BSN, CCRP
Deanna Welsh, RN, CCRP

External Scientific Collaborators
Paul Sondel MD, PhD
Departments of Pediatrics and Human Oncology,
University of Wisconsin School of Medicine and Public Health, Madison, Wisconsin

St. Jude Children's Research Hospital
262 Danny Thomas Place
Memphis, Tennessee 38105-3678
Telephone: (901) 595-3300

Protocol Summary

NEUROBLASTOMA PROTOCOL 2012: THERAPY FOR CHILDREN WITH ADVANCED STAGE HIGH-RISK NEUROBLASTOMA
Principal Investigator: Wayne Furman, MD
IND holder: St. Jude Children's Research Hospital
Brief overview: The efficacy (response: CR+PR) to two initial courses of cyclophosphamide and topotecan combined with hu14.18K322A (4 doses/course with GMCSF) in previously untreated children with high-risk neuroblastoma will be evaluated.
Intervention: Four doses of hu14.18K322A will be added to each course of induction chemotherapy in newly diagnosed children with high-risk neuroblastoma.
Brief outline of treatment plan: All children will receive fixed doses of intravenous hu14.18K322A with each course of chemotherapy (two courses each of cyclophosphamide and topotecan; cyclophosphamide, doxorubicin and vincristine (CAV); and cisplatin and etoposide (CiE).
Study design: A single institution Phase II study of the addition of hu14.18K322A to induction chemotherapy with cyclophosphamide and topotecan for two courses followed by two courses of alternating cisplatin and etoposide (CiE) and cyclophosphamide, doxorubicin and vincristine (CAV). The major endpoint is response (CR+PR) after the initial two courses of therapy.
Sample size: It is estimated that about 61 evaluable participants (estimate 70 total participants) will be needed for primary objective 1.1.1 (response after 2 courses induction therapy) and primary objective 1.1.2 (event-free survival) to complete this phase II study of hu14.18K322A given with induction chemotherapy for anti-tumor activity in children with high-risk neuroblastoma. **Data management:** Data management and statistical analysis will be provided locally by the Solid Tumor Division, the St. Jude Comprehensive Cancer Center clinical trials staff and Biostatistics Department at St. Jude Children's Research Hospital. **Human subjects:** The risks to participants will be related to the toxicity of the systemic chemotherapeutic and biological agents, radiation therapy and surgical resection. Participants will be informed of this and other potential side effects during informed consent. Adverse events and treatment response will be closely monitored and reported and treated appropriately.

TABLE OF CONTENTS

APPENDICES:

Appendix VI: Prevention and Management of Hypersensitivity Reactions to hu14.18K322A
Appendix VII: Hypotension Management with hu14.18K322A
Appendix VIII: Criteria for Acute & Chronic Graft vs. Host Disease
Appendix IX: Standard of Care and Research Tests/Evaluations/Interventions

1.0 OBJECTIVES

1.1 Primary Objective

1.1.1 To study the efficacy (response: CR+PR) to two initial courses of cyclophosphamide and topotecan combined with hu14.18K322A (4 doses/course followed by GMCSF) in previously untreated children with high-risk neuroblastoma.

1.1.2 To estimate the event-free survival of patients with newly diagnosed high-risk neuroblastoma treated with the addition of hu14.18K322A to each phase of treatment (Induction, Consolidation, Experimental MRD* and MRD Treatment as outlined in Section 4.0, Treatment Plan).

Note: Experimental MRD phase deleted with Amendment 8.0

1.2 Secondary Objectives

1.2.1 To study the feasibility of delivering hu14.18K322A to 6 cycles induction chemotherapy and describe the antitumor activity (CR+PR) of this 6 course induction therapy.

1.2.2 To estimate local control and pattern of failure associated with focal intensity modulated or proton beam radiation therapy dose delivery in high-risk abdominal neuroblastoma.

1.2.3 To describe the tolerability of four doses of hu14.18K322A with allogeneic NK cells from an acceptable parent, in the immediate post-transplant period (day +2 to +5 after PBSC infusion) in consenting participants*

1.2.4 To describe the tolerability of hu14.18K322A with IL-2 and GM-CSF as treatment for minimal residual disease (MRD)

*Note: Experimental MRD phase deleted with Amendment 8.0

1.3 Exploratory Biologic Objectives

1.3.1 To measure natural killer (NK) cell number and function during Induction, Intensification/Expermimental MRD, and MRD/Maintenance.*

1.3.2 To measure T-lymphocyte subset number and function during Induction, Intensification/Experimental MRD*, and MRD/Maintenance.

1.3.3 To genotype natural killer (NK) cell receptors and measure their phenotype at diagnosis and after the initial six weeks of therapy, and to associate these features with treatment outcome.

Note: Experimental MRD phase deleted with Amendment 8.0

1.3.4 To describe the relative frequency of positive bone marrow and peripheral blood by sensitive minimal residual disease (MRD) methods at diagnosis, after the initial six weeks of therapy, at the time of stem cell harvest, and at several time points following the completion of intensification. These results will be compared with timing and pattern of disease recurrence.

1.3.5 To describe whether or not human anti-human antibodies (HAHA) develop in participants receiving hu14.18K322A.

1.3.6 To procure tumor samples for construction of tissue microarray blocks that will be utilized in further biologic characterization of these tumors.

1.3.7 To assess the feasibility of measuring plasma catecholamine metabolites in patients with known neuroblastoma and to obtain preliminary data on the possible utility of plasma catecholamine metabolite analyses in patients with neuroblastoma.

1.3.8 To determine the spectrum of host immune response against tumor by measuring the amount of CD8+ cytotoxic T-cells relative to Foxp3+ regulatory T-cells in tumor specimens.

1.3.9 To assess the expression of PD-L1 by neuroblastoma tumor cells by applying antibodies directed against PD-L1 using fully automated immunohistochemical techniques.

1.4 Exploratory Diagnostic Imaging Objective

To determine if there is a correlation between the number of Image Defined Risk Factors (as defined in the International Neuroblastoma Risk Group Staging System[1]) and outcome of patients with high risk neuroblastoma.

1.5 Exploratory Radiation Oncology Objective

To perform a simple descriptive comparison of renal radiation doses and other adjacent normal tissues between photon IMRT techniques and proton beam radiation technique.

1.6 Exploratory Pharmacokinetic Objective

To assess the ability to achieve the target systemic exposure of intravenous busulfan in patients with neuroblastoma undergoing stem cell transplant, and to explore possible associations between busulfan pharmacokinetic parameters and patient's outcome (e.g., response, event-free survival and overall survival) and specific covariates (e.g., age, sex, race, weight).

2.0 BACKGROUND AND RATIONALE

Neuroblastoma is the most common extracranial solid tumor in childhood, with nearly 50% of patients presenting with widespread metastatic disease. The current treatment for this group of high-risk patients includes intensive multi-agent chemotherapy (induction) followed by myeloablative therapy with stem-cell rescue (consolidation) and then treatment of minimal residual disease (MRD) with isotretinoin.[2] Recently a new standard of care[3] was established by enhancing the treatment of MRD with the addition of a monoclonal antibody (ch14.18) which targets a tumor-associated antigen, the disialoganglioside GD2, which is uniformly expressed by neuroblasts.[4] Despite improvement in 2-yr EFS of 20%, more than one-third of children with high-risk neuroblastoma (HR defined in[5]) still cannot be cured by this approach.[2] Therefore novel therapeutic approaches are needed for this subset of patients. This study will be a pilot Phase II study of a unique anti-disialoganglioside (anti-GD2) monoclonal antibody (mAb) called hu14.18K322A, given with induction chemotherapy.

2.1 Rationale for Evaluation of hu14.18K322A with Chemotherapy

The clinical evaluation of various anti-GD2 monoclonal antibodies (mAbs) in children with neuroblastoma has been exclusively focused on treatment of patients after recovery from consolidation, in a state of minimal residual disease. This is because traditionally chemotherapy has been thought to be too immunosuppressive to combine with monoclonal antibodies. However recent studies suggest, even in the setting of "bulky" solid tumors, the combination of chemotherapy with monoclonal antibodies can be synergistic.[6–9] For example, in three different preclinical models (murine mammary carcinoma, Lewis lung carcinoma and murine colon epithelial cell line) of subcutaneously implanted tumors, cyclophosphamide combined with an antibody-interleukin 2 fusion protein, augmented the antitumor activity of the antibody by lowering the diffusion barriers for antibody penetration of established tumors.[9] In another study, C57BL/6 mice with established 9464D neuroblastoma were treated with a three drug combination and an anti-CD40 monoclonal antibody, resulting in synergistic anti-tumor effects.[8] In other preclinical studies chemotherapy can increase the efficacy of immunotherapy by depleting immunosuppressor cells such as regulatory T-cells which are known to suppress NK cell-mediated immunotherapy[10–12][NK cells are the presumed major effector cells of anti-GD2 mAb induced ADCC in neuroblastoma].[13,14] Also chemotherapy-induced tumor cell death can trigger tumor antigen release, uptake by antigen processing cells and an enhanced antitumor immune response.[6,11,15] These data have led to the evaluation of several different mAb's given with chemotherapy (rituximab,[16,17] trastuzumab,[18] bevacizumab,[19] and ipilimumab[20]) in a variety of adult cancers, all resulting

in significant enhancement of anticancer activity over chemotherapy alone.[17–19,21]

With anti-GD2 specific mAb's, there are preclinical studies demonstrating synergistic activity with chemotherapy in small cell lung cancer cell lines,[22] and a neuroblastoma cell line.[23] In this latter study an anti-GD2 14G2a mAb showed at least additive (carboplatin) and some synergistic (doxorubicin, topotecan) effects on cell killing.[23] In addition to synergistic effects of anti-GD2 mAbs with chemotherapy, there is also evidence that anti-GD2-specific antibodies can suppress tumor growth, independent of immune system involvement, by directly inducing apoptosis in lung cancer cells,[24,25] and suppress growth in melanoma xenografts[26,27] and a neuroblastoma cell line.[23]

Combining different cytotoxic drugs with different mechanism of action is a successful clinical strategy. The addition of mAbs to combination chemotherapy is a logical extension of this approach and as mentioned previously, has been successful in several different adult cancers with several different mAbs (e.g. rituximab,[16,17] trastuzumab,[18] bevacizumab,[19] and ipilimumab[20]).[28] These mAb-chemotherapy combinations have all shown benefit in newly diagnosed patients, presumably some with bulky disease. These data that 1) mAbs combined with agents such as cyclophosphamide,[9,10] paclitaxel[9] or gemcitabine[6] in various preclinical models are synergistic, 2) mAbs with combination chemotherapy are synergistic in a variety of adult cancers,[17–19,21] 3) anti-GD2 mAbs can suppress tumor cell growth independent of immune system involvement,[23,25,29] 4) anti-GD2 mAbs are synergistic with some chemotherapeutic agents in preclinical models,[9,22,23] and 5) anti-GD2 mAbs and chemotherapy have non-overlapping toxicities[30–32] are compelling reasons to evaluate the addition of a novel anti-GD2 mAb, hu14.18K322A, to chemotherapy, outside the setting of minimal residual disease, in children with relapsed or refractory neuroblastoma. The fact that different chemotherapeutic agents cannot be assumed to have the same effects on the immune system[7] justifies the evaluation of this approach with the different chemotherapy combinations (section 4.2).

2.1.1 Rationale for hu14.18K322A dosage

A single agent Phase I study (SJGD2) of the daily x 4 schedule of hu14.18K322A was just completed.[31] In this study 35 patients (22 males, median age 6.7 years) received a total of 94 courses. Patients received a median of 2 courses. Dose-limiting toxicities occurred in 4 of 32 evaluable patients and were characterized by grade 3 cough, asthenia, pain, anorexia and serum sickness and grade 4 hypertension. The most common grade 3 or 4 toxicities were pain (64% of patients), fever (21%) and hyponatremia (12%). Pain was well managed and predominately

occurred on the first day of course 1. Grade 1 or 2 ocular abnormalities were noted in 14 patients and appear to be dose related. No objective responses by RECIST have been observed; however, 4 patients had responses documented by ^{123}I-MIBG (2CR, 2PR). Based on these results the MTD and recommended phase II dose of hu14.18K322A is 60 mg/m^2/dose, given over 4-hrs daily for 4 consecutive days, repeated every 28 days.[31] The toxicities noted above are significantly different and non-overlapping with those of standard chemotherapy and should be readily discernible from those observed with this induction chemotherapy.[33] We have chosen a dose-level of hu14.18K322A, two dose-levels below the recommended Phase II dose, to combine with this induction chemotherapy (40 mg/m^2/dose, given over 4-hrs daily for 4 consecutive days). This dosage of anti-GD2 antibody is > 1.5 x more than that used in Yu's study[3] and should be sufficiently active and tolerable. Additional tolerability data of this combination has been provided by an ongoing safety/feasibility trial of the addition of hu14.18K322A to chemotherapy (GD2NK). To date, five patients have been enrolled and received 10 courses of cyclophosphamide/topotecan chemotherapy with 40 mg/m^2/d x 4 days of hu14.18K322A (identical doses and schedule) to those proposed in this trial. These relapsed patients have tolerated this therapy well (4/10 courses with addition of parental-derived NK cells).

2.2 Rationale for Standard Chemotherapy Plan

Although a wide variety of induction chemotherapy regimens have been used, there are only two randomized trials comparing different regimens.[34,35] and no regimen has been demonstrated to be superior.[36] Most regimens use combinations of cyclophosphamide, etoposide, cisplatin (with or without carboplatin), doxorubicin and vincristine[35,37-40] and more recently topotecan has been added.[33,41,42] The feasibility of administering these agents in the combinations proposed for the induction phase is well documented by our own experience and that of others.[33,37,38,42,43] Prior, mostly single-agent Phase II investigational "window" evaluations,[44,45] have seen response rates (CR+PR) ranging from 17% (epirubicin) to 54% (carboplatin). In our own NB97 study, the use of two courses of pharmacokinetically guided topotecan as initial therapy resulted in responses of nearly 60%.[42] Recently COG investigators added cyclophosphamide and topotecan to an intense induction regimen originally developed at Memorial Sloan Kettering[46] and reported an 84% response rate (CR+PR) at the end of induction.[33]

This has become the induction regimen for the recent COG trial for newly diagnosed children with high-risk neuroblastoma, ANBL0532 that recently completed accrual. ANBL0532 compares favorably to dose-intensity of other recent/ongoing high-risk protocols (Table1). In order to better evaluate the role that the addition of hu14.18K322A might have in

improving response to induction, we will use this same induction chemotherapy backbone in this trial.

Table 1 – Chemotherapy Dose Intensity (mg/m²/week*): Comparison of Induction Regimens^

Drug	COG 3891[3][7]	POG 9341[3][8]	COG ANBL00P1[39]	COG A3973[40]	COG ANBL02P1[3][3] /ANBL0532 / NB2012	Rapid COJEC[35]	OJEC[3][5]	NB2005[43]
Length of Induction (weeks)	15	15	15	18	18	12	21	33
Oxazophosphorine equivalents+	500	434	700	933	688	350	200	273
Doxorubicin	7.5	10	8	16.6	8.3	0	0	4.5
Platinum equivalents#	15	29.9	27.3	22.2	22.2	26.7	15.2	24.2
Etoposide	50	110	100	66.7	66.7	116.7	66.7	80
Topotecan	0	0	0	0	0.67	0	0	2.45
Vincristine					0.11	8.75	0.5	0
Irinotecan	0	0	0	0	0			9.1
Gefitinib	0	0	0	0	0			81.8

^Dose intensities are calculated as mg/m²/week administered over the entire induction regimen.
+oxazaphosphorine = cyclophosphamide + ifosfamide. The dose intensity of ifosfamide is calculated in cyclophosphamide equivalent using a conversion of 4 mg Ifosfamide is biologically equivalent to 1 mg of cyclophosphamide[59]
#Platinum equivalents = cisplatin + carboplatin. The dose intensity of carboplatin is calculated in cisplatin equivalent using a conversion of 4 mg carboplatin is biologically equivalent to 1 mg of cisplatin.

2.2.1 Rationale for Additional Patient Accrual per Amendment 5.0

At the interim analysis of 20 evaluable participants, (10 male; median age 34 m (range 6 – 180 m), 16 INSS stage 4, 12 *MYCN* amplified), all participants had significant benefit. The response rate (CR+PR) after the first two courses of therapy was 80% (95% CI 56%–94%) with 15 PRs, 1 VGPR. This is compared to 12/30 (40%) > PR reported by Park, et. al.[33] in a similar group of patients who received two courses of CTX/TPT alone (p=0.0057). Although four patients had SD by the International Criteria for Neuroblastoma Response[47,48] their primary tumor volume decreased by 47%, 75%, 81% and 96% and was only coded as stable disease because of persistent elevations in urine catecholamines HVA or VMA. These early responses are very encouraging, but we do not know if these will translate into improvements in event-free survival (EFS). Therefore, we are amending this study to evaluate this more thoroughly.

2.3 Rationale for Addition of Granulocyte Macrophage Colony-Stimulating Factor (GM-CSF)

2.3.1 Granulocyte Macrophage Colony-Stimulating Factor

GM-CSF stimulates the proliferation and differentiation of hematopoietic progenitors to monocytes and granulocytes, and decreases the incidence of febrile neutropenia and duration of severe neutropenia after myelosuppressive chemotherapy.[49–56] GM-CSF augments the antibody-dependent cellular cytotoxicity (ADCC) of peripheral blood granulocytes and monocytes against a variety of human tumors,[56,57] including neuroblastoma.[58–60] In the most recent study, GM-CSF given with the murine anti-GD2 mAb 3F8 was more effective activating granulocytes when given subcutaneously and resulted in improved patient outcome.[60] For these reasons we propose to use daily subcutaneous GM-CSF after each course of chemotherapy and hu14.18K322A as prophylaxis for febrile neutropenia and to augment ADCC induced by hu14.18K322A against neuroblastoma.

2.3.2 Interleukin-2

Tumor cell killing by hu14.18K322A is primarily mediated by antibody dependent cellular cytotoxicity (ADCC). The immune effector cells which mediate ADCC are F_c-receptor bearing cells such as natural killer (NK) cells, monocytes and macrophages. rIL-2 administration at low-dose given either by continuous infusion or SQ every 48-72 hours is very well tolerated and results in preferential expansion of NK cells.[61–67] IL-2 also enhances the monoclonal antibody-mediated cellular cytotoxicity against human neuroblastoma cell lines *in vitro*.[68] A low-dose regimen of IL-2 (1 million units/m^2/dose SQ) every other day has been used in the GD2NK

study as well as four other clinical trials using haploidentical NK cell transplantation. This regimen has been very well tolerated. Because low-dose IL-2 is very well tolerated, expands and activates NK cells and enhances ADCC, we will administer this low dose regimen (1 million units/m^2/dose SQ every other day for six doses) after every cycle of induction chemotherapy/hu14.18K322A.

2.4 Role of Surgery in the Management of High-Risk Neuroblastoma

The role of surgery in the management of children with high-risk neuroblastoma is uncertain. Although some reports of patients with stage 3 or 4 disease have found that gross total resection of the primary tumor and metastatic locoregional disease has been associated with improved local tumor control and increased overall patient survival,[69,70] other reports have not.[71,72] Nevertheless, the Children's Oncology Group as well the major European cooperative group, SIOPEN,[35] currently recommend gross total resection of the primary tumor and regional disease in patients with high-risk neuroblastoma. Most children undergo delayed surgery following the completion of both “window” and “induction” neoadjuvant chemotherapy. The current COG protocol dictates that surgery should be performed after the fifth course of chemotherapy, even though tumor volume reduction appears to plateau after the second or third course of chemotherapy.[73]

One of the implications of the "Goldie-Coldman Hypothesis" is that the likelihood of developing chemotherapy resistance during treatment is related, in part, to the number of tumor cells being treated.[73] In other words, earlier gross tumor volume reduction may decrease the likelihood of developing drug resistant clones. Therefore, the experience of treating children on NB-97 was reviewed to determine the feasibility of earlier resection. Patients were evaluated at various time points during the course of their treatment to determine the resectability of their primary tumor and regional disease. Resectability was assessed prospectively by the attending surgeon following a careful evaluation of the CT scan and/or MRI. Tumors were considered to be unresectable if the tumor had significant involvement with major vascular structures or contiguous organs, or would likely require nephrectomy to remove the entire tumor. Evaluation time points were at diagnosis, after the initial six weeks of therapy, and after induction therapy. Seven of 24 (29%) patients were felt to be resectable at diagnosis, of these 2 were thoracic primaries; 9 more patients (another 37%) were felt to be resectable after the initial six weeks of therapy, while only 4 more (an additional 17%) were felt to be resectable after completion of induction. All but 1 of these 20 resectable patients had complete gross tumor resection, 4 at the completion of the initial six weeks of therapy and 15 after induction therapy. Importantly, overall 16 of 24 (67%) patients were felt to be resectable following the

initial six weeks of therapy with only 4 more patients becoming resectable after completing induction therapy. The 4 patients who underwent surgery after the initial six weeks of therapy were able to have a complete gross tumor resection. No complications occurred in this group and there was no delay in the re-initiation of chemotherapy (mean recovery time from surgery 6.7 days). Thus, it appears that complete resection of the primary tumor and regional disease in children with high-risk neuroblastoma can be performed safely after the initial six weeks of therapy in the majority of patients.[74] Since earlier removal of this disease may decrease the chance for the development of chemotherapy resistant clones, we are proposing to perform surgery in patients as soon as the tumor appears to be resectable, with the expectation that over two-thirds of the patients will have this performed prior to the consolidation phase of their therapy.

2.5 Rationale for Consolidation Therapy with Busulfan and Melphalan with Peripheral Blood Stem Cells

Early studies using high-dose chemotherapy showed strong correlation with progression free survival in metastatic neuroblastoma.[75,76] The critical importance of dose intensity ultimately led to the integration of high-dose chemotherapy and autologous stem cell transplant (ASCT) into modern treatment regimens for high-risk neuroblastoma.[77-79] Although with it comes unique challenges and risks, the use of myeloablative ASCT allows for the delivery of chemotherapeutic doses not usually accessible due to the usual dose-limiting toxicity of myelosuppression. ASCT for neuroblastoma has been performed using various combinations of chemotherapy and total body irradiation (TBI). While the optimum chemotherapeutic combination has not been conclusively demonstrated, the incorporation of TBI has not been shown to improve survival yet increases transplant-related complications.[80,81] There is an unequivocal survival benefit for ASCT, demonstrated in both randomized and non-randomized studies. [82-84]

One of the most common non-TBI myeloablative regimens used worldwide is a combination of busulfan and melphalan (BuMel). Recent retrospective analysis of nearly three decades of European experience with ASCT for neuroblastoma revealed a survival benefit for a BuMel approach over other melphalan-based conditioning regimens.[80] The 343 patients treated with this regimen in first remission had a 5-year overall survival rate of 48%. Specifics regarding transplant-related complications were not reported. A recent, smaller retrospective study from Spain using a BuMel conditioning reported a progression free survival of 57% among 36 patients at a mean follow-up of 55 months.[85] There were no toxic deaths reported and no reported veno-occlusive disease (VOD). Risk of complications from this regimen may be age dependent, as a report of infants treated with a busulfan-melphalan regimen (with or without cyclophosphamide) reported VOD in 9 of 12 children treated- including one death.[86] However, this regimen utilized oral busulfan, which has been shown to have a higher risk of complications.

Another common myeloablative regimen is a combination of melphalan, etoposide and carboplatin or cisplatin (MEC), and it has been used in some of the largest reported US trials.[84] The SIOP Europe Neuroblastoma Group (SIOPEN) randomized patients to receive BuMel or MEC in the HR-NBL1 trial with the primary aim to demonstrate superiority based on EFS. This randomization was closed early due to superiority of the BuMel regimen. A significant difference in EFS in favor of BuMel (3-years EFS 49% vs. 33%) was observed as well as for overall survival (3-years OS 60% vs. 48%, p=0.004). This difference was mainly related to the relapse and progression incidence, which was significantly (p<0.001) lower with

BuMel (48% vs. 60%). The severe toxicity rate up to day 100 (ICU and toxic deaths) was below 10%, but was significantly higher for MEC (p=0.014). The acute toxic death rate was 3% for BuMel and 5% for MEC (NS). The acute toxicity profile also favored the BuMel regimen despite an 18% incidence of VOD (grade 3:5%).

2.5.1 Rationale for Peripheral Blood Progenitor Cell Collection

The ability to provide bone marrow stem cells for patient rescue following intensive therapy can be limited by marrow contamination with tumor cells, and by the lack of adequate marrow reserves to harvest sufficient number of stem cells.[87,88] Rescue with peripheral blood stem cells (PBSC) has several advantages over autologous bone marrow. These include the ability to perform progenitor cell collection without general anesthesia and a significant reduction in the duration of cytopenia post-myeloablative therapy. Moreover, higher yields of stem cells can be obtained from PBSC harvests than from conventional bone marrow harvests.[89-91]

We have gained significant experience with PBSC harvest in children with neuroblastoma and other solid tumors. It is clear from these studies that early initiation of collection, after 1-3 courses of chemotherapy, results in adequate products in the majority of cases. Moreover, the procedure does not appear to be limited by patient age or weight.[89-92] In patients with neuroblastoma, the incidence of tumor contamination has been reported to be lower in PBSC harvest than in bone marrow grafts.[93] In addition, recent studies suggest that peripheral blood can be rendered free of circulating tumor cells after as few as 2 courses of chemotherapy and tumor-free PBSC harvest can be collected at this time.[94-96] We propose to collect PBSC in all patients after the second course of induction chemotherapy (TPT).

2.6 Rationale for Additional Treatment Course of hu14.18K322A with Adoptive NK Cell Infusion during Consolidation / Intensification*

*Note: Experimental MRD phase deleted with Amendment 8.0

Despite optimal therapy with intensive multiagent induction chemotherapy, consolidation with BuMel, radiation therapy, and treatment of MRD with ch14.18, GM-CSF, IL-2 and isotretinoin, less than half of children with high-risk neuroblastoma remain disease free.[3] Novel therapies are still needed. In the most recent advance in treatment, COG investigators (Yu et al[3]), added the chimeric anti-GD2 antibody, ch14.18, together the cytokines listed above, but did not begin this "MRD treatment" until hematopoietic recovery from consolidation, because there were no effector cells during this period of recovery. Additionally, in preclinical models, the efficacy of anti-GD2 treatment is influenced by the timing of the initiation of treatment relative to the injection of tumor in these animals.[97] These data suggest that earlier initiation anti-GD2 treatment could have improved efficacy. We are proposing an additional course of MRD treatment with hu14.18K322A with the addition of NK

cells (major effector cells of hu14.18K322A) from a suitable parent, prior to hematopoietic recovery. In the event that there is not a suitable parental donor we will give this additional course of hu14.18K322A, without parental NK cells, in consenting patients.

Natural killer (NK) cells represent 5% to 20% of human lymphocytes.[98] NK cells are able to lyse virally-infected and transformed cells without previous sensitization, giving them important physiological roles in controlling infection and preventing cancer.[99] The ability of NK cells to perceive alterations in target cells is mediated by a balance of signals through many surface receptors, including inhibitory receptors such as killer immunoglobulin-like receptors (KIRs) and activating receptors such as CD16a. CD16a is a low-affinity transmembrane GD2NK Fragment c gamma receptor IIIA (FcγRIIIA) found predominantly in NK cells and is essential for ADCC. The FcγRIIIA binds clustered IgG molecules bound to antigens on the cell surface of target cells and does not bind circulating monomeric IgG. Therefore, ADCC is highly specific and occurs only when the target cells are coated with antibody. Moreover, NK cells are the presumed major effector cells of anti-GD2 mAb induced ADCC in neuroblastoma.[13,14] Because the mechanism of action of hu14.18K322A is primarily through ADCC rather than complement activation, strategies to optimize NK cell mediated ADCC will be crucial to improve the outcomes of patients treated with hu14.18K322A.

However, NK cells interact with other hematopoietic cell types, each of which has the potential to alter this activity.[100] NK cell cytotoxicity, including through ADCC, is known to be inhibited by normal peripheral blood mononuclear cells, especially monocytes.[101,102] While therapeutic monoclonal antibodies such as hu14.18K322A promote NK cell-mediated ADCC, this killing can be also inhibited by acellular components of human serum including endogenous IgG and complement by interfering with binding to CD16 on the surface of NK cells.[103,104] NK cells also have a high affinity for platelet-derived growth factor (PDGF), a chemokine secreted by platelets.[105] Signaling through PDGF significantly inhibits NK cell activity, and defective NK cell activity has been demonstrated in patients with abnormal platelet release and high levels of plasma PDGF.[106] The use of chemotherapy can increase the efficacy of immunotherapy by depleting immunosuppressor cells such as regulatory T-cells, which are known to suppress NK cell-mediated immunotherapy.[10-12]

In efforts to avoid potential inhibition by other cell types but to maximize antibody benefit, we are proposing that hu14.18K322A be given together with their major effector cells (NK cells) via an adoptive NK cell infusion prior to early hematopoietic cell recovery, when endogenous NK cells are anticipated to be very low. This approach has been both safe and

effective in several other St. Jude protocols. For example, in the St. Jude NKCELL protocol, Dr. Leung and colleagues performed 12 consecutive purifications of NK cells from 12 normal volunteers. The system uses a two-step procedure.[107] First, mononuclear cells obtained by leukapheresis are depleted of T cells by CD3+ cell depletion using the CliniMACS. Second, the CD3-depleted product is enriched for CD56+ cells using the CliniMACS. The final products contained a median of 1.6 x 10^8 mononuclear cells and 91% CD3-CD56+ cells. In addition, the final products had minimal contamination with T cells (at or below the lower limit of detection in 10 of the 12 products) or B cells (median 0.2%). Thus, recipients are expected to be at low risk for GVHD or EBV lymphoproliferative disease. We also found that the expression of KIRs, adhesion molecules, intracellular cytokines, perforin, and granzyme B in NK cells was not significantly different before and after cell purification. Extensive proliferative capacity and potent anti-neuroblastoma activity of the NK cells were demonstrated by using an immunodeficient mouse model.[108] In addition, GVHD developed in all mice transplanted with unpurified mononuclear cells, but in none of the 10 mice transplanted with purified NK cells.

Based on these preclinical data, four clinical protocols have been opened at St. Jude to transplant normal NK cells from parents into leukemia participants as well as an additional protocol in children with refractory neuroblastoma. The data from the first cohort of 10 patients from the NKAML protocol (FDA IDE 11533) were published recently.[109] This study demonstrated that haploidentical NK cell infusions were safe, feasible, and efficacious. The conditioning, IL-2, and NK cell transplantation were all well tolerated. The median NK cell dose was 27 x 10^6/kg (range, 5 to 80 x 10^6/kg). The average hospital stay was only 2 days. None of the participants have acute or chronic graft-versus-host disease. Correlative laboratory studies showed that all patients had transient donor NK cell engraftment for a median of 10 days. NK cell cytotoxicity against K562 cells normalized in all patients by Day 7 after NK cell transplantation. Importantly, there was a significant expansion of KIR mismatched cells in the blood, from a median of only 210/ml on Day 2 to a median of 5,800/ml on Day 14.

2.6.1 Rationale for eliminating additional treatment course of hu14.18K322A / IL-2/Parental NK cells/ GM-CSF during Consolidation/Intensification

Two participants treated on this study during Consolidation experienced a severe side effect called Hemophagocytic Lymphohistiocytosis (HLH) / Macrophage Activation syndrome (MAS). HLH / MAS is a syndrome resulting from abnormal immune activation and excessive inflammation and has been reported as a rare complication of stem cell

transplantation.[110–116] It usually manifests with fevers, hepatosplenomegaly, hyperferritinemia, hypertriglyceridemia, hypofibrinogenemia and coagulopathy.[113,117] The excessive activation of immune cells including T-cells and macrophages results in the release of various inflammatory cytokines and hematopoietic growth factors resulting in vascular endothelial damage, myelosuppression, severe coagulopathy, cytopenias, infection and multi-organ failure.[118–121] HLH has also been reported to be exacerbated by GM-CSF.[122,123] Although HLH is a known side effect after stem cell transplantation, we think that it has occurred more than expected on this study (2/42). Although it is impossible to determine if the experimental phase of MRD treatment in the immediate post-consolidation period consisting of 4 doses of hu14.18K322A beginning on day+2, parentally derived NK cells on day +4, 4 doses of low dose IL-2 (day +3, 5, 7 and 9) and GM-CSF have contributed to this apparent increased risk. However, in evaluating the timing of the onset of the signs/symptoms of HLH in these two patients, in particular the peak serum ferritins occurred 21 and 24 days after parental NK cell administration and NK chimerism studies revealed 0% donor NK cells prior to HLH development. Furthermore, as mentioned above, we have given parentally derived NK cells with low dose IL-2 on several other St. Jude protocols, and in one prior protocol we gave this combination with hu14.18K322A. In these protocols combined we have not observed HLH/MAS. These data taken together make parental NK cells with low dose IL-2 unlikely as causative agents for this HLH-like picture. Timing of administration Hu14.18K3222 also makes this agent unlikely: last dose of hu14.18K322A was administered 20 and 23 days and low-dose IL-2, seventeen and 19 days prior to peak ferritin ($t_{1/2}$ of hu14.18K322a 1.74 days[124]), and as mentioned above, no detectible donor NK cells remained present upon HLH/MAS diagnosis. However, since the efficacy of this portion of therapy is unknown and we are unable to determine with certainty, whether any of the agents used in this 'experimental phase' contribute to the development of HLH we will eliminate this part of therapy for all subsequent patients.

2.6.2 Rationale for hu14.18K322A Dosage

As pointed out in section 2.1.1, the toxicities observed with hu14.18K322A are significantly different and non-overlapping with those of chemotherapy and should be readily discernible from those observed from the consolidation regimen, other than possibly liver dysfunction. With the BuMEL consolidation regimen in newly diagnosed children with high-risk neuroblastoma, 24% experience veno-occlusive disease (VoD), only 3% grade 3 or higher.[125] Additionally, nine patients on the Phase I study of hu14.18K322A (SJGD2) had ≥ grade 2 ALT/AST elevations; only two of these were grade 3, (both treated at 60 mg/m^2/day). All LFT elevations were transient and resolved to baseline by the next course of

therapy. Therefore, in addition to monitoring for severe unique toxicities observed with anti-GD2 therapy we will also carefully monitor for VoD as described in section 16.3.3.

2.7 Rationale for Radiation Therapy

Primary site failure persists as a component of tumor progression in recently published studies of high risk neuroblastoma and commonly occurs within the first 27 months post-irradiation.[126-128] Dose-escalation of radiotherapy (RT) was recently incorporated into protocols to address this issue.

Primary site irradiation has been used as part of consolidation therapy for patients with high risk neuroblastoma,[126,127,129,130] including those enrolled on the recent Children's Oncology Group (COG) ANBL0532 study. [131] A recent retrospective study has demonstrated that radiotherapy independently affects the rates of local control and overall survival for patients with neuroblastoma, even when controlling for extent of resection.[128] In the last decade, radiation doses of 21-26 Gy were used to treat microscopic disease and higher doses were used for residual macroscopic disease. Dose escalation further improved outcomes of patients with gross residual disease.[130,132] Small trials on patients with high risk neuroblastoma have shown that local control rates of more than 90% are attainable if radiation therapy is used after induction chemotherapy and surgery; however, large randomized trials have demonstrated much lower local control rates, especially for patients with post-operative gross residual disease.[132,133] In CCG-3891 the estimated 5-year loco-regional recurrence rate was 33% $\pm$ 7%, for patients treated with autologous bone marrow transplant and total body irradiation (TBI).[134] Despite the non-randomized application of primary site irradiation in addition to total body irradiation, the best local control rate obtainable in the unplanned subgroup receiving both TBI and focal boost irradiation was 22% ± 12%. In a study by Kushner et al., 99 patients with high risk neuroblastoma treated with an accelerated, hyper-fractionated radiation regimen of 21 Gy (twice daily in 1.5 Gy fractions) had a 3 year local failure rate of 10.1 ± 5.3%.[127] George et al. subsequently reported that of 97 patients who received tandem transplant with TBI of 12 Gy followed by loco-regional irradiation of 10.8-18 Gy, only 3 developed loco-regional failures in a 7-year post-treatment follow-up period.[135] Although these data were encouraging, the mortality rate for patients was high at nearly 5%. Of the 41 patients administered local irradiation only to sites of gross residual disease in a CCG-321P2 study, 22 showed disease progression at the primary site.[136] All recent studies on local control of single transplant regimens in neuroblastoma have reported local failure rates of at least 16% at a

median follow-up of 5 years, with most locoregional failures occurring within the first 2 years after radiotherapy.[128,132,134]

A St. Jude institutional study has retrospectively evaluated improved conformality with intensity modulated radiotherapy (IMRT) and noted a 100% local control rate with a median follow-up of 2.2 years in 20 children with high risk abdominal neuroblastoma. This data suggests that our novel RT volumetric targeting is necessary and sufficient to control local disease. Furthermore, the imaging assessment practices and/or radiotherapeutic coverage of anatomic pathways of loco-regional disease spread appears necessary and sufficient to sterilize the adjacent cancer field. The acquisition of proton beam radiation therapy capabilities at St. Jude now allows us to even further limit radiation doses outside of the target volume. Use of this modality with the well-defined target volumes described in this study should further our goals of minimizing adjacent organ (kidney) normal tissue doses. These ICRU 62 compliant techniques are proposed for protocol therapy to enhance local control and outcome.

2.8 Rationale for NK Cell Receptor Study*

*Note: Experimental MRD phase deleted with Amendment 8.0

Natural killer (NK) cells are the only immune cells that have immunosurveillance capability against human cancer, as demonstrated in a prospective cohort study of healthy persons.[137] NK cells express killer immunoglobulin-like receptors (KIRs) which interact with their cognate ligands expressed as HLA class I molecules on target cells.[138,139] Based on the theory of KIR ligand mismatch, the lack of receptor-ligand interaction will result in a "missing self" signal and target cell killing.[139] This finding was illustrated in patients with acute myeloid leukemia who received bone marrow transplants and were noted to have better outcomes if they were lacking HLA class I ligands for donor-inhibitory KIRs.[140,141] Neuroblastoma cells are hypothesized to be particularly susceptible to NK cell killing because they demonstrate a down-regulation of HLA class I molecules.[142] In these patients, it was noted that lack of the HLA-C1 ligand for the most common KIR was associated with the highest 3-year survival rate after autologous bone marrow transplantation.[143] Another study demonstrated that certain KIR subtypes and HLA-B content convey better outcome in selected patients.[144]

To further study the KIR repertoire in neuroblastoma patients, the goal of this NK cell receptor study is to evaluate the KIR expression before and during therapy in patients enrolled on NB2012. Specifically, we will look at their expression before and after exposure to chemotherapy, anti-GD2 antibody, and interleukin-2. As part of this characterization, we will also study the expression of natural cytotoxicity receptors (NCRs) which

correlate with the activation status of the NK cells and evaluate their functionality by performing standardized kill assays *in vitro*. Ultimately, NK cell KIR/NCR phenotype will be correlated with patient survival.

2.9 Generation of NK Cells*

Note: Experimental MRD phase deleted with Amendment 8.0

Differentiation of NK cells from CD34+ hematopoietic stem cells has been performed and described in the past.[145,146] Conceptually, murine stromal cells provide a microenvironment as feeder cells[147] and are co-cultured with hematopoietic stem cells using NK cell differentiation media. These procedures yield a generous number of NK cells when derived from umbilical cord blood and slightly less cells when using mobilized peripheral blood CD34+ cells.[145,148]

As per protocol, patients on NB2012 are already undergoing phenotype and functional testing of their peripheral blood NK cells at different time points during therapy. One of these time points is after bone marrow mobilization with G-CSF and prior to apheresis to collect the CD34+ hematopoietic progenitor cells. We want to sort the residual sample that would otherwise be discarded for CD34+ cells and use the sorted cell population to generate NK cells ex vivo. These cells will then be used to test ADCC in vitro against established neuroblastoma cell lines and patient-derived xenograft (PDX). If enough cells are available, we will also conduct in vivo testing in mice that were orthotopically injected with PDX. Collectively, these experiments will allow us to compare the function and phenotype of mature peripheral blood NK cells of the patients with neuroblastoma with NK cells that have been differentiated from their progenitor cells and are naïve to tumor cells. We will also be able to assess feasibility of ex vivo NK cells differentiation using patient-derived CD34+ cells.

2.10 Rationale for T-Cell Monitoring

The role and potential for T-lymphocytes in solid tumor immunity is unclear. Significant anti-tumor activity has been observed in some settings, such as the reports of LAK and TIL cells in malignant melanoma.[149,150] Moreover, immunoscore, based on the presence of T cells within solid tumors has been shown to have a prognostic significance in many solid tumors.[150,151] Dendritic cell vaccines, which are associated with T cell activation, have been shown to produce at least limited clinical response in patients with neuroblastoma and other pediatric solid tumors.[152,153] At the same time, regulatory T cells may play a role in tumor permissive immunologic suppression, including suppression of NK cells.[99] These simple flow cytometric survey assays will

assist in assessing the potential for different T cell subtypes to be playing a role in the different phases of treatment and associated MRD, including the potential to be interacting with both endogenous and administered NK cells.

2.11 Rationale for Minimal Residual Disease Monitoring

The use of molecular and immunological methods to detect residual disease in the marrow and blood of children with leukemia has become an important tool in determining prognosis and treatment options.[154] In contrast, less is known about the validity and usefulness of such strategies in the context of neuroblastoma. Seeger[2] and colleagues have pioneered the used immunocytochemistry to detect residual neuroblastoma cells. Using a cocktail of 5 antibodies with a reported sensitivity of 1 in 10^5 cells, these investigators identified a cohort of patients who respond poorly to multicourse chemotherapy.[2,155] These patients have either increasing numbers of residual tumor cells in the bone marrow during therapy or positive blood immunocytology at diagnosis. Others have utilized antisera specific for the neuroblastoma antigen GD2,[2,155,156] which is expressed on greater than 95% of neuroblastoma cells.

Immunocytology, flow cytometry and PCR are sensitive methods for detection of minimal involvement of peripheral blood in neuroblastoma patients. The International Neuroblastoma Risk Group has proposed the application of standardized immunocytologic and PCR methods for detection of minimal neuroblastoma in bone marrow, blood, and stem cell preparations.[157] Advantages of these methods over flow cytometric methods include higher sensitivity and decreased interpretive subjectivity. Conversely, the advantages of flow cytometry include the potential for multi-parametric assessment of antigen expression profiles (GD2, CD81, CD45 and CD56) as well as capacity for direct enumeration of tumor cells. We propose to use flow cytometric methods to assess the relative level and frequency of bone marrow and blood involvement by neuroblastoma at diagnosis, prior to initiation of course three, after course six (end of induction), prior to initiation of MRD therapy (see figure 11.2.2). This assay when correlated with the timing and pattern of disease recurrence will provide a platform for the development of rational treatment designs for future high-risk patients.

2.12 Rationale for Procuring Tumor Samples for Tissue Microarray

Neuroblastomas show significant biological heterogeneity. Expression profiling data can be a powerful tool in revealing the existence of up-regulated and down-regulated gene clusters into favorable and unfavorable tumors, and could provide insight into key genes for cell

growth and regression, as well as help identify diagnostic and therapeutic targets for future treatment of neuroblastomas.

Tissue microarrays (TMA) represent a highly efficient method for studying protein expression patterns (by immunohistochemistry) and DNA gains and losses (by fluorescence *in situ* hybridization, FISH). Therefore, representative regions from fixed, wax embedded tumor blocks of each case will be incorporated into a TMA in an attempt to gain further insight into the biologic characterization of these tumors.

2.13 Rationale for Measuring Plasma Catecholamine Metabolites

New plasma assays, developed by at the NIH by Dr. Graeme Eisenhofer, involve measurements of plasma normetanephrine and metanephrine, the O-methylated metabolites of norepinephrine and epinephrine, and methoxytyramine and O-methy-dopa. These metabolites are produced within chromaffin cells, including pheochromocytoma tumor cells on a continuous basis.[158] This continuous production within pheochromocytoma cells provides the basis for the superiority of plasma metanephrines over catecholamines for diagnosis of pheochromocytoma.[159,160] In patients with familial pheochromocytoma, measurements of plasma normetanephrine and metanephrine are also superior to other available biochemical tests.[161,162] Urine catecholamine metabolites are usually elevated in patients with neuroblastoma. They can be used to confirm the diagnosis and monitor the course of disease. We wish to extend the application of plasma catecholamine metabolites to patients with neuroblastoma. The utility of this test for diagnosis of neuroblastoma has not been established.

Neuroblastoma cells produce 3,4-dihydroxyphenylalanine (L-DOPA), because they express the enzyme, tyrosine hydroxylase.[163-165] They usually also produce, take up, retain, and metabolize catecholamines, from expression of L-aromatic-amino-acid decarboxylase, the cell membrane norepinephrine transporter, the vesicular monoamine transporter, and catechol-O-methyltransferase. Based on these relatively specific properties, we propose to evaluate novel biochemical and imaging biomarkers, to aid in the diagnosis of neuroblastoma, quantify responses to experimental treatments, and determine prognosis. If the results are promising, then the study can be expanded to include other patients subsequently participating in this protocol, and prospective multicenter trials can be designed to assess the utility of plasma catecholamine metabolite measurements for monitoring patients with neuroblastoma. Patients without neuroblastoma could be included in order to establish an age related normal range for this test. If such plasma tests are shown useful in monitoring patients with neuroblastoma, then routine diagnostic imaging could be avoided in many patients, sparing them from additional radiation and sedation, and saving the institution resources from

expenses imaging tests.

2.14 Rationale for Evaluation of T-cell, Myeloid-cell and PD-1 Pathway Expression in Neuroblastoma

The immunotherapy of neuroblastoma has had a major impact on the treatment of children with high-risk disease, resulting in a 20% improvement in 2-yr EFS. There is accumulating evidence that tumor infiltrating immune cells such as regulatory T-cells and myeloid derived suppressor cells (MDSC) suppress anti-tumor immune responses and may promote tumor growth. Relatively little is known about the extent of T cell, regulatory T cells, and MDSC infiltration in neuroblastoma.

The programmed death 1 (PD-1) receptor is an inhibitory T-cell receptor that has two known ligands, PD-L1 (also known as CD274 or B7-H1) and PD-L2 (also known as CD273 or B7-DC). Recent clinical trials of an anti-PD-1 antibody and an anti-PD-L1 antibody resulted in significant durable tumor responses across a spectrum of adult refractory solid tumors, including lung cancer. In these trials preliminary evidence suggests that the expression of PD-L1 in tumor may predict response to inhibition of this pathway.

Using immunohistochemical assays, we will evaluate the extent of tumor infiltrating T lymphocytes and subtypes of T cells in neuroblastoma tissue samples. To determine the spectrum of host immune response against tumor, we will measure the amount of CD8+ cytotoxic T-cells relative to Foxp3+ regulatory T-cells in each tumor specimen. Importantly, we will assess the expression of PD-L1 by neuroblastoma tumor cells by applying antibodies directed against PD-L1 using fully automated immunohistochemical techniques available in the lab.

2.15 Feasibility of Assessing Tumor-Derived GD2 on White Blood Cells Including NK Cells

GD2 is known to be secreted from mucosa and present in serum of patients with neuroblastoma.[166] In patients with hepatocellular carcinoma, reduced natural killer (NK)-cell numbers and function have been shown to be related to the tumor-derived AFP (tAFP) that can directly impair NK-cell activity.[167] tAFP negatively modulates human NK-cell activity and longevity in vitro through the hydrophilic components within the low molecular mass cargo that copurified with tAFP.[167] From our ongoing studies, we have noticed that patient-derived NK cells are functionally inferior compared to NK cells from healthy adults because they are less potent in eradicating tumor cells in vitro. We want to conduct the following molecular and cellular testing to better understand the underlying mechanism of functional exhaustion. For molecular characterization, single-cell sequencing will be performed on diagnostic blood samples. To

study the role of tumor-derived GD2 on white blood cells including NK cells, we plan to utilize liquid chromatography/tandem mass spectrometry for quantification of gangliosides in serum and in white blood cells.[168] All proposed tests will be performed with left-over serum and white blood cells from samples that are already being provided for NK cell phenotyping.

2.16 Rationale for Assessing Image Defined Risk Factors in Children with High Risk Neuroblastoma

The International Neuroblastoma Staging System (INSS) was developed in 1988[48] and modified in 1993[47] and is still used by most cooperative groups. However, the INSS is not suitable for pre-treatment risk classification of patients with extensive localized disease as staging is based on the extent of tumor removal.[1] Actually, the same tumor can be either INSS stage 1 or 3 depending on the extent of surgical excision,[169] making direct comparison of clinical trials based on INSS difficult. Assessment of lymph node involvement is also difficult to apply uniformly.[1] In 2004, investigators from the major cooperative groups, i.e., COG (Children's Oncology Group) from North America and Australia/New Zealand, GPOH (German Pediatric Oncology and Hematology Group), JNBSG (Japanese Neuroblastoma Study Group), SIOPEN (International Society of Pediatric Oncology Europe Neuroblastoma Group) and China formed the International Neuroblastoma Risk Group (INRG) Task Force who developed the INRG Staging System (INRGSS) and the INRG risk classification system for neuroblastoma.[1,170] The INRGSS, published in 2009, is designed for staging *before* surgery or any other treatment (Appendix III, Table 2).[1] Localized tumors are classified L1 or L2 dependent on whether one or more of 20 Image Defined Risk Factors (IDRF) are present (Appendix III, Table 3). IDRF are surgical risk factors detected on imaging that make total tumor excision risky or difficult at the time of diagnosis.[171,172] Stage M is disseminated disease (comparable to the INSS stage 4), and stage MS metastatic disease similar to INSS stage 4S (except for the size of the primary tumor and age range). Compared with the INSS, focus has been shifted from surgico-pathological findings to imaging. Since imaging data can be retrospectively reviewed, a system based on preoperative diagnostic images will be more robust and reproducible than one based on operative findings.

This new staging system is not intended to substitute for the INSS and it is recommended that both systems are used in parallel. We will investigate whether the number of IDRFs as defined by the INRGSS predict surgical outcome at our institution and survival in children with high-risk neuroblastoma.

2.17 Treatment of Minimal Residual Disease (MRD)

In a recent publication by the Children's Oncology Group (Yu et al[3]) immunotherapy with ch14.18 (a chimeric anti-GD2 monoclonal antibody), GM-CSF and interleukin-2 in children who had a response to induction chemotherapy and stem-cell transplantation, resulted in a 20% improvement in EFS. This antibody, now called dinutuximab, is commercially available. Patients can choose to receive this anti-GD antibody, off study. For patients who elect to continue treatment of MRD on this study, we will use a novel anti-GD2 monoclonal antibody produced by Children's GMP, LLC, hu14.18K322A. This antibody was specifically engineered to be less toxic and more effective and has been tested in a single-agent Phase I trial (SJGD2) and is currently being utilized in a combination study with chemotherapy and allogeneic NK cells in children with refractory NB (GD2NK). In SJGD2, 35 patients (22 males, median age 6.7 years) received a total of 94 courses. Patients received a median of 2 courses. Dose-limiting toxicities occurred in 4 of 32 evaluable patients and were characterized by grade 3 cough, asthenia, pain, anorexia and serum sickness and grade 4 hypertension. The most common grade 3 or 4 toxicities were pain (64% of patients), fever (21%) and hyponatremia (12%). Pain was well managed and predominately occurred on the first day of course 1. Grade 1 or 2 ocular abnormalities were noted in 14 patients and appear to be dose related. No objective responses by RECIST have been observed; however, 4 patients had responses documented by ^{123}I-MIBG (2CR, 2PR). Based on these results the MTD and recommended phase II dose of hu14.18K322A is 60 mg/m^2/dose, given over 4-hrs daily for 4 consecutive days, repeated every 28 days.[31] This dose-level is almost 2½ times the dosage given by Yu et al.,[3] although the SJGD2 study is using antibody alone in contrast to Yu's study which includes GM-CSF, IL-2 and 13-cis retinoic acid.

As pointed out above, hu14.18K322A was "specifically engineered to be less toxic and more effective". Although the relative effectiveness of hu14.18K322A vs. ch14.18 would require a randomized study of a large number of patients, we think the single agent responses in the Phase I study (SJGD2)[31] and an MTD that is 2½ times the dosage given in the COG study of Yu et al,[3] are encouraging preliminary data and suggest that hu14.18K322A should be no less active than ch14.18 and may provide an advantageous toxicity spectrum. Thus, to ensure hu14.18K322A is as tolerable with the addition of GM-CSF and IL-2 we will treat an initial cohort of ten children with 25 mg/m^2/day x 4 of hu14.18K322A, identical to the dose of anti-GD2 antibody ch14.18 used in current open COG trial (ANBL0032) for similar patients. If this is tolerable we will treat the remaining patients with 40 mg/m^2/day x 4. The schedule and dose of the remaining agents (GM-CSF, IL-2 and 13-cis retinoic acid) will be identical to that reported by Yu et al[3] and to the dosages being utilized in

ANBL0032 (see sections 4.1.7, 4.9.3 and 16.3.4).

2.18 Rationale for Busulfan Pharmacokinetic Evaluation

Busulfan pharmacokinetic studies in the pediatric population have shown a wide variability in busulfan serum concentrations. There is also a shift in clearance of busulfan as age changes resulting in doses ranging from 0.8 mg/kg to 1.2 mg/kg in the pediatric population in order to achieve optimal serum concentrations.[173] Additional studies have shown that younger children receiving intravenous busulfan have markedly higher clearance than adolescents and adults.[174] This is important as elevated plasma concentrations are associated with a greater risk of veno-occlusive disease, while lower concentrations may result in poor response to therapy.

It may be that the current dosing model for all patients > 12 kg is resulting in a proportion of patients who are under-dosed based on average steady-state concentrations. Thus, to gain better understanding of the pharmacokinetic variability in the pediatric population and to better target goal steady-state concentrations, it is necessary to study the pharmacokinetics of busulfan in this population.

3.0 RESEARCH PARTICIPANT ELIGIBILITY CRITERIA

3.1 Inclusion Criteria

3.1.1 Participants < 19 years of age (eligible until 19th birthday)

3.1.2 Newly diagnosed, advanced stage, high-risk neuroblastoma defined as one of the following:

a. Children < 1 year with International Neuroblastoma Staging System (INSS) stage 2a, 2b, 3, 4 or 4S disease and MYCN amplification (>10 copies, or greater than four-fold increase in MYCN signal as compared to reference signal).

b. INSS 2a or 2b disease and MYCN amplification, regardless of age or additional biologic features

c. INSS stage 3 and:
   1) MYCN amplification (>10 copies, or greater than four-fold increase in MYCN signal as compared to reference signal, regardless of age or additional biologic features
   2) Age > 18 months (> 547 days) with unfavorable pathology, regardless of MYCN status

d. INSS stage 4 and:
   1) MYCN amplification, regardless of age or additional biologic features
   2) Age > 18 months (> 547 days) regardless of biologic features
   3) Age 12 – 18 months (365 – 547 days) with any of the following three unfavorable biologic features (MYCN amplification, unfavorable pathology and/or DNA index =1) or any biologic feature that is indeterminant/unknown
e. Children ³ 365 days initially diagnosed with: INSS stage 1, 2, 4S who have progressed to a stage 4 without interval chemotherapy.

3.1.3 Histologic proof of neuroblastoma or positive bone marrow for tumor cells with increased urine catecholamines.

3.1.4 Adequate renal and hepatic function (serum creatinine < 3 x upper limit of normal for age, AST < 3 x upper limit of normal).

3.1.5 No prior therapy, unless an emergency situation requires local tumor treatment (discuss with PI).

3.1.6 Written, informed consent according to institutional guidelines.

3.2 Exclusion Criteria

3.2.1 Any evidence, as judged by the investigator, of severe or uncontrolled systemic disease (e.g., unstable or uncompensated respiratory, cardiac, hepatic, or renal disease).

3.2.2 Pregnant or breast feeding (female of child-bearing potential).

3.2.3 Children with INSS 4 disease, age < 18 months with all 3 favorable biologic features (non-amplified MYCN, favorable pathology and DNA index > 1).

3.3 Research Participant Recruitment and Screening

Research participants will be recruited from the principal investigator's and co-investigators' clinical practice according to standard methods within the Division of Solid Tumor and following institutional guidelines. The NB2012 screening consent must be signed before any research studies are performed.

3.4 Enrollment On Study

A member of the study team will confirm potential participant eligibility, complete and sign the 'Participant Eligibility Checklist'. The study team will enter the eligibility checklist information into the St. Jude centralized enrollment 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 595–6265 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 6:00 pm. If you have a prospective research enrollment and need assistance releasing your consent, please call the MILLI helpline (901–338–0596) on call number.

4.0 TREATMENT PLAN

4.1 General Overview

4.1.1 General Assumptions about Chemotherapy Administration

The term "every" used is an approximate term meaning that these medications will be administered approximately "every" 12 hours. The drug administration timing in the case of "every 12 hours" may be modified by approximately ± 4 hours or as clinically indicated such as in the case of surgical procedures or to accommodate other necessary medication, blood product delivery or procedures (such as a needed CT scan). The term "day" does not refer to an absolute calendar day. It refers to a general 24-hour period.

Medication dosing may be modified for research recipients based upon actual body weight or adjusted ideal body weight when clinically indicated. Criteria for medication calculations based on body weight/body surface area can be found in any version of the St. Jude Formulary. Medication doses may be rounded to the nearest integer or to the nearest appropriate quantity when clinically or pharmaceutically indicated as per the MD and PharmD.

4.1.2 Initial Treatment with Cyclophosphamide and Topotecan

Participants enrolled on this study will first receive cyclophosphamide, topotecan, and hu14.18K322A to study the response to this combination. Cyclophosphamide will be given intravenously daily x 5 and topotecan will be administered intravenously daily x 5 days. Hu14.18K322A will be given intravenously daily x 4, beginning on day 2 of each course of chemotherapy (See table in Section 4.2). Peripheral blood stem cell harvest (PBSCH) and surgical resection of the primary tumor will be performed after these two initial courses of chemotherapy, if feasible (see section 6.0 for PBSC collection guidelines and 7.0 for surgical guidelines). PBSC harvesting will be performed using the current standard operating procedure of the apheresis center SJCRH.

<u>First course</u>:

The first course will begin on day 1 and end on day 21.

<u>Second course</u>:

The second course will begin on as close to day 22 as possible, in the absence of unacceptable toxicity or progressive disease (section 4.10 & 4.11).

4.1.3 Additional Induction Chemotherapy

After evaluation of the initial six weeks of treatment, participants will receive four additional courses of induction chemotherapy with hu14.18K322A: cisplatin and etoposide (CiE), (course 3); cyclophosphamide, doxorubicin, and vincristine (CAV), (course 4). CiE will be repeated again in course 5and CAV again for course 6. Also refer to tables 4.2-4.5.1 for treatment schema.

4.1.4 Resection of Primary Tumor

Patients who were unable to have their primary tumor resected after the initial two courses of induction chemotherapy will undergo surgery for resection of the primary tumor mass and careful lymph node staging (see sections 4.6 and 7.0 for surgical guidelines).

4.1.5 Intensification

Following chemotherapy and surgery, as outlined above, patients will receive an intensification course of high-dose busulfan and melphalan along with PBSC support.

4.1.6 Consolidative Radiation Therapy

Radiation therapy to the primary and metastatic disease sites will follow peripheral blood stem cell transplant with the exception of any patient requiring emergent radiotherapy. External beam radiotherapy will be delivered to the primary site and select metastatic and bulky nodal sites as outlined in section 8.0.

4.1.7 Minimal Residual Disease Treatment

Patients will be offered MRD treatment in one of two ways: 1) if ch14.18 becomes commercially available, patients can come off study and be treated with this antibody or, 2) following completion of the above outlined treatment, consenting patients will be treated identical to the Children's Oncology Groups ANBL0032 study[3], with the exception that hu14.18K322A will be substituted for the ch14.18 anti-GD2 antibody. An initial cohort of ten patients will be treated with an identical dose, on a mg/m^2 basis, of hu14.18K322A and identical dosages and schedules of GM-CSF, IL-2 and isotretinoin. If this initial cohort tolerates this treatment (defined in section 16.3.4), then subsequent participants will receive 40 mg/m^2 of hu14.18K322A.

4.2 Overall Treatment Schema

[α]See Section 4.6, Surgical removal of primary tumor

Course	Week	
1** Cyclo/TPT+GD2+IL-2	0	Cyclo 400 mg/m² I.V. day 1-5; TPT 1.2 mg/m²/d 1-5 IV hu14.18K322A 40 mg/m² I.V d 2-5 Low-dose IL-2 SQ 1 million units/m² for 6 doses (days 6, 8, 10, 12, 14 and 16)
2** Cyclo/TPT+GD2+IL-2	3	Cyclo 400 mg/m² I.V. day 1-5; TPT 1.2 mg/m²/d 1-5 IV hu14.18K322A 40 mg/m² I.V d 2-5 Low-dose IL-2 SQ 1 million units/m² for 6 doses (days 6, 8, 10, 12, 14 and 16)
Evaluate response /PBSCH/ Resect tumor if possible[α]		
3**	6	CISplatin 50 mg/m²/day x 4 I.V. over 1 hour (days 1-4) Etoposide 200 mg/m²/day x 3 I.V. over 1 hour (days 1-3) hu14.18K322A 40 mg/m² I.V d 2-5 Low-dose IL-2 SQ 1 million units/m² for 6 doses (days 6, 8, 10, 12, 14 and 16)
4**	9	Cyclophosphamide 2.1 gm/m² daily x 2 I.V. day 1 and 2 DOXOrubicin 25 mg/m² I.V. day 1 - 3 MESNA: 525 mg/m² I.V. immediately prior to each cyclophosphamide infusion and again at 4, 8 and 12 hours after each cyclophosphamide *VinCRIStine 0.67 mg/m²/dose, daily x 3 hu14.18K322A 40 mg/m² I.V d 2-5 Low-dose IL-2 SQ 1 million units/m² for 6 doses (days 6, 8, 10, 12, 14 and 16)
5**	12	CISplatin 50 mg/m²/day x 4 I.V. over 1 hour (days 1-4) Etoposide 200 mg/m²/day x 3 I.V. over 1 hour (days 1-3) hu14.18K322A 40 mg/m² I.V d 2-5 Low-dose IL-2 SQ 1 million units/m² for 6 doses (days 6, 8, 10, 12, 14 and 16)
6**	15	Cyclophosphamide 2.1 gm/m² daily x 2 I.V. day 1 and 2 DOXOrubicin 25 mg/m² I.V. day 1 - 3 MESNA: 525 mg/m² I.V. immediately prior to each cyclophosphamide infusion and again at 4, 8 and 12 hours after each cyclophosphamide *VinCRIStine 0.67 mg/m²/dose, daily x 3 hu14.18K322A 40 mg/m² I.V d2-5 Low-dose IL-2 SQ 1 million units/m² for 6 doses (days 6, 8, 10, 12, 14 and 16)
Evaluate response / complete surgical resection[α] (if not done already)		
Intensification	24	BuSulfan and Melphalan, followed by stem cell rescue
Radiation therapy		
MRD Treatment	Hu14.18K322A, GM-CSF, IL-2, isotretinoin (see section 4.9)	

**Total dose of vincristine may not exceed 2 mg in 72 hours or 0.67 mg/day for any patient.*
***Interleukin-2 will be given SQ, beginning the day following the last dose of chemotherapy at a dose of 1 million units/m² and continued every other day for 6 doses during induction courses 1-6.*
GM-CSF will begin on day 7 of each chemotherapy course at a dose of 250 mcg/m²/day and continued daily through the nadir until ANC ≥ 2000/mm³ (section 5.5)

4.3 Treatment Administration: Course 1 and 2

Day of treatment	Chemotherapeutic agent		Hu14.18K322A Antibody	Cytokine
1	Cyclophosphamide (30 min)	Topotecan (30 min)		
*2	Cyclophosphamide (30 min)	Topotecan (30 min)	4 hours*	
*3	Cyclophosphamide (30 min)	Topotecan (30 min)	4 hours*	
*4	Cyclophosphamide (30 min)	Topotecan (30 min)	4 hours*	
*5	Cyclophosphamide (30 min)	Topotecan (30 min)	4 hours*	
6				rIL-2†
***Day 7-21				rhu-GM-CSF daily***
Course 2				
1	Cyclophosphamide (30 min)	Topotecan (30 min)		
2	Cyclophosphamide (30 min)	Topotecan (30 min)	4 hours*	
3	Cyclophosphamide (30 min)	Topotecan (30 min)	4 hours*	
4	Cyclophosphamide (30 min)	Topotecan (30 min)	4 hours*	
5	Cyclophosphamide (30 min)	Topotecan (30 min)	4 hours*	
6				rIL-2†
***7-21				rhu-GM-CSF qd*** **G-CSF qd

Cyclophosphamide- 400 mg/m^2 I.V. day 1-5; topotecan 1.2 mg/m^2/d 1-5 IV [dosages identical to COG ANBL0532 induction].[33]
* hu14.18K322A 40 mg/m^2 IV over 4 hours
**G-CSF at a dose of 5 mcg/kg/day will be added to GM-CSF only for course in which PBSCH will be done (preferably course 2 above)
***GM-CSF will begin on day 7 of each chemotherapy course at a dose of 250 mcg/m^2/day and continued daily through the nadir until ANC ≥ 2000/mm^3 (section 5.5).
†-Interleukin-2 will be given SQ, beginning on day 6 of each chemotherapy course at a dose of 1 million units/m^2 and continued every other day for 6 doses (day 6, 8, 10, 12, 14 and 16).

Participants ≤ 12 kg: Cyclophosphamide 13.3 mg/kg/dose; topotecan 1.2 mg/m^2/dose.

4.4 Courses 3 and 5 – High Dose CISplatin and Etoposide (CiE)

**** Urine specific gravity does NOT need to be < 1.010 to start chemotherapy ****

Day of treatment	Chemotherapeutic agent		Hu14.18K322A Antibody	Cytokine
1	CISplatin (60 min)	Etoposide (60 min)		
2	CISplatin (60 min)	Etoposide (60 min)	4 hours*	
3	CISplatin (60 min)	Etoposide (60 min)	4 hours*	
4	CISplatin (60 min)		4 hours*	
5			4 hours*	
6				rIL-2†
***Day 7-21				rhu-GM-CSF daily**

Cisplatin-50 mg/m^2/dose (if ≤ 12 kg see below), once daily x 4 doses
Etoposide-200 mg/m^2/dose (if ≤ 12 kg see below), once daily x 3 doses
* hu14.18K322A 40 mg/m^2 IV over 4 hours
†-Interleukin-2 will be given SQ, beginning on day 6 of each chemotherapy course at a dose of 1 million units/m^2 and continued every other day for 6 doses (day 6, 8, 10, 12, 14 and 16).
**GM-CSF will begin on day 7 of each chemotherapy course at a dose of 250 mcg/m^2/day and continued daily through the nadir until ANC ≥ 2000/mm^3 (section 5.5).

4.4.1 Participants ≤ 12 kg – CiE Courses 3 and 5

Agent	Dose	Route	# Doses	Schedule
CISplatin	1.66 mg/kg/day	IV over 1 hour	4	Days 1-4
VP16 (etoposide)	6.67 mg/kg/day	IV over 1 hour	3	Days 1,2 and 3
hu14.18 K322A	40 mg/m^2/day	IV over 4 hours	4	Days 2-5
GM-CSF (sargramostim)	250 mcg/m^2/day	SQ	Start Day 7	Daily through the nadir until ANC ≥2000/mm^3
Interleukin-2 (Aldesleukin,	1 million units/m^2	SQ	6	qod, beginning day 6 for six

Proleukin[a])				doses

4.4.2.1 *Recommended* hydration guidelines CiE

Pre-hydration on day 1 with D5W ½ NS and 20 mEq/L KCL at 125 ml/m^2/hour x 6 hours. Mannitol 8 g/m^2 immediately prior to CISplatin.

Days 1-5 hydration:

Hours 1-7: D5W 0.45% NS and 20 mEq/L KCL and 8 g/m^2 mannitol at 125 ml/m^2/hour.
Hours 7-23: D5W 0.45% NS and 10 mEq/L KCL at 125 ml/m^2/hour

4.5 Courses 4 and 6 -Cyclophosphamide, Doxorubicin, Vincristine

Day of treatment	Chemotherapeutic agent			Hu14.18K322A Antibody	Cytokine
1	Cyclophosphamide (6 hours)	DOXOrubicin (continuous infusion over 72 hours)	VinCRIStine (IV minibag)		
2	Cyclophosphamide (6 hours)		VinCRIStine (IV minibag)	4 hours*	
3			VinCRIStine (IV minibag)	4 hours*	
4				4 hours*	
5				4 hours*	
6					rIL-2†
***Day 7-21					rhu-GM-CSF daily***

Cyclophosphamide - 2100 mg/m^2/dose (if < 12 kg see below), once daily x 2 doses.
DOXOrubicin -25 mg/m^2/dose (if < 12 kg see below), once daily (CI x 24 hours) for 3 doses.
VinCRIStine-0.67 mg/m^2/dose or 0.022 mg/kg/dose (whichever is LOWER), once daily x 3 doses.
Total dose of vincristine may not exceed 2 mg in 72 hours or 0.67 mg/day for any participant.
MESNA 525 mg/m^2/dose will be given immediately prior to each cyclophosphamide dose and again at 4, 8 and 12 hours after the cyclophosphamide infusion.

*hu14.18K322A 40 mg/m^2 IV over 4 hours. †-Interleukin-2 will be given SQ,

beginning on day 6 of each chemotherapy course at a dose of 1 million units/m^2 and continued every other day for 6 doses (day 6, 8, 10, 12, 14 and 16). ***GM-CSF will begin on day 7 of each chemotherapy course at a dose of 250 mcg/m^2/day and continued daily through the nadir until ANC ≥ 2000/mm^3 (section 5.5). Interleukin-2 will be given SQ, beginning on day 6 of each chemotherapy course at a dose of 1 million units/m^2 and continued every other day for 6 doses (day 6, 8, 10, 12, 14 and 16).

4.5.1 Participants ≤ 12 kg – Courses 4 and 6

Agent	Dose	Route	# Doses	Schedule
CYCLO (cyclophosphamide)	70 mg/kg/day	IV over 6 hours	2	Days 1 and 2
Mesna	17.5 mg/kg/dose	IV	4	Pre, @ 4, 8 and 12 hours after CYCLO
DOXOrubicin (doxorubicin)	0.83 mg/kg/day	IV continuous infusion over 24 hours	3	Day 1 after 1st dose of CYCLO
VinCRIStine*	0.017 mg/kg/dose (< 12 months of age); ≥ 12 months: 0.67 mg/m^2/dose or 0.022 mg/kg/dose– *whichever is lower);* ≥ 12 months and ≤ 12 kg: 0.022 mg/kg/dose	IV minibag	3	
hu14.18 K322A	40 mg/m^2	IV over 4 hours	4	Day 2 – 5
GM-CSF (sargramostim)	250 mcg/m^2/day	SQ	Start Day 7	Daily through the nadir until ANC ≥2000/mm^3
Interleukin-2 (Aldesleukin, Proleukin®)	1 million units/m^2	SQ	6	qod, beginning day 6 for six doses

MESNA will be given will be given immediately prior to each cyclophosphamide dose and again at 4, 8 and 12 hours after the cyclophosphamide infusion.
**Total dose may not exceed 2 mg in 72 hours or 0.67 mg/day for any participant.*

4.5.1.1 Recommended hydration – [cyclophosphamide, DOXOrubicin, vinCRIStine]

3000 ml/m^2/day using fluid containing at least 0.45% NS and 10 mEq/L KCl will begin 2 hours prior to the cyclophosphamide infusion and continue for at least 24 hours after the last dose of cyclophosphamide. Monitor urine output and specific gravity closely.

4.6 Surgical Removal of Primary Tumor

Surgical resection should be performed when, in the judgment of the operating surgeon, the primary tumor and regional disease can be completely resected based on the radiographic appearance (CT scan and/or MRI) of the tumor. Tumors will be considered unresectable if there is significant involvement of major vascular structures or contiguous organs, or if it would likely require a nephrectomy to remove all gross disease. Surgical resection may be performed at any time during the treatment course, but should occur prior to the Consolidation phase, even if the tumor appears to remain unresectable. In these cases, tumor debulking will be performed with every attempt being made to remove the primary site.

4.7 Intensification Phase

Day	Agent	Dosage and route	# Doses	Schedule
-7	Levetiracetam±	10 mg/kg dose PO or IV	2	Every 12 hours
-6	Busulfan	0.8 mg/kg for >12kg and 1.1mg/kg for ≤12kg	Total doses to be determined by therapeutic drug level monitoring and dose adjustment (see section 4.7.1)	Every 6 hours
	Levetiracetam	10 mg/kg dose PO or IV	2	Every 12 hours
-5	Busulfan	0.8 mg/kg for >12kg and 1.1mg/kg for ≤12kg	Total doses to be determined by therapeutic drug level monitoring and dose adjustment (see section 4.7.1)	Every 6 hours
	Levetiracetam	10 mg/kg dose PO or IV	2	Every 12 hours
-4	Busulfan	0.8 mg/kg for >12kg and 1.1mg/kg for ≤12kg	Total doses to be determined by therapeutic drug level monitoring and dose adjustment (see section 4.7.1)	Every 6 hours
	Levetiracetam	10 mg/kg dose PO or IV	2	Every 12 hours
-3	Busulfan	0.8 mg/kg for >12kg and 1.1mg/kg for ≤12kg	Total doses to be determined by therapeutic drug level monitoring and dose adjustment (see section 4.7.1)	Every 6 hours
	Levetiracetam	10 mg/kg dose PO or IV	2	Every 12 hours
-2	Melphalan	70 mg/m^2 IV for > 10 kg; 2.3 mg/kg IV for ≤ 10 kg	1	Once daily
	Levetiracetam	10 mg/kg dose PO or IV	2	Every 12 hours
-1	Melphalan	70 mg/m^2 IV for > 10 kg; 2.3 mg/kg IV for ≤ 10 kg	1	Once daily
	Levetiracetam	10 mg/kg dose PO or IV	2	Every 12 hours
0	**Autologous stem cell infusion**			

+6	Filgrastim	5 mcg/kg/day	Until ANC > 2,000 for 2 days (or as instructed by BMT team)	Daily

± Alternative anticonvulsant prophylaxis (e.g. phenytoin, fosphenytoin) may be considered at the discretion of attending M.D.
**in consenting patients only*

4.7.1 General Assumptions Regarding Chemotherapy Administration and Clinical Management during Intensification Phase.

- Busulfan dosing will be as follows: 0.8 mg/kg for > 12kg and 1.1mg/kg for ≤ 12 kg approximately every 6 hours for the first 2 doses, with subsequent doses to be based on pharmacokinetics to provide 16 total doses with an approximate every 6 hour dosing schedule. For patients ≤ 12 kg total body weight, consideration will be made to use increased dosing of busulfan of approximately 1.1 mg/kg/dose IV every 6 hours.
- Dosing for busulfan and melphalan may be modified for research recipients based upon actual body weight or adjusted ideal body weight when clinically indicated. Criteria for medication calculations based on body weight/body surface area and other medication related information can be found in the St. Jude Formulary or the St. Jude Department of Pharmaceutical Sciences intranet website. (**http://www.crlonline.com/crlsql/servlet/crlonline**).
- Clinically indicated changes in conditioning regimen and supportive medications may be done for safety reasons when indicated. These changes will be reported as clinically indicated and in the continuing review reports where applicable.
- Anticonvulsant prophylaxis for busulfan therapy should start 12 to 24 hours prior to the start of busulfan and continue for approximately 48 hours after completion of busulfan. Levetiracetam will be the preferred agent. Alternatives may be considered at the discretion of the attending MD (e.g. fosphenytoin / phenytoin), as per institutional clinical practice.
- Medication doses may be rounded to the nearest integer or to the nearest appropriate quantity when clinically or pharmaceutically indicated as per the MD and PharmD.
- The term “every” used in this table is an approximate term, meaning that these medications noted will be administered approximately “every” hourly interval listed. The drug administration timing in the case of “every 12 hours” may be modified by approximately +/- 4 hours or as clinically indicated such as in the case of surgical procedures or to accommodate other necessary medication, blood product delivery, or procedures.
- The term “day” does not refer to an absolute calendar day. It refers to a general 24-hour period. Moreover, the cell infusions may be delayed by approximately 24 hours in order to accommodate stem cell processing, the Blood Donor Center

and/or Human Applications Laboratory as well as the research participant clinical condition.

4.7.2 Dosage Escalation Schema for hu14.18K322A:

Stage 1: Accrue 10 patients at an initial 25 mg/m^2/dose of hu14.18K322A, daily for 4 days (day+2 – +5). If 1 or no patient experiences at least one unacceptable DLT (see section 16.3.3) during this phase treatment (immediate post-PBSC infusion period), then continue to Stage 2. If 2 or more experience at least one unacceptable DLT during this phase treatment, the regimen will be considered to have unacceptable toxicity, and the dosage of hu14.18K322A will be decreased to 17.5 mg/m^2/dose (identical to dose use in United Therapeutics protocol DIV-NB-201). An additional 10 patients will be accrued at this reduced dosage level. If 2 or more experience at least one unacceptable DLT, the regimen will be considered to have unacceptable toxicity, and will be temporarily closed, in order to review all relevant data and consider modifying the regimen to improve safety. If 1 or no patients have an unacceptable DLT at this reduced dosage level of hu14.18K322A, then all subsequent patients will receive this reduced dosage level of hu14.18K322A of 17.5 mg/m^2/dose.

Stage 2: Increase dose of hu14.18K322A to 40 mg/m^2/dose and accrue 10 patients. As in stage 1, if 2 or more experience at least one unacceptable DLT, the regimen will be considered to have unacceptable toxicity at this increased dosage level of hu14.18K322A (40 mg/m^2/dose), and the remaining patients will be given the initial dosage of 25 mg/m^2/dose. If 1 or no patients have an unacceptable DLT, then it is reasonable to assume that the combination therapy is safe and accrue all of the remaining patients to this dose-level of hu14.18K322A (40mg/m^2/dose).

4.8 Radiation Therapy

Radiation therapy (RT) to the primary and metastatic disease sites will follow peripheral blood stem cell transplant with the exception of any patient requiring emergent radiotherapy. External beam radiotherapy will be delivered to the primary site, select metastatic sites, and bulky nodal disease as outlined below. Adequate bone marrow recovery, renal and hepatic function (ANC > 300/mL, platelets > 40,000/mL, serum creatinine <3 x upper limit of normal for age, AST < 3 x upper limit of normal) should be established prior to start of radiation therapy. If there are compelling reasons to initiate RT prior to "recovery" as defined above these should be discussed with the radiation oncologist and study PI and the reasons clearly documented in the medical record.

4.8.1 Timing and Duration of Irradiation

Radiotherapy should be initiated within 42 days of stem cell re-infusion. However, patient specific factors may dictate variations from these recommendations; organ toxicity within potential radiation fields should be resolved. For patients with microscopic residual, a total dose of 23.4 Gy will be delivered in 13 fractions of 180 cGy each. The duration of the treatment plan will be 2 weeks and 3 days. Patients with gross residual disease will receive 30.6 Gy in 17 fractions of 180 cGy each, requiring 3 weeks and 2 days for delivery (see section 8.0 for further details and guidelines during radiation therapy).

4.9 Minimal Residual Disease (MRD) Treatment

4.9.1 Trial participants will be offered treatment with dinutuximab, off protocol, identical to that reported by Yu et al[3] as an alternative to this study, now that this drug is commercially available.

4.9.2 Upon recovery from PBSC rescue, local radiation and disease re-evaluation children will be treated as outlined below. Every effort should be made to begin MRD treatment prior to day +100 after PBSC infusion.

The initial ten patients will be treated with 25 mg/m^2/day x 4 days of hu14.18K322A in all courses of antibody plus cytokine as outlined below. Many of the dose-limiting toxicities (DLTs) that occur due to the hu14.18K322A with IL2 therapy are not unexpected. However, as in the ANBL0322 protocol, we plan to consider a subset of the DLTs unacceptable. If during the second course of mAb therapy (with IL-2), > 1 participant develops an unacceptable toxicity attributable to combination therapy (see section 16.3.4), then an additional 10 patients will be enrolled at 17.5 mg/m^2/day x 4 days of hu14.18K322A. If 2 or more experience at least one unacceptable DLT, the regimen will be considered to have unacceptable toxicity, and will be temporarily closed, in order to review all relevant data and consider modifying the regimen to improve safety. If 1 or no patients have an unacceptable DLT at this reduced dosage level of hu14.18K322A, then all subsequent patients will receive this reduced dosage level of hu14.18K322A of 17.5 mg/m^2/dose.

If 1 or no patient's treated with 25 mg/m^2/day x 4 days of hu14.18K322A have an unacceptable DLT in the first treatment cycle containing IL-2, the remaining patients will be given 40 mg/m^2/day x 4 days of hu14.18K322A.

Patients who experience an unacceptable toxicity judged to be a result of hu14.18K322A during consolidation (section 4.7.2), will receive hu14.18K322A, during "standard" MRD treatment at one dose-level

below the dosage they received in this prior phase of treatment (e.g. if receive 25 mg/m^2 of hu14.18K322A during consolidation, then they will receive 17.5 mg/m^2 of hu14.18K322A during standard MRD treatment; if receive 40 mg/m^2 during consolidation, then they will receive 25 mg/m^2).

4.9.3 MRD Treatment Schema and Guidelines for Therapy

Prior to each course of MRD treatment:

a. ALT < 5 x normal
b. Skin toxicity no greater than Grade 1
c. No evidence of serious infection, or infection under control with no active disease and negative blood culture
d. Serum creatinine < 1.5 mg/dL
e. Platelet count ≥ 20,000/µL (may be transfused). Patients with a history of neuroblastoma metastatic to the central nervous system should receive platelet transfusion support to maintain platelet count ≥ 50,000/µL.

Schema for the administration of 5 courses of hu14.18K322A, isotretinoin and cytokines

Course 1	Course 2	Course 3	Course 4	Course 5	Course 6
hu14.18K322A	hu14.18K322A	hu14.18K322A	hu14.18K322A	hu14.18K322A	–
GM-CSF	Aldesleukin (IL-2)	GM-CSF	Aldesleukin (IL-2)	GM-CSF	–
Isotretinoin (RA)	RA	RA	RA	RA	RA

Hu14.18K322A treatment will be administered every 28 days (±7 days) at 25 mg/m^2/day for 4 days for all 5 courses for the first 10 patients. See section 4.9.2 and 16.3.4 for hu14.18K322A dosages for subsequent patients; GM-CSF at 250 mcg/m^2/day for 14 days; aldesleukin (IL-2) at 3 MIU/m^2/day for the first week, 4.5 MIU/m^2/day for the second week.

All patients will receive 6 cycles of isotretinoin (13-cis retinoic acid) therapy at 160 mg/m^2/day PO divided bid for 14 days. For patients' ≤ 12 kg, dose is 5.33 mg/kg/day, in two divided doses for 14 days. Round up to the nearest 10 mg.

4.9.3.1 Therapy outline for Courses 1, 3 and 5.

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14-23	24
GM-CSF**	X	X	X	X	X	X	X	X	X	X	X	X	X	X		Begin course 2 and
hu14.18K322A				X	X	X	X									

Isotretinoin (RA)										*RA	RA	RA	RA	RA	4

*For course 1 only, isotretinoin (RA) should start on day 11 and continue through Day 24 (courses 3 and 5: RA Day 10–23). It is suggested that day 0 of courses 1, 3 and 5 be on a Friday; Day 0 of courses 2 and 4 be on a Monday, and should fall on day 24 and day 80 respectively, from the start of MRD treatment (see calendar).

**GM-CSF should be given prior to hu14.18K322A and held if WBC > 50,000/μL. GM-CSF will be held until WBC < 20,000/μL and then resumed at 50% dose (125 μg/m^2/day for the remainder of that course. Full dose GM-CSF (250 μg/m^2/day) if criteria for holding GM-CSF are met. Hu14.18K322A should be given without GM-CSF.

4.9.3.2 Therapy Outline for Courses 2 and 4.

Day	**0**	**1**	**2**	**3**	**4–6**	**7**	**8**	**9**	**10**	**11–13**	**14**	**15**	**16**	**17**	**18–27**
Aldesleukin (IL-2)	X	X	X	X		X	X	X	X						
hu14.18K322A						X	X	X	X						
Isotretinoin (RA)											RA	RA	RA	RA	RA

It is suggested that day 0 of course 2 and 4 be on a Monday. **Hu14.18K322A** treatment will be administered every 28 days (±7 days). Day 0 of course 2 and 4 will be on day 24 of course 1 and 3, respectively (see calendar). **Aldesleukin (IL-2) 3 MIU/m^2/day** will be given by continuous infusion (using a CADD or similar infusion pump) for 4 days during the first week of each course given on Days 0 – 3. The first week of therapy has been very well tolerated and typically given as an outpatient on ANBL0032.[3] During the second week of each course, aldesleukin (IL-2) 4.5 MIU/m^2/day for 4 days will be given on days 7–10 (with the infusion of hu14.18K322A).

MRD Treatment Calendar

SUN	MON	TUES	WED	THURS	FRI	SAT
Pre-ASCT	Enter start date			Pre-immunotherapy	0 GM	1 GM
2 GM	3 GM hu14.18	4 GM hu14.18	5 GM hu14.18	6 GM hu14.18	7 GM	8 GM
9 GM	10 GM	11 GM RA	12 GM RA	13 GM RA	14 RA	15 RA
16 RA	17 RA	18 RA	19 RA	20 RA	21 RA	22 RA
23 RA	24/0 IL-2 RA	25/1 IL-2	26/2 IL-2	27/3 IL-2	28/4	29/5
30/6	31/7 IL-2 hu14.18	32/8 IL-2 hu14.18	33/9 IL-2 hu14.18	34/10 IL-2 hu14.18	35/11	36/12
37/13	38/14 RA	39/15 RA	40/16 RA	41/17 RA	42/18 RA	43/19 RA
44/20 RA	45/21 RA	46/22 RA	47/23 RA	48/24 RA	49/25 RA	50/26 RA
51/27 RA	52/28	53/29	54/30	55/31	56/0 GM	57/1 GM
58/2 GM	59/3 GM hu14.18	60/4 GM hu14.18	61/5 GM hu14.18	62/6 GM hu14.18	63/7 GM	64/8 GM
65/9 GM	66/10 GM RA	67/11 GM RA	68/12 GM RA	69/13 GM RA	70/14 RA	71/15 RA
72/16 RA	73/17 RA	74/18 RA	75/19 RA	76/20 RA	77/21 RA	78/22 RA
79/23 RA	80/0 IL-2	81/1 IL-2	82/2 IL-2	83/3 IL-2	84/4	85/5
86/6	87/7 IL-2 hu14.18	88/8 IL-2 hu14.18	89/9 IL-2 hu14.18	90/10 IL-2 hu14.18	91/11	92/12
93/13	94/14 RA	95/15 RA	96/16 RA	97/17 RA	98/18 RA	99/19 RA
100/20 RA	101/21 RA	102/22 RA	103/23 RA	104/24 RA	105/25 RA	106/26 RA
107/27 RA	108/28	109/29	110/30	111/31	112/0 GM	113/1 GM
114/2 GM	115/3 GM hu14.18	116/4 GM hu14.18	117/5 GM hu14.18	118/6 GM hu14.18	119/7 GM	120/8 GM
121/9 GM	122/10 GM RA	123/11 GM RA	124/12 GM RA	125/13 GM RA	126/14 RA	127/15 RA
128/16 RA	129/17 RA	130/18 RA	131/19 RA	132/20 RA	133/21 RA	134/22 RA
135/23 RA	136/0	137/1	138/2	139/3	140/4	141/5
142/6	143/7	144/8	145/9	146/10	147/11	148/12
149/13	150/14 RA	151/15 RA	152/16 RA	153/17 RA	154/18 RA	155/19 RA
156/20 RA	157/21 RA	158/22 RA	159/23 RA	160/24 RA	161/25 RA	162/26 RA
163/27 RA	164/28 (Day 220)	165/29	166/30	167/31	168/32	169/33
170/34	171/35	172/36	173/37	174/38	175/39	176/40

177/41	178/42					

IRB approval date: 08-01-

4.10 Contingency Plans for Progressive Disease

Participants with progressive disease at any time during induction chemotherapy (evidence of increase in metastatic disease or development of new sites of disease) will proceed immediately to the next course of treatment. For example, progressive disease after the first cyclophosphamide and topotecan cycle will proceed directly to course #3 (CiE; see section 4.2 for overall treatment schema).

4.11 Dose Modifications for Chemotherapy

Full dose therapy will be given to all participants. If the absolute neutrophil count is <500/µL at the due date for any chemotherapy course, chemotherapy will be *delayed until the ANC is ³ 500/µL*. GM-CSF will be continued until these values are achieved and chemotherapy can be resumed.

If BUN > 40 mg/dL or creatinine > 2 mg/dL – delay chemotherapy and hydrate; resume as soon as levels fall below 40 mg/dL and 2 mg/dL, respectively.

5.0 DRUG AND BIOLOGIC INFORMATION

5.1 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 participants 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.

Supplier: Commercially available, see package insert for more information.

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: 400 mg/m^2/day by 30 min intravenous infusion (course 1 and 2); Participants ≤ 12 kg, 13.3 mg/kg/dose. Courses 4 and 6: 2100 mg/m^2/dose; participants ≤ 12 kg, 70 mg/kg/dose.

5.2 Topotecan (Hycamtin^{Ò})

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 participants 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.

Supplier: Commercially available, see package insert for more information.

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 paresthesia.

Dosage and route of administration: 1.2 mg/m^2/dose.

5.3 Humanized anti-GD2 Antibody (hu14.18 K322A mAb) (IND #100617)

Hu14.18K322A is a humanized chimeric human/mouse monoclonal antibody (mAb) that recognizes the disialoganglioside GD2. The hu14.18K322A is identical to the ch14.18, except the Fab ends involve fully human amino acid sequences for IgG1 heavy and kappa light chains, and the complementarity determining regions correspond to the antigen binding sequences of the murine 14.18 mAb. The resulting hu14.18 mAb is approximately 98% derived from human genes, yet maintains the specificity of the murine ch14.18 mAb. In addition, hu14.18K322A also has a single point mutation (K322A) reducing complement-dependent lysis. Hu14.18K322A mAb is produced in the rat YB2/0 cell line. YB2/0 is a non-immunoglobulin secreting rat myeloma that was derived from the hybrid rat myeloma YB2/3HL.[175,176] Monoclonal antibody produced in YB2/0 cells has been shown to have increased antibody-dependent cellular toxicity (ADCC) due to the lack of fucosylation activity.[177]

Stability of the final diluted product: Hu14.18K322A mAb diluted to a final concentration of 0.1 mg/ml to 2 mg/ml is stable for at least 72 hours at 21°C. Solutions of hu14.18K322A diluted in 0.9% Sodium Chloride to final concentrations of 0.02 mg/ml to 2 mg/ml are stable for 48 hours at room temperature or under refrigeration in glass containers, polyolefin bags or polypropylene syringes.

Preparation of the final diluted product: Aseptic technique must be used to prepare the hu14.18K322A mAb infusions. Preparation of the infusions must be done on the day of administration for each subject. Stock vials of hu14.18K322A are at a concentration of 10 mg/ml in 2.1 ml or 5.1 ml vials kept at 4°C. The vials of hu14.18K322A will be brought to room temperature. Once at room temperature, the vials will be gently mixed by inverting the vials 10 times. It is important that vials not be shaken or handled roughly as this may lead to aggregation or degeneration of the protein.

Using standard pharmacy procedure, an appropriate volume based on the table below of 0.9% Sodium Chloride Injection (Normal Saline, NaCl) will be

added to a glass container, polyolefin bags or polypropylene syringes. After verifying the volume of diluent, using a large bore needle (i.e. 18 gauge) draw up an appropriate amount of hu14.18K322A and gently add the drug to the diluent in the appropriate container for IV administration. The final diluted product must be transported in a manner that will minimize aggregation of the protein. Transportation via a pneumatic tube system is prohibited.

Final calculated dose of hu14.18K322A mAb	Volume of diluent (0.9% NaCl)	Final concentration (mg/mL)
≤ 25 mg	25 mL	≤ 1 mg/mL
25.1 mg – 100 mg	50 mL	0.5 – 2 mg/mL
100.1 – 200 mg	100 mL	1 – 2 mg/mL
≥ 200.1 mg	250 mL*	≤ 2 mg/mL*

**Stability testing of concentrations > 2 mg/ml has not been performed. Although doses > 200 mg are not anticipated, an appropriate amount of diluent should be added to ensure the final concentration is between 0.02 – 2 mg/mL*

Administration: The diluted antibody will be administered within 48 hours of dilution. Antibody that is not infused prior to 48 hours after dilution will be discarded. The intravenous infusion will run over approximately four hours. A low protein binding 0.2 – 0.22 micron filter will be used. If the recipient experiences unacceptable side effects as defined in section 5.0 the rate of the infusion can be slowed down.

Potential toxicities: The potential or anticipated toxicities of the antibody are based on the toxicities described in the table below for the ch14.18 mAb. The risks of receipt of hu14.18K322A antibody to an unborn or nursing child are unknown.

Likely – pain, paresthesia, mild hypotension or mild hypertension, tachycardia, urticaria, pruritis, fever, nausea, mild hyponatremia, fatigue, mild hypokalemia, anorexia and cough

Less likely – moderate hypotension, moderate hypertension, vomiting, diarrhea, moderate hypokalemia, moderate hyponatremia, hypoalbuminemia, somnolence, weight loss, serum sickness, elevated creatinine, elevation liver transaminases, thrombocytopenia, sensory or motor neuropathy, ptosis, and impaired accommodation of the eye

Rare – severe hypertension and hypotension, anaphylaxis, angioedema, disseminated intravascular coagulation (DIC), bronchospasm, seizures, tachyarrhythmia, angina, cardiac ischemia/infarction, cardiac or respiratory arrest, acute vascular leak syndrome, rash with desquamation, stomatitis, papilledema, photophobia, optic atrophy, central catheter thrombosis, pericardial effusion and dyspnea.

Dosage and route of administration: see section 4.2 and 4.7.2.1.

5.4 Interleukin-2 (IL-2, Aldesleukin, Proleukin®)

Source and pharmacology: Aldesleukin is a biosynthetic cytokine (a lymphokine) of recombinant DNA origin. It differs from human interleukin-2 by the absence of an N-terminal alanine, the replacement of cysteine with serine at position 125 of the sequence, and the absence of glycosylation. It is a biologic response modifier with complex antineoplastic and immunomodulating activities.
Formulation and stability: Aldesleukin vials contain 22 million units of lyophilized recombinant interleukin. Each single use vial is reconstituted with 1.2 ml of sterile water for injection to give 18 million units/ml. Aldesleukin should be admixed with D5W for infusion, with albumin 0.1% added to decrease adsorption. Do not use an in-line filter. Do not mix in saline.

Supplier: Commercially available, see package insert for more information

Toxicities: Aldesleukin is a highly toxic drug. Most adverse effects are dose related and schedule dependent, with fewer toxicities associated with low dose, subcutaneous or continuous IV infusions as compared to high dose, rapid IV infusions. Most adverse effects are self-limiting and reversible within 2 to 3 days of drug discontinuance. Many of the adverse effects of aldesleukin are related to capillary leak syndrome, which has been associated with this drug. The most frequently reported serious adverse effects include hypotension, renal dysfunction with oliguria/anuria, dyspnea or pulmonary congestion, and mental status changes (lethargy, somnolence, confusion, agitation). Additional serious adverse effects reported include myocardial ischemia, myocarditis, gangrene, respiratory failure leading to intubation, GI bleeding, intestinal perforation, ileus, coma, seizures, sepsis, and renal impairment requiring dialysis. Most patients receiving aldesleukin develop some degree of a flu-like syndrome that may include fever, chills, rigors, fatigue, weakness, malaise, arthralgia and myalgia.

Dosage and route of administration: subcutaneously or intravenously (see treatment plan sections for details).

5.5 GM-CSF (Sargramostim) (Leukine®)

Source and pharmacology: GM-CSF (granulocyte macrophage colony stimulating factor) is a biosynthetic hematopoietic agent prepared using recombinant DNA technology and a yeast expression system that utilizes *Saccharomyces cerevisiae.* It stimulates proliferation, differentiation and functional activity of neutrophils, eosinophils, monocytes and

macrophages.

Formulation and stability: GM-CSF is available in vials containing 250 mcg and 500 mcg of GM-CSF as a lyophilized powder and 40 mg of mannitol. The intact vials should be stored under refrigeration and protected from freezing. The vials can be reconstituted with 1 ml of either sterile water for injection or bacteriostatic water for injection to give a final concentration of 250 mcg/ml and 500 mcg/ml respectively. If reconstituted with sterile water (containing no preservative), the solution should be used within 6 hours of preparation. If reconstituted with bacteriostatic water for injection, the solution may be stored for up to 20 days under refrigeration. Do not shake the vial to assist in dissolution of the drug. GM-CSF can be further diluted in 0.9% NaCl for IV administration. If the final concentration of GM-CSF is < 10 mcg/ml, albumin at a final concentration of 0.1% should be added to the saline prior to addition of the GM-CSF.

Supplier: Commercially available, see package insert for more information.
Toxicity: GM-CSF causes marked leukocytosis, eosinophilia, and monocytosis. Adverse reactions reported commonly include bone pain, diarrhea, nausea, rash, alopecia, anorexia and pain or bruising at the injection site. A flu-like syndrome including headache, muscle aches, fever and chills is also common. Edema, capillary leak, pleural and pericardial effusions have all been reported. Allergic reactions and reversible supraventricular arrhythmias have been reported rarely.

Dosage and route of administration: Beginning at least 24-hr after last dose of chemotherapy at 250 mcg/m^2/day subcutaneously, and continued daily through the nadir until ANC ≥ 2000/mm^3 (See Treatment Plan Section).

5.6 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, see package insert for more information

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 participants with allergy to E. coli derived products.

Dosage and route of administration: G-CSF at a dose of 5 mcg/kg/day will be added to GM-CSF only for course in which PBSCH will be done.

5.7 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. Diluted solutions are physically and chemically stable for at least 24 hours under refrigeration.

Supplier: Commercially available, see package insert for more information

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: IV infusion, after cyclophosphamide.

5.8 DOXOrubicin (Adriamycin®)

Source and pharmacology: Doxorubicin is an anthracycline antibiotic produced by *Streptomyces peucetius*. Doxorubicin exerts its anti-tumor effects in several different ways. Doxorubicin intercalates between base pairs of DNA causing steric obstruction, disruption of DNA function and inhibition of RNA synthesis. In addition, doxorubicin inhibits topoisomerase II, an enzyme responsible for allowing strands of DNA to pass through one another as they unwind. Lastly, doxorubicin undergoes enzymatic electron reduction to generate highly reactive species, including the hydroxyl free radical, which is thought to be responsible for the drug's cardiac toxicity, but may play a role in its anti-tumor activity as well. Doxorubicin is cell-cycle, phase non-specific. Doxorubicin is widely distributed in the tissues and plasma, but does not cross the blood brain barrier to an appreciable extent. It is metabolized to doxorubicinol, which is thought to be the major active metabolite, and aglycones. Doxorubicin and its metabolites are excreted mainly in the bile and feces (»80%). The remainder is excreted in the urine. Dosage should be reduced in patients with liver dysfunction (bilirubin > 1.2 mg/dl) or renal dysfunction (creatinine > 3 mg/dl).

Formulation and stability: Doxorubicin is available in vials containing 10 mg, 20 mg, 50 mg and 200 mg as a 2mg/ml red-orange solution. It is also available in vials containing 10 mg, 20 mg, 50 mg, 100 mg and 150 mg of doxorubicin as a red-orange lyophilized powder. Intact vials of doxorubicin solution should be stored under refrigeration while the lyophilized product should be stored at room temperature. Both products should be protected from light. Lyophilized doxorubicin can be reconstituted by adding 5, 10, 25, 50 or 75 ml of 0.9% NaCl respectively to the 10, 20, 50, 100 and 150 mg vials to produce a final concentration of 2 mg/ml. Bacteriostatic diluents are not recommended. After reconstitution, the resultant solution should be protected from light and is stable for 7 days at room temperature and 15 days if refrigerated.

Supplier: Commercially available, see package insert for more information

Toxicity: Dose-limiting toxicities include myelosuppression and cardiotoxicity. Two forms of cardiac toxicity can occur. Acute toxicity may take the form of arrhythmias, heart block or pericarditis and may be fatal. The chronic form of cardiotoxicity is related to total cumulative dose and

is characterized by heart failure. Mediastinal radiotherapy and/or other cardiotoxic drugs may increase the risk of cardiotoxicity. In general, total lifetime dosages of 450-550mg/m^2 should not be exceeded. Other toxicities include nausea and vomiting, mucositis, alopecia, diarrhea and red discoloration of the urine and other body fluids. Severe tissue damage and necrosis can occur upon extravasation. Radiation recall reactions can occur and can be severe. Rarely, allergic reactions have occurred. Typhilitis can occur when combined with cytarabine.

Dosage and route of administration: 25 mg/m^2/dose; if ≤ 12 kg. 0.83 mg/kg/dose.

5.9 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 participants 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, see package insert for more information.

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 bronchospasm has 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. NOTE: dose reduction may be necessary in participants < 1 year of age. Dosing on a per kg (rather than per m^2) basis has been advocated for infants in order to decrease toxicity.

Dosage and route of administration: Participants < 12 months of age 0.017 mg/kg/dose; ≥12 months; 0.67 mg/m^2/dose or 0.022 mg/kg/dose-*whichever is lower).* The total dose may not exceed 2 mg in 72 hours or 0.67 mg/day for any patient.

5.10 Etoposide (VP-16) (Vepesid®)

Source and pharmacology: Etoposide is an epipodophyllotoxin derived from *Podophyllum pelatatum.* 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, see package insert for more information

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: 200 mg/m^2/dose; participants ≤ 12 kg, 6.67 mg/kg/dose.

5.11 Etoposide Phosphate (Etopophos®)

To be used in case of etoposide reactions.

Source and pharmacology: Etoposide is an epipodophylotoxin derived from *Podophyllum pelatatum.* 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 participants with liver dysfunction, kidney dysfunction or hypoalbuminemia.

Formulation and stability: Etoposide phosphate is a water-soluble ester of etoposide. The higher water solubility of etoposide phosphate than that of etoposide lessens the potential for precipitation following dilution and during administration. Etoposide phosphate is available in single-dose vials containing etoposide phosphate equivalent to 100mg etoposide. The intact vials of etoposide solution should be stored at 2 to 8 degrees Celsius. Etoposide phosphate solution should be diluted in D5W or 0.9% NaCl prior to administration. Solution is stable at room temperature for 24 hours.

Supplier: Commercially available, see package insert for more information.

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: Used in substitution for etoposide in participants that experience allergic reaction.

5.12 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, see package insert for more information

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 > 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: 50 mg/m^2/dose; participants ≤ 12 kg, 1.66 mg/kg/dose.

5.13 Busulfan Injection (Busulfex®)

Source and pharmacology: Busulfan is a non-cell cycle specific bifunctional alkylating agent. In aqueous media, busulfan hydrolyzes to release methanesulfonate groups. This produces reactive carbonium ions that interact with cellular thiol groups and nucleic acids to form DNA cross-links. Busulfan injection is 100% bioavailable by definition of intravenous administration. The elimination of busulfan appears to be independent of renal function, presumably reflecting the extensive metabolism of the drug in the liver, since less than 2% of the administered dose is excreted in the urine unchanged within 24 hours. The drug is metabolized by enzymatic activity to at least 12 metabolites, among which tetrahydrothiophene, tetrahydrothiophene 12-oxide, sulfolane, and 3-hydroxysulfolane were identified. These metabolites do

not have cytotoxic activity. Irreversible binding to plasma proteins (primarily albumin) is approximately 32.4%. Busulfan has a plasma terminal elimination half-life (t1/2) of about 2.6 hours and demonstrates linear kinetics. It is rapidly distributed into tissue and crosses the blood-brain and the placental barriers. CSF concentrations are approximately equal to those in plasma. Itraconazole reduced busulfan clearance by up to 25% in patients receiving itraconazole compared to patients who did not receive itraconazole. Higher busulfan exposure due to concomitant itraconazole could lead to toxic plasma levels in some patients. Fluconazole had no effect on the clearance of busulfan.

Formulation and stability: Each ampoule of busulfan injection contains 60 mg (6 mg/mL) of busulfan, N,N-dimethylacetamide (DMA) 33% vol/vol and Polyethylene Glycol 400, 67% vol/vol. Store refrigerated at 2°-8°C, (36°-46°F).

Supplier: Commercially available, see package insert for more information.

Toxicities: Nausea, vomiting, fever, electrolyte, changes (hypokalemia, hypomagnesemia, hypocalcemia, hypophosphatemia, and hyponatremia), hyperglycemia, dizziness, rash, pruritus, urticaria, injection site pain and inflammation, back pain, tachycardia, chest pain, edema, insomnia, anxiety, depression, headache, abdominal pain, diarrhea or constipation, anorexia, rectal discomfort, dyspnea, epistaxis, weight gain, confusion, seizures (rare with phenytoin prophylaxis), hematemesis, hyperuricemia, arrhythmias other than tachycardia, pleural effusion, alveolar hemorrhage, myelosuppression, asthenia, immunosuppression (L), mucositis, hyperbilirubinemia, hepatotoxicity, sinusoidal obstruction syndrome (SOS, formerly VOD), mild alopecia (L), arthralgia, myalgia, hemorrhagic cystitis, hyperpigmentation, elevated creatinine and BUN reduced adrenal function, esophagitis, and radiation recall reactions.
Guidelines for administration: Dilute busulfan injection to a final concentration of approximately 0.5 mg/mL with 0.9% sodium chloride or 5% dextrose in water. The drug should not be infused with any other drug or IV solution other than 0.9% sodium chloride or 5% dextrose. Only the 5-micron filter provided should be used to prepare the dose, using one filter per ampoule. (If using the enclosed syringe filter in the forward flow direction, the calculated volume of busulfan injection should allow for approximately 0.16 mL of residual busulfan in the filter.) Always add the busulfan to the diluent, not the diluent to the busulfan injection. Mix thoroughly by inverting several times. Do not use polycarbonate syringes or filter needles with busulfan injection. Busulfan injection diluted in 0.9% Sodium Chloride Injection, *USP,* or 5% Dextrose Injection, *USP,* is stable at room temperature (25°C) for up to 8 hours but the infusion must be

completed within that time. Busulfan injection diluted in 0.9% Sodium Chloride Injection, *USP*, is stable at refrigerated conditions 2°–8°C (36°–46°F) for up to 12 hours but the infusion must be completed within that time. Busulfan injection should be administered by IV infusion through a central venous catheter. Patients receiving busulfan in a conditioning regimen for bone marrow transplant should receive anticonvulsant prophylaxis to prevent seizures. Anticonvulsants should be given 12–24 hours prior to the start of busulfan, then daily during busulfan administration and for 48 hours after completion of busulfan. After an initial dose of busulfan injection, blood levels are monitored with bone marrow transplant patients in order to achieve a target area-under-the-curve (AUC) plasma concentration.

5.14 Melphalan (L-phenylalanine mustard, phenylalanine mustard, L-PAM, L-sarcolysin, Alkeran®)

Source and pharmacology: Melphalan, a phenylalanine derivative of nitrogen mustard, is a bifunctional alkylating agent. Melphalan forms covalent cross-links with DNA or DNA protein complexes thereby resulting in cytotoxic, mutagenic, and carcinogenic effects. The end result of the alkylation process results in the misreading of the DNA code and the inhibition of DNA, RNA, and protein synthesis in rapidly proliferating tumor cells. It is cell cycle non-specific. After IV administration, melphalan plasma concentrations decline rapidly in a bi-exponential manner with distribution phase and terminal elimination phase half-lives of approximately10 and 75 minutes, respectively. Plasma melphalan levels are highly variable after oral dosing, both with respect to the time of the first appearance of melphalan in plasma (range approximately 0 to 6 hours) and to the peak plasma concentration achieved. These results may be due to incomplete intestinal absorption, a variable "first pass" hepatic metabolism, or to rapid hydrolysis. The oral dose averages 61% ± 26% of that following IV administration. The terminal elimination plasma half-life of oral melphalan is 1.5 ± 0.83 hours. The steady-state volume of distribution of melphalan is 0.5 L/kg. The extent of melphalan binding to plasma proteins ranges from 60–90%. Melphalan is eliminated from plasma primarily by chemical hydrolysis to monohydroxymelphalan and dihydroxymelphalan. The 24-hour urinary excretion of parent drug is approximately 10% suggesting that renal clearance is not a major route of elimination of parent drug. Penetration into CSF is low. Despite the fact that the contribution of renal elimination to melphalan clearance appears to be low, one pharmacokinetic study suggests dosage may need to be reduced in patients with renal impairment.
Formulation and stability: Melphalan for Injection is supplied as a sterile, nonpyrogenic, freeze-dried powder. Each single-use vial contains

melphalan hydrochloride equivalent to 50 mg melphalan and 20 mg povidone. Melphalan for Injection is reconstituted using the sterile diluent provided. Each vial of sterile diluent contains sodium citrate 0.2 g, propylene glycol 6.0 mL, ethanol (96%) 0.52 mL, and Water for Injection to a total of 10 mL. Store at controlled room temperature 15°–30°C (59°–86°F) and protect from light.

- Reconstitute to a concentration of 5 mg/mL by rapidly injecting 10 mL of the supplied diluent directly into the vial of lyophilized powder using a sterile needle (20–gauge or larger needle diameter) and syringe. Immediately shake vial vigorously until a clear solution is obtained. Rapid addition of the diluent followed by immediate vigorous shaking is important for proper dissolution.
- Immediately dilute the dose to be administered in NS to a final concentration not to exceed 2 mg/mL for IV central line administration or 0.45 mg/mL for peripheral IV administration
- A precipitate forms if the reconstituted solution is stored at 5°C. Do not refrigerate the reconstituted product. (The time between reconstitution/dilution and administration of melphalan should be kept to a minimum because reconstituted and diluted solutions of melphalan are unstable. Over as short a time as 30 minutes, a citrate derivative of melphalan has been detected in reconstituted material from the reaction of melphalan with the sterile diluent for melphalan. Upon further dilution with saline, nearly 1% label strength of melphalan hydrolyzes every 10 minutes).

Toxicity: Anorexia, nausea, vomiting, hyponatremia (high dose) anaphylaxis, hypotension, diaphoresis, pruritus atrial fibrillation (high dose), extravasation (rare) but if occurs= local ulceration, SIADH, seizures, myelosuppression, mucositis, diarrhea, alopecia, abnormal liver function tests, jaundice, hepatitis.

Supplier: Commercially available, see package insert for more information.

Guidelines for administration: administer by IV infusion through a peripheral or a central line. Complete infusion within 1 hour of product reconstitution.

5.15 Levetiracetam (Keppra®)

Source and pharmacology: KEPPRA is an antiepileptic drug. The chemical name of levetiracetam, a single enantiomer, is (–)–(S)–α–ethyl–2–oxo–1–pyrrolidine acetamide, its molecular formula is C8H14N2O2 and its molecular weight is 170.21. Levetiracetam is chemically unrelated to

existing antiepileptic drugs (AEDs). It is rapidly and extensively absorbed orally, and is primarily eliminated in the urine unchanged.

Formulation and stability: Levetiracetam is a white to off-white crystalline powder with a faint odor and a bitter taste. It is very soluble in water (104.0 g/100 mL). It is freely soluble in chloroform (65.3 g/100 mL) and in methanol (53.6 g/100 mL), soluble in ethanol (16.5 g/100 mL), sparingly soluble in acetonitrile (5.7 g/100 mL) and practically insoluble in n-hexane. (Solubility limits are expressed as g/100 mL solvent.)

Available as 250 mg (blue), 500 mg (yellow), 750 (orange) and 1000 mg (white) tablets and as a clear, colorless, grape-flavored liquid (100 mg/mL) for oral administration, or as an intravenous solution (500 mg/5 mL), which must be diluted prior to administration.

Store at 25°C (77°F); excursions permitted to 15-30°C (59-86°F) [see USP Controlled Room Temperature].

Supplier: Commercially available, see package insert for more information.

Toxicity: The most common adverse events are asthenia, headache, infection, pain, anorexia, somnolence, dizziness, depression, nervousness, ataxia, vertigo, amnesia, hostility, paresthesias, emotional labiality, pharyngitis, rhinitis, increased cough, sinusitis, and diplopia. See package insert for additional information.

Dosage and administration: See protocol section 4.7.

5.16 13-cis-Retinoic Acid (Isotretinoin, Accutane®)

Source and pharmacology: The exact mechanism of retinoic acid-induced maturation of tumor cells is not known. Isotretinoin is classified as a retinoid, which is a synthetic analogue of vitamin A. The principal pharmacologic effect of isotretinoin appears to be regulation of cell proliferation and differentiation. The drug also affects the function of monocytes and lymphocytes, resulting in modulation of cellular immune responses. The bioavailability of orally administered isotretinoin has not been determined in humans, but studies in animals indicate that about 25% of an oral dose of the drug reaches systemic circulation as unchanged isotretinoin. Food and/or milk increase GI absorption of isotretinoin. Distribution of isotretinoin into human body tissues and fluids has not been fully characterized. The elimination half-life of the parent compound is 10-20 hours. Isotretinoin is metabolized in the liver

principally via oxidation to 4-oxo-isotretinoin; it is not known if this metabolite is pharmacologically active. Empirical dose reductions are indicated in hepatic disease due to extensive metabolism of the drug by the liver.

Formulation and stability: Isotretinoin is available for oral administration in liquid-filled capsules in the following strengths: 10 mg, 20 mg & 40 mg. Store the medicine at room temperature, away from heat and direct light.

Supplier: Commercially available, see package insert for more information.

Cautions: Isotretinoin is a known human teratogen, and can cause severe, life-threatening birth defects or fetal death if taken during pregnancy. Therefore, isotretinoin has been labeled as being contraindicated during pregnancy, with warnings that use in women of childbearing potential be initiated only after advising them of the teratogenic risks, excluding the possibility of pregnancy, and ensuring the use of an effective means of contraception during isotretinoin therapy.
Toxicity: Reported adverse reactions include: dry mouth and nose, dry eyes, depression and suicidal thoughts, changes in vision, decreased night vision, blurred vision, headache, unusual tiredness, alopecia, photosensitivity, skin fragility, dry skin, chelitis, arthralgia, nausea or vomiting, inflammatory bowel syndrome, leukopenia, neutropenia, hepatotoxicity, hypertriglyceridemia and hyperglycemia.

Dosage and route of administration: Administer orally 160 mg/m^2/day po divided bid for 14 days. For patients' ≤ 12 kg, dose is 5.33 mg/kg/day, in two divided doses for 14 days. Round up to the nearest 10 mg.

5.17 Guidance during Times of Drug Shortages and Unavailability

Treating investigators are urged to consult with the PI or co-PI and use their best clinical judgment in optimizing therapeutic intent and ensuring patient safety in managing the protocol-specified therapy. Although these decisions may constitute "protocol violations," they are unavoidable and made in consideration of the best interest of an individual patient. These will not be considered monitoring/audit findings if appropriately documented. All protocol deviations must be noted in the research database and the alterations in therapy due to the agent shortage will be captured. This should be accomplished by entering "dose modified" and details noted in the comments field. These deviations will also be noted in the Deviation Log with the notation "Drug substitution/reduction due to unavoidable drug shortage or unavailability".

6.0 PBSC COLLECTION AND ADMINISTRATION

6.1 Eligibility

All St. Jude participants enrolled on the protocol will be presented at the weekly transplant new patient meeting. The presentation should be done at the meeting following enrollment and include a brief medical history.

6.2 Evaluation

Pre-evaluation for the collection procedure shall include the following:

- Review of the pretreatment evaluation by a member of the transplant staff.
- Participant visit to the donor room for the purpose of explaining the procedure, reviewing infectious disease testing results, assess adequacy of venous access, completion of the consent form for the procedure, and a signed physician order to initiate the PBSC collection.

6.3 Mobilization and Collection of PBSC

Although collections can be performed after any course of induction therapy, patients will typically be monitored on or after course 2. During this course G-CSF at a dose of 5 mcg/kg/day will be added to the daily GM-CSF. Collection of PBSC will begin once their absolute peripheral blood $CD34^+$ number is greater than or equal to 15/mL. If this is unsuccessful, harvesting will be done with subsequent chemotherapy courses in consultation with transplant staff. Patients who do not meet Blood Donor Center criteria for apheresis (i.e., low body weight) will not be monitored and will have a bone marrow harvest performed.

- G-CSF is to be continued until all the PBSC collections are complete for that course.
- WBC count prior to beginning the procedure after initiation of G-CSF should not exceed 75,000/µL.
- Each leukapheresis will have the goal of processing three to four times the patient's blood volume.
- If an inadequate product is obtained after the first day's pheresis, additional days of pheresis may be performed.

6.4 PBSC Specimen Collection

Once monitoring for PBSC collection has commenced (timing in consultation with Transplant Coordinator), collect the following specimens prior to the daily administration of G-CSF (after day 1 of G-CSF) when ordered by the transplant staff:

- Peripheral blood for CD 34^+ cell analysis. Collect 5 ml of whole blood in green top tube and notify Transplant Coordinator or Human Applications Laboratory (ext. 2920).
- Complete blood count and differential.

6.5 PBSC Processing and Storage

- PBSCs are to be processed with or without purging and stored as per standard operating procedures of the Human Applications Laboratory.
- PBSCs are to be stored in aliquots defined according to Human Applications Laboratory policies and procedures.
- If PBSCs are unable to be collected, a bone marrow harvest will be performed.

7.0 SURGICAL GUIDELINES

The ultimate goal of surgery is gross total resection of residual tumor in the primary site as well as tumor in areas of regional dissemination (usually lymph nodes). Resection with microscopically negative margins may not be feasible because of proximity to major vascular structures and the spine. Instead, the surgeon should concentrate on removing, as completely as possible, all gross disease. It is acceptable, and often necessary, to incise the tumor and remove it in a segmental fashion. All attempts should be made to preserve organs, especially the kidney.

7.1 Initial Surgery

The primary purpose of the first surgical procedure is to establish the diagnosis and stage, and obtain tissue for biological studies. A diagnosis frequently can be made by bone marrow evaluation; occasionally a needle or open biopsy of the most accessible site will be required. Biopsy of either the primary tumor or an accessible metastatic site is acceptable. It may also be feasible and efficient to place a vascular access device and obtain a bone marrow aspiration and biopsy during the same anesthetic. Complete excision of the primary tumor can occasionally be performed if the tumor is easily resectable without a lengthy procedure or extensive dissection. A resection should not be undertaken if it would result in a serious delay in the initiation of chemotherapy or great morbidity, however. The great majority of high-risk patients will undergo an initial diagnostic biopsy without resection. The goal of the biopsy procedure is to obtain enough tissue for a histopathologic diagnosis as well as *MYCN* determination, cytogenetics, and other biological studies. The surgeon should try to obtain at least one cm^3 of viable tumor tissue, if feasible.

7.2 Delayed Resection

Surgical resection should be performed when, in the judgment of the operating surgeon, the primary tumor and regional disease can be completely resected based on the radiographic appearance (CT scan and/or MRI) of the tumor. Tumors will be considered unresectable if there is significant involvement of major vascular structures or contiguous organs, or if it would likely require a nephrectomy to remove all gross disease. In addition, at the time of surgery the ANC should be $\geq$ 500/µL and the patient medically stable. Surgical resection may be performed at any time during the treatment course when these conditions have been met, but should occur prior to Consolidation therapy, even if the tumor appears to remain unresectable. In these cases, tumor debulking will be performed with every attempt being made to remove the primary site. It is expected that approximately two-thirds of the resections will be performed prior to the initiation of Induction therapy. However, if four patients have a delay of greater than 14 days in the re-initiation of their chemotherapy due to a complication of the surgical procedure, the remainder of the patients on this protocol will undergo their delayed resection following completion of Induction therapy, regardless of when, radiographically, their tumor appears to be resectable. In addition, if one patient suffers a surgically related mortality, the remainder of the patients on this protocol will undergo their delayed resection following completion of Induction therapy.

8.0 RADIATION THERAPY GUIDELINES

Participants with high-risk neuroblastoma will receive conformal radiotherapy (IMRT preferred for abdomino-pelvic primary sites) with application of evidence for organ/target motion from a 4DCT dataset. All patients will undergo a treatment planning CT & 4DCT in treatment position. Tumor volumes and proximally appropriate normal tissues will be contoured, accounting for physiologic motion as measured by 4D imaging data.

The primary site will be irradiated even if complete resection is achieved. Given the excellent local control in an institutional retrospective series with IMRT for high risk abdominal neuroblastoma, 2340 cGy will be delivered to all patients, and those with macroscopic residual after induction therapy will receive an additional 720 cGy for a total dose of 3060 cGy. Radiation therapy to the primary and bulky nodal disease sites will follow peripheral blood stem cell transplant. External beam radiotherapy may be delivered to select metastatic sites as outlined below, but is not required and left to physician discretion. Emergent palliation of metastases will not preclude protocol eligibility.

8.1 Guidelines and Requirements for the Use of IMRT or Proton Beam Radiation Therapy

8.1.1 Timing and Duration of Irradiation

Radiotherapy should be initiated within 42 days of stem cell re-infusion. However, patient specific factors may dictate variations from these recommendations; organ toxicity within potential radiation fields should be resolved. For patients with microscopic residual, a total dose of 2340 cGy will be delivered in 13 fractions of 180 cGy each. The duration of the RT treatment plan will be 2 weeks and 3 days. Patients with gross residual disease will receive 3060 cGy in 17 fractions of 180 cGy each, requiring 3 weeks and 2 days for delivery.

8.1.2 Equipment

Modality: X-rays with a nominal energy of ≥ 4 MV and ≤ 6MV or MeV proton beams are allowed on this trial. In the unusual circumstance of a superficial lesion, electron fields may be used. Conventional conformal and IMRT photon techniques as well as proton (scanned single field uniform dose [SFUD] and intensity modulated proton therapy [IMPT]) techniques are allowed in this study.

CT treatment planning: All patients will undergo CT-based treatment

planning for this protocol. Slices no more than 5 mm thick (2–3 mm is recommended) shall be taken throughout the extent of the irradiated volume.

Immobilization: Immobilization devices are used for daily positioning for treatment. Alpha cradle, vac-loc bags, skin demarcation and thermoplastic systems are to be used at the discretion of the treating radiation oncologist. Either cone-beam MV CT, kV in-beam line imaging or some other method of imaging localization is highly recommended to verify accuracy of treatment positioning prior to the delivery of each fraction, particularly for IMRT.

Field shaping: Field shaping shall be done with either customized cerrobend blocking or multileaf collimation.

8.1.3 Techniques

Only external beam radiotherapy techniques are allowed on this study. Aside from CT-based beam's eye view planning, MR simulation or fusion of MR datasets (diffusion weighted imaging, MP-RAGE and other gradient echo T1 techniques), with and without contrast, are highly recommended for definition of normal tissues and surrounding vasculature, draining nodal regions and tumor bed/tumor residual. All treatment fields will be irradiated daily and weekly quality assurance for target localization is required. In-beam line imaging CT may be used at the option of the treating radiation oncologist to verify target and avoidance structure position. The treating radiation oncologist may choose to perform localization verification more frequently depending on the requirements of treatment for a specific case.

For proton beam radiation single or multi field techniques using apertures or other beam shaping devices are allowed. Daily imaging with electronic portal imaging or cone-beam imaging is required and all fields will be treated daily.

8.1.4 4-DCT Acquisition & Volume Definition

8.1.4.1 Technique

Baseline simulation and 4D imaging should occur within a 30 day window prior to start of radiation, however, various scheduling or patient specific factors may require variation from this recommendation and delays in starting radiotherapy will not be considered a deviation. At that time, positioning will be aided by a behind-the-knee block which is designed to flatten the lumbo-sacral spine against the treatment table. Skin demarcation near the level of the xiphoid process and at the anterior superior iliac spine are highly recommended in order to enhance verification of accurate positioning. The CT scan will obtain axial images through the entire post-induction, pre-surgical extent of tumor with sufficient margin to account for loco-regional extension of disease noted at the time of surgery. Additionally, a 4DCT scan will be performed to assess physiologic motion. MRI should be obtained at the time of simulation on either an MRI simulator or a diagnostic MRI scanner, and should be utilized for fusion with the planning CT dataset.

If a child requires anesthesia for the procedure, the same requirements for data acquisition should be met, with the understanding that minimal diaphragmatic excursion will likely be obtained. Patients on therapy are expected to receive general anesthesia with diprivan without requiring airway management in most instances. Notation of anesthesia during

simulation and therapy must be applied to the digital/paper record associated with the 4DCT. If a child is borderline for anesthesia requirements, necessitating simulation with sedation, they must continue the entire course with general anesthesia.

8.1.4.2 Treatment sites and dose

Site or volume (see section 8.1.4.3–8.1.4.6)	Dose (see Section 8.3)
Primary tumor site (PTV1)	23.4 Gy
Boost Site for residual primary tumor after induction/surgery (PTV2)	7.2 Gy
Metastatic disease	23.4 Gy
Emergent treatment for organ or life-threatening disease	Contact Study RT Coordinators

8.1.4.3 Volume definitions

Target volumes will be defined according to International Commission on Radiation Units and Measurements (www.icru.org) Report 50 and 62 definitions for gross tumor volume (GTV), clinical target volume (CTV), and planning target volume (PTV). One exception is the GTV that may be defined as the gross palpable or visible/demonstrable extent of residual tumor *and/or* tumor bed. This differs from the ICRU 50 definitions where the GTV defines only the gross residual tumor. The CTV is an anatomically confined tissue margin that contains the GTV and is intended to treat subclinical microscopic disease. The PTV is a geometrical expansion of the CTV in three dimensions and is not anatomically confined. The purpose of the PTV is to account for the net effect of all uncertainty in immobilization and daily variability in patient positioning. For this study, the PTV margin will be institutionally defined.

The GTV, CTV, and PTV as well as pertinent adjacent normal tissue structures including heart, lungs, thyroid, kidneys, liver, spleen, bowel, bladder and spinal cord will be delineated for each patient's primary site tumor on a treatment planning imaging study (CT or MRI) prior to undergoing treatment.

Physiologic motion of normal tissues and target volumes is critical, particularly for targets in close proximity to the diaphragm. The internal target volume (ITV) is defined as the CTV surrounded by the internal margin (IM). The IM, which commonly encompasses the CTV asymmetrically, is intended to compensate for all movements and variations in site, size and shape of the organs and tissues contained in or adjacent to the CTV. The planning organ at risk volume (PRV) includes the organ at risk surrounded by a margin to compensate for physiologic change in the target volume. Estimates of margins necessary to construct ITVs and PRVs should be obtained from baseline CT and MRI studies.

For metastatic sites similar target volume definitions are utilized. The

appropriate margins for these volumes are outlined as follows.

8.1.4.4 Target volumes

Although the post-surgical CT is used for treatment planning, the target volume is based on the extent of disease on post-induction, pre-operative CT, with modifications noted below, regardless of extent and timing of the surgical resection or response to chemotherapy. If the primary tumor was grossly resected at diagnosis, radiation therapy should be given to the primary site based on the initial diagnostic tumor volume. No pelvic irradiation should be given prior to bone marrow/PBSC harvest. The GTV, CTV and PTV and normal tissues must be outlined on all CT slices in which the structures exist. Beam's eye view display must be used to design beam apertures.

8.1.4.5 Primary site

Gross tumor volume 1 (GTV1)

- The GTV1 is the volume of tissue containing the highest concentration of residual tumor cells.
- The GTV1 includes disease defined by CT, MR and MIBG imaging PRIOR to surgery.
- The GTV1 includes disease (tumor and lymph nodes) identified intra-operatively.
- The GTV1 is corrected volumetrically after surgical resection but not at the point of attachment.
- The GTV1 does NOT include the extent of disease PRIOR to chemotherapy.
- The GTV1 does NOT include uninvolved draining lymph node regions.

Special circumstances (GTV1)

- If the primary tumor was grossly resected at diagnosis, GTV1 will be based on the initial diagnostic tumor volume.
- In cases where there is discrepancy between imaging studies or intra-operative findings, the larger volume will define GTV1.
- When the primary tumor expands into a body cavity such as the lung or displaces a normal structure such as the liver without infiltration, if following surgical resection the normal structure now occupies the space previously occupied by tumor, normal tissue volume should not be included within GTV1.

Gross tumor volume 2 (GTV2)

- The GTV2 is defined as the volume of residual tumor AFTER induction surgery and chemotherapy measuring >1cm^3
- The GTV2 includes disease defined by CT, MR and MIBG imaging.

Special circumstances (GTV2)
- GTV2 will NOT be altered even when there is a complete response after consolidative chemotherapy.

Clinical target volume 1 (CTV1)
The CTV is defined as the volume of tissue containing subclinical microscopic disease:
- The CTV1 margin should be an expansion of the GTV1 to encompass microscopic disease.
- The CTV1 for this protocol is the GTV1 with an anatomically confined margin of 1.5 cm.
- The CTV1 should be tailored at tissue interfaces where invasion/infiltration is not likely.

Clinical target volume 2 (CTV2)
- The CTV is defined as the volume of tissue containing subclinical microscopic disease surrounding the post-surgical residual tumor (GTV2).
- The CTV2 margin should be an expansion of the GTV2 to encompass microscopic disease.
- The CTV2 for this protocol is the GTV2 with an anatomically confined margin of 1.0 cm.
- The CTV2 should be tailored at tissue interfaces where invasion/infiltration is not likely.

Internal target volume 1/2 (ITV1/2)
- The ITV is defined as the CTV surrounded by the internal margin (IM).
- The IM, which commonly encompasses the CTV asymmetrically, is intended to compensate for all movements and variations in site, size and shape of the organs and tissues contained in or adjacent to the CTV.

Planning target volume (PTV1)
- The PTV1 is a geometric concept and includes a margin surrounding the CTV1 and ITV1.
- The PTV1 should account for physiologic change or motion in the CTV1 and set-up uncertainty.
- The PTV1 is defined as the CTV1 with a geometric margin of 0.5-1.0 cm.

- The PTV1 may vary depending on immobilization and cooperation; 0.5cm is the minimum extent of the margin surrounding CTV1 to form PTV1.
- The PTV1 margin does not have to be uniform in all dimensions; especially if it compromises normal tissue volumes or if directional target or normal tissue motion is assessed and understood.
- For proton planning, PTVs may be eliminated if other methods of assuring set-up in the context of daily uncertainties are employed (such as robustness calculations).

Planning target volume (PTV2)

- The PTV2 is a geometric concept and includes a margin surrounding the CTV2 and ITV2.
- The PTV2 should account for physiologic change or motion in the CTV2 and set-up uncertainty.
- The PTV2 is defined as the CTV2 with a geometric margin of 0.5-1.0 cm.
- The PTV2 may vary depending on immobilization and cooperation; 0.5 cm is the minimum extent of the margin surrounding CTV2 to form PTV2.
- The PTV2 margin does not have to be uniform in all dimensions; especially if it compromises normal tissue volumes or if directional target or normal tissue motion is assessed and understood.
- For proton planning, PTVs may be eliminated if other methods of assuring set-up in the context of daily uncertainties are employed (such as robustness calculations).

8.1.4.6 Metastatic sites

Only metastatic sites of disease active prior to consolidation with stem cell transplant defined by visibility on MIBG, FDG based PET imaging (if done), CT, MRI or bone scan will be considered for irradiation. Radiation therapy for specific active metastatic sites may be eliminated due to a large number of active metastases or limited bone marrow reserve at the discretion of the treating radiation oncologist. Alternatively, metastatic site irradiation may be reserved for post-maintenance therapy in cases of persistent disease.

Criteria for treatment of metastases: While the primary site is always irradiated, radiation is only given to those metastatic sites with persistent active disease demonstrated on the pre-ASCR evaluation. Sites that are negative on the pre-ASCT scans will NOT be irradiated, even if they had enhanced uptake on MIBG and/or bone scan at diagnosis.

For participants with >5 MIBG positive metastatic sites on pre-ASCT evaluation

If the participant had> 5 persistently positive MIBG metastatic sites identified after induction, the MIBG scan should be repeated on Day 28+ post-HSCT. Only sites still MIBG positive post-transplant should be considered. If there are still > 5 MIBG positive sites contact Primary Study Investigator to discuss treatment plan prior to starting radiation. Alternatively, metastatic site irradiation may be reserved for post-maintenance therapy in cases of persistent disease.

The planning target volume for metastatic sites is the area of residual tumor defined on MIBG, CT, or MR scan with a 1 cm margin. In cases where there is a discrepancy in volume between the scans, the larger volume will be irradiated. For osseous metastases, the margin need not extend more than 1 cm outside the bone or across a joint space.

8.2 Normal Tissue Volumes

The following normal tissues should be delineated based on the location of the primary tumor. Delineation of other normal tissues is at the discretion of the radiation oncologist:

Neck/thorax	Abdomen	Pelvis
thyroid, heart, lungs, spinal cord	liver, spleen, kidneys, spinal cord	bladder, bowel, spinal cord

8.3 Target Dose and Fractionation

Prescribed dose and fractionation:

Nominal dose by site	Target volume	Dose/fraction	Number of fractions
Primary site 23.4 Gy	PTV1	1.8Gy	13
Primary site boost 7.2 Gy	PTV2	1.8Gy	4
Metastatic sites 23.4 Gy	MTV	1.8Gy	13

Prescribed dose: If conventional methods are used, the prescription point should be at or near the centroid of the target volume. If 3DCRT, IMRT or proton beam radiation is used, dose should be prescribed to an isodose surface that encompasses the PTV and allows the dose uniformity requirements to be satisfied as noted below.

Dose definition: Dose is to be specified in centigray (cGy)-to-muscle.

Tissue heterogeneity: Heterogeneity corrections are required even when conventional planning methods are used and shall be applied for IMRT in compliance with current guidelines for the use of IMRT in clinical trials.

Prescription dose and fractionation

Primary site: The total dose to the PTV will be 23.4 Gy given in 13 fractions. The patient will be treated with one fraction per day, all fields per day, giving 1.8 Gy per fraction to the prescription volume. For patients undergoing an incomplete surgical resection ($\geq$ 1 cm^3 residual soft tissue density) as assessed at the end of induction scans, a sequential boost of 7.2 Gy will be delivered to the gross-residual volume for a total dose of 30.6 Gy. For proton planning, PTVs may be eliminated if other methods of assuring set-up in the context of daily uncertainties are employed (such as robustness calculations).

Metastatic sites: Sites of persistent active metastatic disease will be irradiated concurrently with the primary site once daily to a total dose of 23.4 Gy given in 13 equal fractions of 1.8 Gy.

Dose uniformity: For 2D treatment, the dose variations shall be within +7%, -5% of the prescription dose. For volume-based treatment plans, the entire PTV should be encompassed within the 95% isodose surface and no more than 10% of the PTV should receive greater than 110% of the prescription dose as evaluated by DVH. For proton planning, PTVs may be eliminated if other methods of assuring set-up in the context of daily uncertainties are employed (such as robustness calculations).

Interruptions, delays and dose modifications: There will be no planned rests or breaks from treatment, and once radiation therapy has been initiated, treatment will not be interrupted except for any life-threatening infection or severe hematologic toxicity defined as ANC < 300/㎖ or platelets less than 40,000/㎖ during the course of treatment. Under these circumstances, radiation therapy shall be delayed until the counts have recovered. Blood product support should be instituted according to institutional/protocol guidelines. The reason for any interruptions greater than 3 treatment days should be recorded in the patient's treatment chart and submitted with the QA documentation. There should be no modifications in dose fractionation due to age or field size. If any area has been previously treated (emergently), care should be taken not to exceed normal tissue tolerance levels.

8.4 Organs at Risk

The organ at risk guidelines in this section are recommendations. If the recommended doses to the organs at risk are exceeded because of target volume coverage requirements notation should be appended to the treatment record documenting the rationale. In most cases, IMRT is envisioned as a suitable treatment method to meet these recommendations and the required target volume coverage guidelines.

Kidneys: The dose to renal structures should be minimized to reduce the risk of chronic renal failure. Both kidneys should always be contoured and designated as left and right kidney, respectively. If the target volume demonstrates laterality, the ipsilateral kidney should receive a mean dose ≤ 1440 cGy and no more than 50% of the ipsilateral kidney should receive a cumulative dose of greater than 1800 cGy. For the contralateral kidney, no more than 50% should receive a dose greater than 800 cGy and no more than 30% should receive a cumulative dose of greater than 1200 cGy.

If the tumor volume does not demonstrate laterality, the kidneys may receive a median dose of 1440 cGy and no more than 50% of the volume of either kidney should receive more than 1800 cGy.

Vertebral bodies: Volumes should be contoured separately and 80% of the total volume should receive prescription dose homogeneity of within +/- 20% to minimize risk of bone growth abnormality. The superior and inferior margins for PTV coverage may be increased if this improves homogeneity within a complete vertebral body bone dose.

Liver: The liver dose should be minimized to reduce the risk of sinusoidal-obstruction syndrome (SOS), formerly veno-occlusive disease. No more than 50% of the liver shall receive a cumulative dose greater than 1800 cGy and no more than 25% of the liver shall receive a dose greater than 2600 cGy.

Pulmonary: When a major portion of both lungs must be treated because of a large intrathoracic tumor volume, effort should be made to minimize the irradiated lung volume. Lung DVH's should be evaluated to determine feasibility of IMRT in superior abdominal primaries. No more than one-third of the entire lung volume shall receive a cumulative dose greater than 1500 cGy.

Cardiac: Heart and left ventricular volumes should be contoured when involved and spared from excess dose, with the whole heart receiving < 2400 cGy and the left heart being completely blocked after doses of 3000

cGy.

Dose limitations: Normal tissues should be maximally shielded as allowed by the target volume. Portions of the target volume may be partially shielded if necessitated by normal tissue tolerance.

8.5 Definitions of Deviation in Protocol Performance

Prescription dose:
Minor deviation: The prescribed dose differs from that in the protocol by between 6% and 10%.
Major deviation: The prescribed dose differs from that in the protocol by more than 10%.

Dose uniformity:
Minor deviation for IMRT treatments: The entire PTV is not encompassed within the isodose surface representing 95% of the prescription dose or more than 10% of the PTV receives more than 110% of the prescription dose.

Volume:
Minor deviation: Margins less than specified or fields excessively large as deemed by the Primary Study Investigator.

Major deviation: A portion of the tumor (GTV) or potentially tumor bearing area (CTV) is not included in the treated volume.

Critical structures:
A minor or major deviation will be assessed at the time of data review (depending on the details of each case) if the critical structure dose limits are exceeded.

For proton therapy the target will be the CTV (no PTV exists for proton beam therapy). We will assess deviations as noted above utilizing robustness assessments available within the planning systems.

9.0 SUPPORTIVE CARE GUIDELINES

These guidelines are provided to help physicians caring for patients treated on this protocol. They are guidelines not protocol requirements. Nothing in these guidelines is intended to supplant the judgment of the treating physician regarding patient management. Institutional policy and custom may dictate other approaches to the management of the areas discussed in this section.

9.1 Anti-Emetics

Secondary to infusion of hu14.18K322A, steroids will NOT be administered as antiemetics

Antiemetic treatment algorithm:

1. For all courses of chemotherapy (EXCEPT CiE) begin with the following:
   - **Ondansetron** 0.15 mg/kg IV Q 8 hours (max dose 8 mg)
   - **Diphenhydramine** 0.5 mg/kg IV (max dose 50 mg) Q 6 – 8 hours prn
   - **Lorazepam** 0.02 – 0.04 mg/kg IV (max dose 2 mg) Q 6 – 8 hours prn
2. For CiE courses begin with the following:
   - **Ondansetron** 0.15 mg/kg IV Q8hrs (Max dose 8 mg)
   - **Diphenhydramine** 0.5 mg/kg IV Q8hr (Max dose 50 mg) – begin 4 hours after 1st dose of ondansetron
   - **Lorazepam** 0.04 mg/kg IV Q8hr (max dose 2 mg)
   - **Aprepitant** 72 mg/m^2 on Day 1 then 46 mg/m^2 on Days 2 and 3.

3. If mild yet persistent N/V, consider the following:
   - Schedule **diphenhydramine** and **lorazepam** around the clock, off-setting each drug by 3-4 hours (participant will then receive an antiemetic every 3 to 4 hours).
   - Increase dose of **diphenhydramine** to 1 mg/kg (max dose 50 mg)
   - Increase dose of **diphenhydramine** to 1 mg/kg and schedule around the clock

4. For uncontrolled emesis, consider the following:
   - Change from ondansetron to **granisetron** 10 mcg/kg/dose IV Q12hr (max dose 1 mg)
   - For participants> 2 years old: **promethazine** 0.25 mg/kg IV Q6 – 8hr (max dose 25 mg) with diphenhydramine "pre-med".
   - **Metoclopramide** 0.1 to 0.5 mg/kg IV Q6 – 8hr with diphenhydramine "pre-med" (***Do NOT use concurrently with promethazine due to the risk of extrapyramidal reactions***).
   - For participants> 12 years old: **scopolamine** if vestibular component to N/V present
   - If compatibility and access allows, consider **diphenhydramine and lorazepam continuous infusion.**

9.2 Other Supportive Care during Chemotherapy Treatment

9.2.1 Antibiotic Prophylaxis

All participants will be placed on trimethoprim-sulfamethoxazole prophylaxis starting with the induction phase at a dose to TMP/SMZ 150/750 mg/m^2 per day in two divided oral doses on Monday, Tuesday, and Wednesday of each week. If a patient cannot tolerate TMP/SMZ due

to allergy, G6PD deficiency or other reasons, another appropriate medication for *Pneumocystis carinii* pneumonia should be used.

9.2.2 Magnesium Levels

If serum magnesium levels drop below normal it is recommended that patients be placed on oral magnesium gluconate (90–150 mg/kg/day) or magnesium oxide (10–30 mg/kg/day) in 2–3 divided doses. Patients NPO for medical or surgical reasons should have magnesium 0.5–1.0 mEq/kg/24 hour added to maintenance IV fluids.

9.2.3 Blood Product Support

All blood products will be irradiated with 1500-3000 cGy. Filters to remove leukocytes should be used to prevent WBC sensitization. CMV-seronegative patients should receive CMV-negative blood products, if possible.

Platelets: Participants will be transfused as necessary with platelets to maintain the platelet count at ³10,000/µL following chemotherapy or as clinically indicated.

Red blood cells: Therapy-induced anemia and reticulocytopenia are expected. Participants will be transfused as necessary with irradiated packed red blood cells to maintain hemoglobin ³ 8.0 g/dL or as clinically indicated. The recommended dose of PRBC's is 10ml/kg.

9.2.4 Management of Febrile Neutropenia

Management of fever in the presence of neutropenia will be according to current institutional guidelines. Hospitalization and treatment with empiric antibiotics will begin when oral temperature is ³38.3°C once or greater than 38.0°C on two or more occasions during a 12-hour period and the absolute neutrophil count is < 500/µL. Selection of antibiotic therapy should be broad-spectrum standard care for neutropenic pediatric patients. Because of potential nephrotoxicity, use of aminoglycosides should be individualized at the discretion of the investigators. Careful monitoring and dosage modification based on peak levels, nadir levels and half-life of the aminoglycoside is recommended.

9.2.5 Nutritional Management

Participant with greater than 10% weight loss over pretreatment baseline should begin nutritional support. Participants may enroll on hyperalimentation protocols, and will be monitored by the Metabolic Support Service.

9.2.6 Venous Access

Participants treated in this protocol will undergo intensive induction chemotherapy, leukapheresis, myeloablation, and stem cell reinfusion. A venous catheter should be inserted at diagnosis to permit all of these treatments. The catheter should have at least two lumens and should be sufficiently rigid to permit leukapheresis.

9.2.7 Hematopoietic Growth Factors

The protocol calls for the use of GM-CSF following each course of myelosuppressive chemotherapy. GM-CSF should begin 24 – 48 hours following the completion of myelosuppressive chemotherapy and must be discontinued for at least 24 hours before the next course of myelosuppressive chemotherapy can begin.

9.3 Supportive Care during hu14.18K322A Infusion

9.3.1 Allergic/Hypersensitivity Reactions

Refer to Appendix VI for guidance on prevention and management of hypersensitivity reactions to hu14.18K322A.

9.3.2 Premedication for Pain

Pre-medication with morphine (or an appropriate narcotic) prior to the first dose of course 1 of hu14.18K322A antibody will be given to all participants with course 1. Premedication with morphine after course 1 will be at the discretion of the treating physician

9.3.3 Hypotension

For symptomatic participants and/or those with a systolic or diastolic blood pressure 15% below baseline, refer to the algorithm outlined in Appendix VIII for management of hypotension. The following supportive care measures are recommended:

1. Stop the hu14.18K322A mAb infusion; place patient in Trendelenburg position.
2. If the patient is receiving IV IL-2, hold the cytokine.
3. If blood pressure does not return to baseline with stopping infusion, give fluid bolus of normal saline 10-20 ml/kg.
4. Decrease narcotic dose if possible.
5. Fluid bolus may be repeated once or twice as needed.
6. Once two fluid boluses are administered without appreciable improvement, consider transfer to ICU.
7. If blood pressure is unresponsive, consider bolus with 25% albumin or packed red blood cells if hemoglobin < 10 m/dL.
8. If responsive to fluid resuscitation, restart infusion at 50% rate at least one hour after blood pressure returns to baseline. If tolerated, all subsequent infusions should be given at 50% rate. If hypotension recurs, follow steps 1-3.

9. If unresponsive to fluid resuscitation, consider IV pressors (adrenergic pressors are preferred over dopamine).
10. If blood pressure is stable for at least two hours following restarting of hu14.18K322A, restart cytokine.
11. If hypotension recurs after restarting cytokine, hold cytokine until the following day. Restart cytokine at 50% dose, starting the next day (it is recommended that GM-CSF be given SubQ). During courses 2 and 4 of MRD Treatment, if IL-2 is tolerated at 50% dose, subsequent cycles should start at 50% dose. If IL-2 is NOT tolerated with hu14.18K322A, substitute GM-CSF for subsequent IL-2 courses.

Every effort should be made to administer hu14.18K322A as scheduled. The above measures are suggested guidelines only.

9.4 Supportive Care during Radiation Therapy

The majority of children undergo adjuvant radiation therapy for neuroblastoma without significant difficulties. Specific patient care issues related to radiation should be managed in conjunction with a radiation oncologist. The following are guidelines for management of acute radiation related treatment effects.

Nausea/emesis – The preferred anti-emetic is ondansetron at 0.15 mg/kg given 30-60 minutes before each radiation treatment fraction and repeated every 6-8 hours thereafter for a total of three doses as necessary.

Neutropenia – Radiation therapy should not be initiated until an ANC of 500 is obtained and preferably an ANC of 1000. Should the ANC fall below 500 during radiation therapy, a treatment interruption should be considered until the ANC recovers to a level above 500.

Thrombocytopenia – Participants with platelet counts below 25,000, either before initiation of radiation or after radiation therapy has been instituted, should be considered for a treatment break or platelet support.

Anemia – Participants with hemoglobin below 8 g/dl should be evaluated for transfusion during radiation therapy.

9.5 Supportive Care for Etoposide Reactions

9.5.1 Cardiovascular Effects

Transient hypotension has occurred in about 1 to 2 % of patients following rapid IV administration of etoposide during clinical trials. However, hypotension has not been associated with cardiac toxicity or electrocardiogram changes. Blood pressure usually normalizes within a few hours after discontinuation of the infusion. To avoid this complication, Etoposide should be infused over 30 – 60 minutes. If hypotension should occur, stop the infusion, and if necessary, give 10 mL/kg NS bolus over 15 minutes. Repeat as necessary. Once symptoms resolve, resume infusion at ½ previous infusion rate until full dose administered. If hypotension recurs, stop infusion and administer 10 mL/kg NS bolus as indicated. Once hypotension resolves, resume infusion at ½ previous infusion rate until complete. Consider infusing NS at 1 – 1.5 x maintenance during remainder of infusion. For all subsequent doses, further dilute and infuse over 2 hrs.

9.5.2 Sensitivity Reactions

Anaphylactoid reactions consisting principally of chills, rigors, diaphoresis, pruritis, loss of consciousness, nausea, vomiting, fever, bronchospasm, dyspnea, tachycardia, hypertension, and/or hypotension have occurred in 0.7 – 3% of patients receiving etoposide. Other manifestations include flushing, rash, substernal chest pain, lacrimations, sneezing, coryza, throat pain, back pain, abdominal cramps, and auditory impairment. Facial/lingual swelling, coughing, diaphoresis, cyanosis, tightness in the throat, and laryngospasm have also occurred.

If an anaphylactoid reaction should occur:

1. Stop the infusion immediately and notify H/O Fellow or Attending MD
2. Administer the following as indicated:
   a) diphenhydramine 1mg/kg IV (max dose 50 mg)
   b) hydrocortisone 50 – 100 mg/m^2 IV
   c) epinephrine 0.01 mg/kg of a 1:1000 concentration for IM administration
   d) fluid bolus 10 mg/kg NS infused over 15 minutes
3. Once symptoms have resolved, resume infusion at ½ previous rate until infusion complete. Consider infusing NS at 1 – 1.5 x maintenance during remainder of infusion.
4. If anaphylaxis recurs, stop the infusion and re-treat as above. Do not administer remainder of dose. Consider substituting Etoposide with etoposide phosphate (Etopophos®) for all subsequent doses.
5. If anaphylaxis does not recur, pre-medicate all subsequent doses with diphenhydramine 1mg/kg and hydrocortisone 50 – 100 mg/m^2. Consider slowing the loading dose to be administered over 1 hour. Further dilute 72 hour continuous infusion (CA*E* courses) as

appropriate, but do not prolong infusion time more than 72 hours. For bolus courses (CiE), may prolong infusion over 2 – 6 hours as required. If anaphylaxis does not recur, pre-medicate all subsequent doses with diphenhydramine 1mg/kg and hydrocortisone 50 – 100 mg/m^2. Further dilute and infuse over 2 – 6 hours as required.

6. If anaphylaxis occurs during continuous infusion Etoposide, stop infusion, treat as above, further dilute and infuse at same rate. Piggyback NS at 1 – 1.5 x maintenance with Etoposide. If symptoms recur, stop infusion and consider substitution with Etoposide phosphate for all subsequent doses.
7. Have at bedside all of the following for all subsequent infusions:
   a) Diphenhydramine 1mg/kg IV
   b) Hydrocortisone 50 – 100 mg/m^2 IV
   c) Epinephrine 0.01 mg/kg of a 1:1000 concentration for IM administration

Please note: anaphylactoid reactions are still possible with etoposide phosphate. If the patient cannot tolerate the substitution, drug is contraindicated and must be discontinued.

10.0 REQUIRED EVALUATIONS, TESTS AND OBSERVATIONS

Notes pertaining to testing and evaluation schedules:

- In general, standard volumes will be obtained for blood testing. Minimal volumes will be used whenever possible and when clinically indicated such as in cases of small children, difficulty obtaining blood return from intravenous catheters, or for safety reasons such as significant anemia, thrombocytopenia, etc.
- Research tests may be omitted based on volume of blood collected. Prioritization will be per BMTCT Immune Core Lab.
- Minor adjustments in timing or scheduling of tests for reasons of medical management or clinical scheduling will not be considered protocol deviations; this will be at the discretion of the PI and treating physician.
- The bone marrow studies for disease status and MRD will be obtained simultaneously.
- To avoid unnecessary bone marrow testing procedures, MRD and disease status results done prior to enrollment may be used as the baseline values if they were obtained within approximately 2 weeks of enrollment.
- All clinical and laboratory studies to determine eligibility must be performed within 14 days prior to enrollment unless otherwise indicated.

10.1 Pre-Treatment and First Two Courses

	Pre-Treatment/Prior to Course 1	During Courses 1& 2	Prior to Course 2
History/PE	X	--	X
Weight/Height	X	--	X
CBC, diff, platelets	X	2 x per week	X
CMP, Mg++, PO_4, LDH, uric acid	X	--	X
Serum ferritin	X	--	--
Neuron specific Enolase	X	--	--
Pregnancy test	X		
Urine HVA, VMA	X	--	--
Bilateral BMA/Biopsy	X	--	--
CT chest, abdomen, pelvis (and/or whole body MRI†)	X†	--	X†
MIBG scan*	X	--	--
Bone scan*	X	--	--
EKG/ECHO**	X	--	--
Ophthalmology exam, including visual acuity°	X	--	--
Surgery consultΩ	X	--	--
Tumor for MYCN and Shimada histologic classification	X	--	--
MRD (peripheral blood and BM) (Section 11.1)	X	--	--
HLA, KIR genotype (Section 11.2)	X††	--	--
BMTCT NK cell phenotype and functional studies (Section 11.2)	X	X	--
Fresh tumor for biology studies (section 11.3)	X *(pretreatment and second look if possible)*	--	--
HAHA (Section 11.4)	X	--	X
hu14.18K322A serum level (Section 11.4)	--	X *(1 hour after first dose only)*	--
PB for catechol metabolites (to be done while in Nuclear Med for MIBG scan) (section 11.5)	X *(during MIBG)*	--	--

CMP-comprehensive metabolic panel (Na+, K+, Cl, CO_2, Ca++, BUN, Cr, blood sugar, total protein, albumin, Alk Phos, AST, ALT, bilirubin
**May do one or the other (MIBG is preferred). May be performed within 2 weeks of starting chemotherapy if is not possible to obtain scan prior to starting chemotherapy*
***Does not need to be done prior to initiating first course of chemotherapy with cyclophosphamide and topotecan.*
†Whole body MRI in situations where clinically indicated
ΩTo evaluate for resectability of primary tumor; not required but suggested for good clinical care
††HLA, KIR genotype may be drawn any time prior to consolidation, as long as adequate ANC

°Do not hold chemotherapy for ophthalmological exam; may be done any time during first course.

10.2 Induction Chemotherapy Courses 3 – 6 and Pre-Intensification

	Prior to Course 3/ Completion Course 2©	**Prior to** each chemotherapy Courses 4-6	**During** each chemotherapy Courses 3-6	End of Induction / Prior to Intensification
History/PE	X	X	--	X
Weight/height	X	X	--	X
CBC, diff, platelets	X	X	--	X
CMP, Mg++, PO_4, LDH, uric acid	X	X	--	X
Urine HVA, VMA	X	--	--	X
Bilateral BMA/biopsy	X	--	--	X
CT chest, abdomen, pelvis (and/or whole-body MRI‡)	X	--	--	X
MIBG scan or bone scan*	X	--	--	X
EKG/echocardiogram**	X	--	--	X
Surgery consultΔΩ	X	--	--	X
Dental evaluationΔ	--	--	--	X
Radiation Oncology consultΔ	--	--	--	X
Audiogram or BAER†	X	X (Course 5 only)	--	X
Ophthalmology exam (including visual acuity)	X	--	--	--
MRD peripheral blood and marrow (section 11.1)	X	--	--	X
BMTCT NK cell phenotype and functional studies (Section 11.2)	X	X	X	X
HAHA (section 11.4)	X	X	--	X
hu14.18K322A serum level (section 11.4)	--	--	X (1 hr after 1st dose hu14.18K322A each course)	
PB for catechol metabolites (while in Nuclear Med for MIBG scan) (section 11.5)	X (during MIBG)	--	--	X

©If there is a significant delay between the end of cycle 2 and beginning of cycle 3, some or all of these tests may need to be repeated prior to cycle 3 for safety; this will be at the discretion of the treating investigator. Research tests will not be repeated. CMP-comprehensive metabolic panel (Na+, K+, Cl, CO_2, Ca++, BUN, Cr, Blood sugar, total protein, albumin, Alk Phos, AST, ALT, bilirubin
**May do one or the other (MIBG is preferred)*
***Ideally, the first ECHO should be completed prior to first dose of doxorubicin. It may be completed any time during pre-treatment, week 0 – 6,*
†Recommended to complete sometime prior to course #3, preferably prior to first dose of cisplatin (do not hold chemotherapy for audiogram/BAER; Audiogram/BAER recommended to be repeated prior to each cycle of cisplatin and prior to Consolidation (do not hold chemotherapy for audiogram/BAER).

ǂWhole body MRI in situations where clinically indicated
ΔNot required but suggested for good clinical care
ΩTo evaluate for resectability of primary tumor.
Obtain other tests as needed for good clinical care

10.3 Evaluations during Intensification & Consolidation Radiation Therapy

To be performed as clinically indicated

	Daily	Day +7 & 21
Physical exam**	X	--
CBC, diff, platelets**	X	--
BMTCT NK cell phenotype and functional studies (section 11.2)	--	X

***PE and CBC daily until neutrophil engraftment, then weekly during radiation therapy*

Obtain other tests as needed for good clinical care per discretion of BMTCT and Radiation Oncology

Samples for busulfan pharmacokinetics research will be taken from samples already being collected for clinical care during Intensification. No additional samples are needed for this research.

10.4 Evaluations During Maintenance or Minimal Residual Disease (MRD) Treatment

	Prior to course 1	Prior to course 2	Prior to course 3	Prior to course 4	Prior to course 5	Prior to course 6	End of Treatment
Physical exam	X	X	X	X	X	X	X
CBC, diff, platelets	X	X	X	X	X	X	X
CMP, Mg++, PO_4, LDH, uric acid	X	X	X	X	X	X	X
Urine HVA/VMA	X	X	X	X	X	X	X
Ophthalmology exam, including visual acuity							X
HAHA (section 11.4)	X	X	X	X	X	X	--
hu14.18K322A serum level (section 11.4)	1 hour after first dose of hu14.18k322a on each course						--
MRD bone marrow (section 11.1)	X	--	--	--	--	--	X
MRD peripheral blood (section 11.1)	X	--	--	--	--	--	X
BMTCT NK cell phenotype and functional studies (section 11.2)	X	X	X	--	--	X	--
CT chest, abdomen, pelvis (and/or whole-body MRI‡)	X	--	--	--	--	--	X
MIBG scan or bone scan*	X	--	--	X	--	--	X

Pregnancy test	X	X	X	X	X	X	X
Plasma catecholamine metabolites (section 11.5)	X	--	--	X	--	--	X
Bilateral BMA/Biopsy	X	--	--	--	--	--	X

CMP-comprehensive metabolic panel (Na+, K+, Cl, CO_2, Ca++, BUN, Cr, blood sugar, total protein, albumin, alk phos, AST, ALT, bilirubin)
**May do one or the other (MIBG is preferred)*
‡Whole body MRI in situations where clinically indicated for good clinical care

Obtain other tests as needed

10.5 After Completion of Treatment and Long-Term Follow-Up

First year: Physical examination, CBC with differential and platelet count, chemistry profile, and urinary VMA and HVA, bilateral bone marrow aspirates and biopsies and samples for MRD (peripheral blood and BM), CT scan or MRI, MIBG, or bone scan of sites of previous disease every 4 months.

Second year: Physical examination, CBC with differential and platelet count, chemistry profile, urine VMA, HVA, bilateral bone marrow aspirates and biopsies and samples for MRD, and imaging of primary site every 6 months.

Third, fourth and fifth years: Physical examination, CBC with differential and platelet count, urinary VMA and HVA, and most appropriate imaging of disease sites a minimum of once yearly.

	Every 4 months x 3	Every 6 months x 2	Yearly x 3
History/PE	X	X	X
Weight/Height	X	X	X
CBC, diff, platelets	X	X	X
CMP, Mg++, PO_4, LDH, uric acid	X	X	X
Urine HVA, VMA	X	X	X
Bilateral BMA/Biopsy	X	X	
CT chest, abdomen, pelvis (and/or whole-body MRI)	X	X	X
MIBG scan or bone scan*	X	X	X
MRD (peripheral blood & bone marrow)	X#		

CMP-comprehensive metabolic panel (Na+, K+, Cl, CO_2, Ca++, BUN, Cr, Blood sugar, total protein, albumin, Alk Phos, AST, ALT, bilirubin
**May do one or the other (MIBG is preferred)*
‡Whole body MRI in situations where clinically indicated
Obtain other tests as needed for good clinical care
#With clinical bone marrows months 4, 8 and 12 (±1 month) first year only.

11.0 CORRELATIVE PHARMACOLOGY AND BIOLOGY STUDIES

11.1 Assessment of Minimal Residual Disease (MRD)

11.1.1 MRD Data: Results of the MRD analysis will be provided by Dr. Mead's laboratory for entry into the CRIS database for analysis.

11.1.2 Assessment of Bone Marrow and Peripheral Blood

	Prior to Start of Treatment	Prior to Start of Course 3	After Course 6 (End of Induction)	Prior to Start of MRD Treatment	End of MRD Treatment	After Completion of Treatment Months 4, 8 and 12 (±1month)
MRD peripheral blood*	X	X	X	X	X	X
MRD bone marrow	X	X	X	X	X	X
Bilateral BMA/biopsy	X	X	X	X	X	X

**Peripheral blood MRD should be collected once for each time point. Bone marrow for MRD will be collected at same time clinical BMA/biopsy procedure is done (see Section 10.5). If bone marrow procedures are delayed, DO NOT recollect peripheral blood MRD sample. Duplicate samples will be rejected.*

11.2 NK Cell Phenotype and Functional Studies

Therapy		NK studies		
		Time points	Sample	Tests
At diagnosis		Prior to therapy	PB or BM	HLA Typing BMTCT NK cell Phenotype Research Calcein kill assay#
Prior to surgery		Day of surgery	PB	BMTCT NK cell Phenotype Research
Course 1	CYCLO/TOPO d 1–5 Hu 14.18 K322A d 2–5 LD–IL2 d 6, 8, 10, 12, 14, 16 GM–CSF day 7 until ANC ≥ 2000	Day 2 pre–antibody Day 6 pre–IL2 Day 7 pre–GM–CSF Day 21	PB	BMTCT NK cell Phenotype Research Day 7: Calcein kill assay#
PBSC harvest	G–CSF	Day of harvest	PB	HLA (if not done at baseline) BMTCT NK cell Phenotype Research
Course 3	CISplatin d 1–4/VP16 d 1–3 Hu14.18K322A d 2–5 LD–IL2 d 6, 8, 10, 12, 14, 16 GM–CSF day 7 until ANC ≥ 2000	Day 2 pre–antibody Day 6 pre–IL2 Day 7 pre–GM–CSF Day 21	PB	BMTCT NK cell Phenotype Research Day 7: Calcein kill assay#
Course 4	CYCLO d 1–2/DOXO d 1–3/VCR d 1–3 Hu14.18K322A d 2–5 LD–IL2 d 6, 8, 10, 12, 14, 16 GM–CSF day 7 until ANC ≥ 2000	Day 2 pre–antibody Day 6 pre–IL2 Day 7 pre–GM–CSF Day 21	PB	BMTCT NK cell Phenotype Research Day 7: Calcein kill assay#
Course 5				
Course 6				
Intensification	HD chemo w/ auto HSCT Hu14.18K322A d 2–5 Haplo NK cell transfer d 4 LD–IL2 d 3, 5, 7, 9	Prior HSCT conditioning Day +7, +21	PB PB	BMTCT NK cell Phenotype Research BMTCT NK cell Phenotype Research Day 7: Calcein kill assay#
MRD Treatment	Course 1: GM–CSF d 0–14, Hu14.18K322A d 3–6, RA d 10–23 Course 2: LD–IL2 d 0–3, HD–IL2 d 7–10, Hu14.18K322A d 7–10, RA d 14–27	Course 1 Day 0 Day 2 pre–antibody Day 7 post–antibody Course 2 Day 0 Day 4 post–IL2 Day 11 post–antibody Course 3&6 D 0	PB	BMTCT NK cell Phenotype Research Course 2 day 4 and 11: Calcein kill assay#

#Patients enrolled prior to amendment 6.0 will not have calcein assay labs drawn; only patients enrolled after activation of Amendment 6.0; these assays will be performed when there are sufficient NK cells.

The role of tumor-derived GD2 on white blood cells (including NK cells) will be performed with left-over serum and white blood cells from samples from NK cell phenotype and functional studies.

11.3 Studies on Resected Tumor Tissue

11.3.1 Sample Acquisition, Processing, and Delivery

When sections of formalin-fixed paraffin-embedded primary tumor tissue are available, either at diagnosis or second look surgery or both, representative regions from fixed, wax embedded tumor blocks of each case will be incorporated into a tissue microarray and sent for T-cell, myeloid-cell and PD-1 pathway expression.

11.4 Pharmacology and Immunological Monitoring

11.4.1 Human Anti-Human Antibodies (HAHA) Testing

Participants will be monitored for the development of human anti-human antibodies (HAHA). Blood samples (~3 ml) will be drawn in a red top tube prior to the start of antibody infusion at the following times:

- Induction phase:
  - Course 1 pretreatment
  - Courses 2-6 - before 1st dose of hu14.18K322A each cycle
  - End of Induction (same as prior to Intensification)

- Intensification
  - Prior to 1st dose of hu14.18K322A during experimental MRD treatment

- Maintenance phase (MRD treatment):
  - Courses 1-6: prior to starting each course.

All serum samples will be assayed for HAHA using an ELISA assay previously reported by Albertini et al.[178] Samples will be kept on ice until centrifugation. Centrifuge for 10 minutes at 4°C at 3000×g. Transfer serum to labeled tube and store at -20°C. Each specimen must be labeled with the unique participant identifier, protocol mnemonic NB2012, date and time the sample was drawn. Serum specimens will be shipped as a batch, on dry ice to the address below.

Samples will only be received Monday through Friday.

Paul Sondel, MD, PhD

Attn: Dr. Jacqueline Hank, MD
UWCCC Immunotherapy Lab
4159 WIMR – UWCCC
1111 Highland Ave
Madison, WI 53705
Phone: 608-263-8919 or 608-263-9069, Email: jgan@wisc.edu

11.4.2 hu14.18K322A mAb Pharmacokinetic Studies (hu14.18K322A serum levels)

Pharmacokinetic studies will be obtained from consenting participants enrolled on this protocol with Induction and Maintenance/MRD Treatment.

Sampling strategy

Serial serum samples for pharmacokinetic studies of hu14.18K322AmAb will be collected with the first dose of all Induction, Intensification (if experimental MRD Treatment is given, and Maintenance (MRD Treatment) phases. Samples should be 1 (one) hour after administration of the study agent during each course of chemotherapy (Courses 1-6 Induction, and Maintenance/ MRD Treatment).

Sample collection and processing instructions

Draw 3 mL of blood into a (red top) vacutainer tube with wax separator. Record the exact time that the sample is drawn along with the exact time that the drug is administered on the Pharmacokinetic Data Collection Form. Place the tube on ice until centrifugation. After the blood has clotted, centrifuge for 10 minutes at 4°C at 3000×g. Aliquot serum from each sample into 2-3 individually labeled tubes and store at -20°C.

Each specimen must be labeled with the unique participant identifier, protocol mnemonic NB2012, date and time the sample was drawn. Serum specimens will be shipped as a batch, on dry ice to the address below.

Samples will only be received Monday through Friday.

Paul Sondel, MD, PhD
Attn: Dr. Jacqueline Hank, MD
UWCCC Immunotherapy Lab
4159 WIMR - UWCCC
1111 Highland Ave
Madison, WI 53705
Phone: 608-263-8919 or 608-263-9069, Email: jhank@wisc.edu

Description of hu14.18K322A assay

Serum samples will be assayed for Hu14.18K322AmAb using an enzyme-linked immunosorbent assay (ELISA). In this assay, material is diluted to achieve a concentration of 100 ng/ml and then 2-fold dilutions are made (down to 3.125 ng/ml). C8 MaxisorpImmuno-modules (NUNC) coated with 1A7 anti-idiotypic to 14.18 antibody (2 µg/ml) are used. Samples are run in triplicates (100 µl/well), incubated overnight at 4C and then after washing, secondary antibody (sheep anti-human IgG1-peroxidase conjugated, The Binding Site) is applied for three hours at room temperature. Signal is generated using TMB substrate (Sigma).

Description of pharmacokinetic data analysis

Hu14.18K322A serum concentration–time data will be fit to a compartmental pharmacokinetic model. Individual pharmacokinetic parameters estimated will include volume of the central compartment (Vc), elimination rate constant (Ke), and half–life ($t_{1/2}$). Systemic clearance (Cl) will be calculated using standard equations and $AUC_{0-¥}$ will be calculated using the log–linear trapezoidal method.

sIL2Rα levels

Serum samples obtained on selected samples collected for PK will also be tested for sIL2Rα levels in the Sondel lab. No extra blood needs to be collected.

11.5 Plasma Catecholamine Metabolites

11.5.1 Procedure:

Participants remain in the supine position during collection of the blood sample (5 mL). The sample is drawn through the patient's indwelling central venous line into a chilled, evacuated, glass collection tube containing heparin in any form as an anticoagulant (e.g., a green top Vacutainer™ tube). The blood sample is stored on ice until centrifuged (4°C) to separate the plasma within 1 hour of blood collection. The plasma is separated into two aliquots — one for catechols, one for metanephrines — (with each aliquot at least 1.5 cc) in plastic tubes legibly and indelibly marked with the date and patient ID and frozen immediately (e.g., on dry ice). The sample is stored at –70°C or colder. Samples will be stored in a –70 freezer in the new cyclotron chemistry facility in CCC. Samples will be obtained the same week as MIBG evaluations – at diagnosis, at completion of the first two courses of induction chemotherapy, end of induction/prior to consolidation, prior to initiation of MRD treatment, after three courses of MRD treatment and after completion of therapy.

Acetaminophen interferes with the plasma metanephrine assay. It is therefore essential that patients inform the investigators of acetaminophen in any form (e.g., Tylenol™, Excedrin™, combination cold medications) taken within 5 days before the sample is drawn. No other drug interferences with the metanephrine assay have been established to date. All medications that the patient is taking will be listed on the accompanying information sheet. It is best if the sample is obtained in

the morning after an overnight fast. Water and non-caffeinated soft drinks are permissible. All samples will be labeled with the patient's ID, name, and date. The samples will be coded and that information stored on the investigator's password protected computer, and accessible to only to investigators. The samples will be identified with only a code once shipped to the laboratory of Dr. Graeme Eisenhofer, the developer of the plasma catecholamine metabolite assay.

11.5.2 Samples will be shipped to:

Prof. Graeme F. Eisenhofer Ph.D
Institut fuer Klinische Chemie und Laboratoriumsmedizin Haus 28/ 3. Etage Universitaetsklinikum Carl Gustav Carus Dresden an der Technischen Universitaet Dresden Anstalt des oeffentlichen Rechts des Freistaates Dresden Fetscherstr. 74
D-01307 Dresden, Germany

12.0 EVALUATION CRITERIA

12.1 Toxicity Criteria

Toxicity for events other than SOS (sinusoidal obstructive syndrome, formerly known as VOD) will be monitored and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0 (CTCAE v4.0). Adverse events not included in the CTCAE v4.0 should be reported and graded under other adverse events within the appropriate category. Copies of the CTCAE v4.0 can be downloaded from the CTEP home page (http://ctep.info.nih.gov) or from the St. Jude Intranet home page.

Severe SOS will be defined as:
Serum total bilirubin > 2.0 mg/dl, PLUS at least 2 of the following findings following transplantation: hepatomegaly with right upper quadrant pain, ascites, or weight gain >5% above baseline PLUS a specific organ failure listed below:

- Hepatic encephalopathy (CTC Grade 4 hepatic failure), OR
- Pulmonary dysfunction: Continuous oxygen support (CTC Grade 3 hypoxia) for >48 hours, ventilator support not clearly attributable to another cause, OR
- Renal dysfunction: serum creatinine > 3 times the ULN for age and sex (CTC Grade 3 creatinine), or the need for dialysis (CTC Grade 4 renal), not clearly attributable to another cause.

12.2 Response Criteria

12.2.1 Chemotherapy Response Parameters

Response will be assessed at five different times in the course of this protocol. The first assessment of response is required after the initial two courses of chemotherapy (post weeks 0 – 6). A second assessment is required at the completion of induction. A third assessment is required at the end of consolidation. Similar response assessments will occur after three courses of MRD treatment and at the completion of all protocol treatment. The same response criteria will be employed at each time point.

12.2.2 Tumor Response Definitions and Criteria

This study will use the International Criteria for Neuroblastoma Response to assess response on this study with some clarification as described below[47,48] (see Appendix IV).

Evaluable disease: Participants who have one or more positive lesions on bone scan or MIBG will be evaluable for response to the initial two courses of therapy.

Measurable disease: Measurable tumor is defined as primary tumor or solid organ metastases ≥ 1.0 cm in greatest diameter and nodal metastases ≥ 2.0 cm. The volume of the primary tumor will be determined using the formula for an ellipsoid model [(anterior–posterior diameter x transverse diameter x length) x 0.52]. For nodal and solid organ metastases we will use the product of the longest x widest perpendicular diameter as measured on axial images by CT or MRI scan. Elevated urine catecholamine metabolite levels and tumor cell invasion of bone marrow also is considered measurable tumor.

Complete response (CR): No evidence of primary tumor, no evidence of metastases (chest, abdomen, liver, bone, bone marrow, nodes, etc.), and HVA/VMA normal.

Very good partial response (VGPR): Greater than 90% reduction of primary tumor volume; no metastatic tumor (as above except bone); no new bone lesions, all pre-existing lesions improved on bone scan or MIBG; HVA/VMA normal, or both decreased >90%.

Partial response (PR): Fifty–90% reduction of primary tumor volume; 50% or greater reduction in sum of products of measurable sites of metastases; 0–1 bone marrow samples with tumor; all pre-existing bone lesions improved, no new lesions, HVA/VMA both decreased 50–90%

Mixed response (MR): Greater than 50% reduction of any measurable lesion (primary tumor volume or sum of products of measurable metastases) with, no new lesions; <25% increase in any existing lesion (exclude bone marrow evaluation).

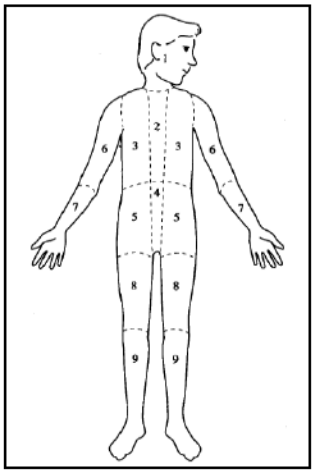
Stable disease (SD): No new lesions; < 25% increase in any lesion; exclude bone marrow evaluation.

No response (NR): No new lesions; <50% reduction but <25% increase in any existing lesion (exclude bone marrow evaluation).

Progressive disease (PD): Any new lesion or increase of a measurable lesion by >25%; previous negative marrow positive for tumor.

12.2.3 Response Criteria for Neuroblastoma Patients with ^{123}I–MIBG positive lesions

Patients who have a positive ^{123}I–MIBG scan at the start of therapy will be evaluable for MIBG response. The response of MIBG lesion will be done using the Curie scale.[179,180]



The body is divided into 9 anatomic sectors for osteomedullary lesions, with a 10th general sector allocated for any extra – osseous lesion visible on MIBG scan. In each region, the lesions are scored as follows:

Absolute Extension Score: graded as:
0= no site for segment
1= 1 site per segment
2= more than 1 site per segment
3= massive involvement (>50% of segment involved)

The absolute score is obtained by adding the score of all of the segments. See segment diagram.

Relative Score is calculated by dividing the absolute score at each time point by the corresponding pre-treatment absolute score. The relative score of each patient is calculated at each response assessment compared to baseline and classified below:

1) Complete response: all areas of uptake on MIBG scan are completely resolved. If morphological evidence of tumor cells in bone marrow biopsy or aspiration is present at enrollment, no tumor cells can be detected by routine morphology on two

subsequent bilateral bone marrow aspirates and biopsies done at least 3 weeks apart to be considered a complete response.

2) Partial response: Relative score ≤ 0.2 (lesions almost disappeared) to ≤ 0.5 (lesions strongly reduced).

3) Stable disease: Relative score > 0.5 (lesions weakly but significantly reduced) to 1.0 (lesions not reduced).

4) Progressive disease: new lesions on MIBG scan.

12.2.4 Response Assessment

Each participant will be classified according to their "best response" for the purposes of analysis of treatment effect. Best response is determined from the sequence of the objective statutes described above. If CR is not attained because of residual primary tumor which is then completely resected at second look surgery or found to be ganglioneuroma only, the patient will be considered for conversion to CR status by surgery.

Duration of disease control – measured from study enrollment to the onset of progressive disease, relapse or death if patient dies in remission.

Duration of survival – measured from date of diagnosis to date of death

12.2.5 Surgical Response Criteria and Definitions

If at presentation a diagnosis cannot be made by bone marrow evaluation and clinical status, a biopsy of the most accessible site will be done. In INSS stage 3 patients, lymph nodes away from primary site must be biopsied and be positive for tumor (exception: large retroperitoneal tumors where nodes are not accessible for biopsy). Unless there is a contraindication, it is highly recommended that all patients have central venous catheter access devices inserted prior to initiation of chemotherapy.

End of first six weeks of therapy surgical evaluation: Prior to commencing first course of CAE, patients will undergo surgical evaluation for potential resection of primary tumor mass and careful lymph node staging.
Post-induction surgery: All patients not able to undergo resection after the first six weeks of therapy will undergo a surgical evaluation after completion of induction for resection of the primary, lymph node biopsies and liver biopsies (if in abdomen) if they have achieved a complete response, a very good – or partial response on the basis of the primary site or improvement of bone scan positive lesions.

Exceptions: Patients with thoracic or neck primaries who by computed tomography scan are CR will not undergo post-induction surgery. Patients who did not have a laparotomy during therapy must undergo a laparotomy at the end of all therapy.

All patients eligible for post-induction surgery and those with thoracic or neck primaries who do not go to surgery will receive intensification chemotherapy.

Surgical definitions:

- Complete resection – complete gross excision of primary tumor
- Near-total resection – > 90% gross excision of primary tumor
- Partial resection – >50% but less than complete gross excision of primary tumor
- Biopsy – <50% excision of primary tumor or biopsy of any site of tumor other than primary

13.0 OFF-STUDY AND OFF-TREATMENT CRITERIA

13.1 Off-Study Criteria

- Death
- Non-compliance
- Lost to follow-up
- Withdrawal of consent for continued follow-up

13.2 Off Treatment Criteria

- Research participant has completed all protocol defined treatment
- Relapse or progression of disease, unless it occurs before the patient has completed all three chemotherapy combinations (Cyclo/topo; CAV, CiE) (see Section 4.2). If patients have progressive disease after exposure to all three combinations, they will come off treatment and be followed until/if they receive other anticancer therapy. At that time, they will be followed for survival status only.
- Development of unacceptable toxicity
- Medical or psychiatric illness, which in the investigator's judgment renders the research participant incapable of further therapy

- Research participant is unwilling or unable to comply with protocol requirements

14.0 SAFETY AND ADVERSE EVENT REPORTING REQUIREMENTS

14.1 Reporting Adverse Experiences and Deaths On Study

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.

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.

- Deaths that occur while on treatment or within 30 days of last protocol therapy meet reporting requirement and will be reported to the IRB regardless of the cause of death. Similarly, all treatment-related deaths will be reported to the IRB, regardless of time frame (i.e., deaths occurring >30 days that are treatment-related).

- Unrelated, expected deaths that occur > 30 days after last protocol therapy do not meet reporting requirements. Though death is "serious", the event must meet the other two requirements of

"related or possibly related" and "unexpected/unanticipated" to meet reporting requirements. These deaths will be reported with annual progress reports to IRB and FDA.

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 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.

14.2 Recording AEs and SAEs

Adverse events (AEs) will be evaluated and documented by the clinical staff and investigators throughout inpatient hospitalizations and each outpatient visit. CRAs are responsible for reviewing documentation related to AEs and entering directly into CRIS protocol-specific database. The data to be recorded are 1) the event description, 2) the NCI CTCAE v4.0 code and grade, 3) the onset date, 4) the resolution date (or ongoing), 4) action taken for event, 5) participant outcome 6) relationship of AE to protocol treatment/interventions, 7) if AE was expected or unexpected, and 8) comments, if applicable. AEs that are classified as serious, unexpected, and at least possibly related will be notated as such in the database as "SAEs". These events will be reported expeditiously to the St. Jude IRB within the timeframes as described above. Cumulative summary of Grade 3-5 events will be reported as part of the progress reports to IRB at the time of continuing review. Specific data entry instructions for AEs and other protocol-related data will be documented in protocol-specific data entry guidelines, which will be developed and maintained by study team and clinical research informatics.

The study team will meet regularly to discuss AEs (and other study progress as required by institutional DSMP). The PI will review Adverse Event reports generated from the research database, and corrections will be made if applicable. Once the information is final the PI will sign and date reports, to acknowledge his/her review and approval of the AE as entered in the research database.

14.3 Reporting to the St. Jude Institutional Biosafety Committee (IBC)

Continuing review reports will be sent to the St. Jude IBC on at least an annual basis using the most current version of the continuing review form found on the St. Jude Biosafety website. Safety reports and related correspondence sent to the IRB will be simultaneously forwarded to the

St. Jude IBC. Therefore, reporting for safety events to this committee will be according to the same timelines as reporting to the IRB. Only those protocol revision and amendments that are directly related to the antibody or related manufacturing will require review and consideration for approval by the IBC. Other revisions and/or amendments will be noted in the IBC continuing review reports where applicable.

14.4 Reporting Requirements to the FDA

Any unexpected fatal or unexpected life-threatening event judged by the PI to possibly be due to the investigational agent, will be reported to the FDA by telephone or fax as soon as possible but no later than seven calendar days after notification of the event and followed by a written safety report as complete as possible within eight additional calendar days (i.e. full report 15 calendar days total after notification of event).

Unexpected, non-fatal and non-life-threatening SAEs, which occur in on-study participants during the time periods specified in Section 9.1 that are considered due to or possibly due to the investigational agent, will be reported to the FDA by written safety report as soon as possible but no later than 15 calendar days of the notification of the occurrence of the event. Expected SAEs, even unexpected fatal SAEs, considered by the PI to be not related to the study, will be reported to the FDA in the Annual Review Report along with non-serious AEs. All FDA correspondence and reporting will be conducted through the St. Jude Office of Regulatory Affairs.

14.5 Summary of Reporting Mechanism by St. Jude Regulatory Affairs

Copies of all correspondence to the St. Jude IRB, including SAE reports, are provided to the St. Jude Regulatory Affairs office. FDA-related correspondence and reporting will be conducted through the Regulatory Affairs office.

14.6 Continuing Review Reports and IND Annual Reports to FDA

Continuing review reports of protocol progress and summaries of adverse events will be filed with the St. Jude IRB, St. Jude IBC, and FDA at least annually.

14.7 Reporting AEs to and from St. Jude Affiliates

Adverse events from collaborating sites will also be reviewed by the PI and discussed in study team meetings as described above. SAE report from

collaborating sites for AEs that are serious, unexpected, and at least possibly related to protocol treatment or interventions will be reported to site IRB and the St. Jude IRB within the reporting requirements described above. The PI will determine if this is an event that will need to be reported expeditiously to all participating sites, considering the following criteria:

- Is the AE serious, unexpected, and related or possibly related to participation in the research?
- Is the AE expected, but occurring at a significantly higher frequency or severity than expected?
- Is this an AE that is unexpected (regardless of severity that may alter the IRB's analysis of the risk versus potential benefit of the research *and*, as a result, warrant consideration of substantive changes in the research protocol or informed consent process/document?

With the submission of the "Reportable Event" in St. Jude TRACKS application, the PI will indicate if all sites should be notified to report to their IRBs, and if the protocol and/or consent should be amended (consent will be amended if event is information that should be communicated to currently enrolled subjects).

Generally, only events that warrant an amendment to the protocol and/or consent will be reported expeditiously to all sites. However, any event may be reported expeditiously to all sites at the discretion of the PI. A cumulative summary of Grade 3–5 events during initial treatment and Grade 3–5 AEs and expected/unrelated deaths that occur more than 30 days off last protocol treatment will be reported to all sites with study progress report at the time of continuing review.

15.0 DATA COLLECTION, STUDY MONITORING, AND CONFIDENTIALITY

15.1 Data Collection

Electronic case report forms (eCRFs) will be completed by the SJCRH Solid Tumor CRAs. Data will be entered directly from record directly into a secure protocol-specific CRIS database, developed and maintained by St. Jude Clinical Research Informatics.

Data Management will be supervised by the Director of Clinical Trials Management, and Manager of Clinical Research Operations for the Solid Tumor Division, working with Dr. Furman and Cancer Center Research Informatics. All protocol-specific data and all grade 3–5 adverse events will be recorded by the clinical research associates into the CRIS

database, ideally within 2-4 weeks of completion of study phase. All questions will be directed to the attending physician and/or PI and reviewed at regularly-scheduled working meetings. The attending physicians (or their designees) are responsible for keeping up-to-date roadmaps in the participant's primary SJCRH medical chart.
Regular (at least monthly) summaries of toxicity and protocol events will be generated for the PI and the department of Biostatistics to review.

15.2 Study Monitoring

Monitoring of this protocol is considered to be in the "High Risk (HR-3)" category. The Eligibility Coordinators will verify 100 % of the informed consent documentation on all participants and verify 100% of St. Jude participants' eligibility status within 5 working days of the completion of enrollment.

The study team will meet at appropriate intervals to review case histories or quality summaries on participants.

The Clinical Research Monitor will assess protocol and regulatory compliance as well as the accuracy and completeness of all data points for the first two participants then 15% of study enrollees every six months. Accrual will be tracked continuously for studies that have strata. All SAE reports will be monitored for type, grade, attribution, duration, timeliness and appropriateness on all study participants *semi-annually.*

The monitor will also verify 100% of all data points on the first two participants and on 15% of cases thereafter. Protocol compliance monitoring will include participant status, eligibility, the informed consent process, demographics, staging, study objectives, subgroup assignment, treatments, evaluations, responses, 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.

15.3 Confidentiality

Study numbers will be used in place of an identifier such as a 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. The medical records of study participants may be reviewed by the St. Jude IRB, FDA, and St. Jude clinical research monitors.

16.0 STATISTICAL CONSIDERATIONS

16.1 Primary Objectives

16.1.1 Primary Objective 1.1.1 – To study the efficacy (response: CR+PR) to two initial courses of cyclophosphamide and topotecan combined with hu14.18K322A (4 doses / course with GM-CSF, in previously untreated children with high-risk neuroblastoma.

Responsible Investigators: Wayne L. Furman, Sara Federico.
Responsible Biostatisticians: Deo Kumar Srivastava, Shenghua Mao
Estimated date for completion of data collection: 12/30/2020

The primary objective is to study the antitumor activity (response: CR+PR) to two initial courses of cyclophosphamide and topotecan combined with humanized anti-GD2 antibody (hu14.18K322A) in previously untreated children with high-risk neuroblastoma. A historical COG trial[33] had an overall response rate 40% (12/30) following two cycles of cyclophosphamide and topotecan induction therapy for newly diagnosed high risk neuroblastoma patients. The current study is designed so that the overall response rate (CP+PR) after two initial courses of chemotherapy (cyclophosphamide and topotecan combined with hu14.18K322A) is no worse than 40%. The hypotheses to be tested are H_0: $p \leq 0.40$ versus H_1: $p > 0.40$, where p is the true response rate. The sample size of study is calculated at an alternative response rate of 60%, the response rate which is considered worthy of further evaluation of the new therapy, with study power of 90% and type I error 5%. A total of 61 patients are needed for the study.

A three-stage group sequential design[181] is used to allow an early stopping if the convincing evidence for lack of antitumor activity is observed and report for efficacy if the treatment shows significant antitumor activity. The study will stop accrual of patients for futility only but not for efficacy. The futility and efficacy boundaries of the three-stage design are shown in the table below. The accrual will not stop for the interim analysis.

Three-stage group sequential design for futility and efficacy

Group Number	Group Size	Total # of patients	Futility boundary if # of response ≤	Efficacy boundary if # of response ≥
1	20	20	4	17
2	22	42	15	29
3	19	61	30	31

All participants who begin course 1 of cyclophosphamide and topotecan combined with hu14.18K322A will be considered evaluable for assessing response, except the rare patient which withdraws from the trial at the request of the parent or patient. Patients who fail to be evaluated for response at the end of the second course due to unacceptable toxicity or progressive disease will be considered as a failure in response evaluation.

If the interim analysis shows futility of the initial two courses treatment, then accrual will be suspended and consideration will be given to amending the protocol.

16.1.2 Primary Objective 1.1.2 – To estimate the event-free survival of patients with newly diagnosed high-risk neuroblastoma treated with the addition of hu14.18K322A to each phase of treatment (Induction, Consolidation, Experimental MRD* and MRD Treatment as outlined in Section 4.0, Treatment Plan).
*Note: Experimental MRD phase deleted with Amendment 8.0

Responsible Investigators: Wayne L. Furman, Sara Federico.
Responsible Biostatisticians: Deo Kumar Srivastava, Shenghua Mao
Estimated date for completion of data collection: 7/30/2022

To estimate the event-free survival of patients with newly diagnosed high-risk neuroblastoma treated with the addition of hu14.18K322A to induction chemotherapy with cyclophosphamide and topotecan for two courses followed by two courses each of alternating cisplatin and etoposide (CiE) and cyclophosphamide, doxorubicin and vincristine (CAV).

Responsible Investigators: Wayne L. Furman, Sara Federico.
Responsible Biostatisticians: Deo Kumar Srivastava, Shenghua Mao
Estimated date for completion of data collection: 07/30/2022

Recently, COG conducted a phase III randomized trial (ANBL0532) of single vs tandem myeloablative consolidation therapy for high-risk neuroblastoma. The study shows the 3-year EFS for all 652 eligible patients (combined two arms) starting from time of study enrollment was 50.9±2.2%. Thus, a 95% confidence interval of 3-year EFS for all patients is (46.5%, 55.3%). Because the 3-year EFS reported in the COG study was combining two arms and the 3-years EFS for tandem transplant is 15% higher than single, thus, we design the study using the lower boundary of the 95% confidence interval as the hypothesis of the 3-year EFS for this trial. Thus, we will test if the 3-year EFS for patients treated on NB2012 is less than 46% or great than 62% (a 16% increasing of 3-year EFS). With type I error 5% and power of 80%, a total of 61 patients are need for the

trial to detect a 16% increasing 3-EFS. The EFS will be estimated at 3 years after the last patient is enrolled on the study.

16.2 Anticipated Timeframe for Completion of Accrual

Accrual on the prior NB2005 study was anticipated to be 13 patients per year. NB2005 accrual was on target as seen in Table 16.1 which shows actual and anticipated accrual by quarter for all 23 patients enrolled on NB2005 and 87% of NB2005 patients received window therapy (20/23). As 61 evaluable patients will be needed for the primary objectives, we anticipate that the study accrual will take approximately 5.5 years based on a historical rate of 13 patients per year (to complete accrual for the primary objective) and 87% (20/23) evaluable rate of NB2005. Thus approximately 61 evaluable patients will be accrued to this study with study duration about 7 years. The EFS and overall survival data will be analyzed in about 8.5 years after the trial activation (5.5 years accrual period plus 3 years follow-up).

The study will be closed to accrual when 61 evaluable patients have been enrolled on the study. We estimate that 70 patients will be enrolled in order to achieve 61 evaluable patients.

Table 16.1: Observed and anticipated accrual on the NB2005 protocol

	Actual Accrual	Estimated Accrual
Recruitment Period		
Year 1: Quarter 1 (August 16, 2005 – November 15, 2005)	4	3.25
Year 1: Quarter 2 (November 16, 2005 – February 15, 2006)	2	3.25
Year 1: Quarter 3 (February 16, 2006 – May 15, 2006)	4	3.25
Year 1: Quarter 4 (May 16, 2006 – August 15, 2006)	5	3.25
Year 2: Quarter 1 (August 16, 2006 – November 15, 2006)	2	3.25
Year 2: Quarter 2 (November 16, 2006 – February 15, 2007)	2	3.25
Year 2: Quarter 3 (February 16, 2007 – May 15, 2007)	4	3.25
Total	**23**	**22.75**

16.3 Secondary Objectives

Responsible Investigators: Wayne L. Furman, Sara Federico
Responsible Biostatisticians: Deo Kumar Srivastava, Shenghua Mao
Estimated date for completion of data collection: 12/30/2020

16.3.1 To study the feasibility of delivering hu14.18K322A to 6 cycles of induction chemotherapy and describe the antitumor activity (CR+PR) of this 6 course induction therapy.

The feasibility of delivering hu14.18K332A to 6 cycles of induction chemotherapy will be monitored in this trial. Historical data for 11 patients treated with same identical induction therapy without antibody had a median of 141 days (range 122–151 days) with a one-sided 95% confidence upper limit of 155 days. Therefore, a patient will be considered as a failure for the 6 cycles of induction therapy if the patient failed to complete induction therapy within 155 days due to toxicity or disease progression, unless delay is a result of non-medical issues (e.g. not due to protocol toxicity).

Based on Park's paper,[33] using identical induction chemotherapy, without anti-GD2 antibody, one of 31 (3.2%) newly diagnosed high-risk NB patients had disease progression during the 6 cycles induction therapy. A 95% confidence interval (Blyth-Still-Casella) upper bound for the induction failure rate is 16.7%. Thus, the feasibility of induction therapy for this study will be target no worse than 75%. A three-stage group sequential design[182] is proposed to monitor induction therapy based on the hypothesis that the induction "success" rate will be no worse than 75% based on the 61 patients enrolled on study. The design is powered at 88% success rate with type I error 5% and power 80%. The three-stage monitoring rule is given in Table 16.2. The accrual will not stop for the interim analysis.

Table 16.2: Induction feasibility three-stage monitoring rule (EAST 6)

Stage	Group size	Total size	Lower bound (induction therapy is rejected if # of patients who do not experience induction failure ("success") is less than or equal to)	Reject the induction feasibility if # of patients who experience induction failure ≥
1	20	20	12	6
2	22	42	32	8
3	19	61	51	10

At the first stage, if 14 or less "success" (do not experience induction failure) of the first cohort of 20 patients, then the study accrual will be closed and consideration will be given to amending the protocol, otherwise the trial will go to Stage 2. At Stage 2, if 32 or less "success" of the first 40 evaluable patients, then the study accrual will be closed and consideration will be given to amending the protocol, otherwise the trial will go to Stage 3. At stage 3, if 51 or less "success", then the induction therapy is not feasible.

The proportion of patients who successfully receive hu14.18K322A with 6 cycles of induction chemotherapy will be estimated together with a 95% confidence interval. The response rate (CR+PR) to 6 cycles of induction chemotherapy will be estimated together with the 95% confidence intervals.

16.3.2 To estimate local control and pattern of failure associated with intensity modulated radiation therapy and proton beam radiation dose delivery in high-risk abdominal neuroblastoma.

Responsible Investigators: Wayne L. Furman, Sara Federico
Responsible Biostatisticians: Deo Kumar Srivastava, Shenghua Mao
Estimated date for completion of data collection: 12/30/2020

Local failure is defined as relapse or progression of disease at the primary site. The cumulative incidence of local failure will be estimated; competing events will include distant failure or death prior to local failure.

16.3.3 To describe the tolerability of four doses of hu14.18K322A with IL-2 and allogeneic NK cells from an acceptable parent, in the immediate post-transplant period in consenting patients*

*Note: Experimental MRD phase deleted with Amendment 8.0

Responsible Investigators: Brandon Triplett, William Janssen
Responsible Biostatisticians: Deo Kumar Srivastava, Shenghua Mao
Estimated date for completion of data collection: 12/30/2020

Participants will be considered evaluable for tolerability if they receive four doses of hu14.18K332A with allogeneic NK cells in the immediate post-transplant period, or if hu14.18K322A is discontinued early for presumed toxicity.

It is expected that a majority of patients will consent for this additional treatment.

Patients after myeloablative doses of busulfan and melphalan can experience significant toxicities. Most toxicity that occurs due to the hu14.18K322A is not unexpected and should be non-overlapping with those of busulfan/melphalan. However, we plan to consider a subset of the toxicities dose-limiting and unacceptable for this additional MRD treatment (hu14.18K322A with allogeneic (parental) NK cells).

Those DLTs designated as unacceptable are:

1. Toxicity requiring the use of pressors, including Grade 4 acute capillary leak syndrome or Grade3 and 4 hypotension.
2. Toxicity requiring ventilation support, including Grade 4 respiratory toxicity.
3. Grade 3 or 4 neurotoxicity with MRI evidence of new CNS thrombi, infarction or bleeding in any subject receiving the hu14.18K322A with NK cell combination.
4. Failure of recovery of ANC > 500/mm^3 by day 35 after PBSC infusion.

We will test for tolerability and monitor for the occurrence of too many unacceptable DLTs **or severe (grade 3 or 4) VoD** during the recovery phase after busulfan/melphalan and PBSC rescue using the following ad hoc two-stage stopping rule:

Stage 1: Accrue 10 patients at an initial 25 mg/m^2/dose of hu14.18K322A. If 1 or no patient out of the first 10 patients experiences at least one unacceptable DLT during this phase treatment (immediate post-PBSC infusion period) and 2 or less patients experience severe (grade 3 or 4) VoD (one-sided lower bounds of 95% Blyth-Still-Casella confidence interval for 2 out of the first 10 and 3 out of the first 10 experience severe VoD are 5.4% and 11.6%, respectively), then continue to Stage 2. If 2 or more experience at least one unacceptable DLT during this phase treatment or 3 or more patients out of the first 10 experience severe VoD, the regimen will be considered to have unacceptable toxicity, and the dosage of hu14.18K322A will be decreased to 17.5 mg/m^2/dose (identical to dose use in United Therapeutics protocol DIV-NB-201). An additional 10 patients will be accrued at this reduced dosage level. If 2 or more experience at least one unacceptable DLT or 3 or more experience severe VoD, the regimen will be considered to have unacceptable toxicity, and will be temporarily closed, in order to review all relevant data and consider modifying the regimen to improve safety. If 1 or no patients have an unacceptable DLT and 2 or less patients experience severe (grade 3 or 4) VoD at this reduced dosage level of

hu14.18K322A, then all subsequent patients will receive this reduced dosage level of hu14.18K322A of 17.5 mg/m^2/dose.

Stage 2: Increase dose of hu14.18K322A to 40 mg/m^2/dose and accrue 10 patients. As in stage 1, if 2 or more experience at least one unacceptable DLT or 3 or more patients experience severe VoD, the regimen will be considered to have unacceptable toxicity at this increased dosage level of hu14.18K322A (40 mg/m^2/dose), and the remaining patients will be given the initial dosage of 25 mg/m^2/dose. If 1 or no patients have an unacceptable DLT and 2 or less patients experience severe (grade 3 or 4) VoD, then it is reasonable to assume that the combination therapy is safe and accrue all of the remaining patients to this dose-level of hu14.18K322A (40mg/m^2/dose).

The accrual will not stop for the interim analyses. Patient who reaches the post-treatment period will be assigned to the current dose of hu14.18K322A before knowing the results of analysis. It is estimated that 1 or 2 patients expected to enroll on the trial before the interim analysis results are known.

16.3.4 To describe the tolerability of hu14.18K322A with IL-2 and GM-CSF as treatment for MRD, in consenting patients.

Responsible Investigators: Wayne L. Furman, Sara Federico
Responsible Biostatisticians: Deo Kumar Srivastava, Shenghua Mao
Estimated date for completion of data collection: 12/30/2020

Participants will be considered evaluable for tolerability if they receive at least one dose of hu14.18K322A with course 2 of MRD treatment (the first course given with IL-2). It is expected that 30 or more patients (about 80% of the total 42 patients) will go through with the course 2 of MRD treatment.

Many of the dose-limiting toxicities (DLTs) that occur due to the hu14.18K322A given with IL2 are not unexpected. However, as in the ANBL0322 protocol, we plan to consider a subset of the DLTs unacceptable for the second course of MRD treatment (hu14.18K322A with IL-2).

Those DLTs designated as unacceptable are:

1. Toxicity requiring the use of pressors, including Grade 4 acute capillary leak syndrome or Grade 3 and 4 hypotension.
2. Toxicity requiring ventilation support, including Grade 4 respiratory toxicity.*

3. Grade 3 or 4 neurotoxicity with MRI evidence of new CNS thrombi, infarction or bleeding in any subject receiving the hu14.18K322A with IL2 + isotretinoin combination.

*If at any time a participant requires ventilator support, new accrual to the study will be temporarily stopped, and the study will be reviewed for safety and dose modification. Based on this review, the study may be reopened.

We will test for tolerability and monitor for the occurrence of too many unacceptable DLTs during the second treatment cycle of MRD treatment using the following two-stage stopping rule:

Stage 1: Accrue 10 patients. If 1 or no patient out of the first 10 patients experiences at least one unacceptable DLT during the second MRD treatment cycle (the hu14.18K322A with IL2 + isotretinoin), then continue to Stage 2. If 2 or more out of the first 10 patients experience at least one unacceptable DLT during the second treatment cycle of MRD treatment (the hu14.18K322A with IL2 + isotretinoin) the regimen will be considered to have unacceptable toxicity, and the dosage of hu14.18K322A will be decreased to 17.5 mg/m^2/dose (identical to dose use in United Therapeutics protocol DIV-NB-201). An additional 10 patients will be accrued at this reduced dosage level. If 2 or more experience at least one unacceptable DLT during the second MRD treatment cycle (the hu14.18K322A with IL2 + isotretinoin) the regimen will be considered to have unacceptable toxicity, and will be temporarily closed, in order to review all relevant data and consider modifying the regimen to improve safety. If 1 or no patients have an unacceptable DLT at this reduced dosage level of hu14.18K322A, then all subsequent patients will receive this reduced dosage level of hu14.18K322A of 17.5 mg/m^2/dose.

Stage 2: Increase dose of hu14.18K322A to 40 mg/m^2/dose and accrue 10 patients. As in stage 1, if more than 1 experience at least one unacceptable DLT during the second treatment cycle (the hu14.18K322A with IL2 + isotretinoin) the regimen will be considered to have unacceptable toxicity at this increased dosage level of hu14.18K322A (40 mg/m^2/dose), and the remaining patients will be given the initial dosage of 25 mg/m^2/dose. If 1 or no patients have an unacceptable DLT, then it is reasonable to assume that the combination therapy is safe and accrue all of the remaining patients to this dose-level of hu14.18K322A.

The accrual will not stop for the interim analyses. Patient who reaches the post-treatment period will be assigned to the current dose of hu14.18K322A before knowing the results of analysis. It is estimated that

1 or 2 patients expected to enroll on the trial before the interim analysis results are known.

16.4 Exploratory Biology Objectives

16.4.1 To measure natural killer (NK) cell number and function during Induction, Intensification/Expermimental MRD, and MRD/Maintenance*.

Responsible Investigator: William Janssen
Responsible Biostatisticians: Deo Kumar Srivastava, Shenghua Mao
Estimated date for completion of data collection: 12/30/2020

NK cell number and function will be measured using phenotypic markers, identified with fluorochrome tagged monoclonal antibodies and enumerated using flow cytometry. The resultant data will be summarized by descriptive statistics.

Specific NK cell markers that will be examined are summarized in the following table:

Cell Surface Marker:	Rationale:
$CD3^{neg}$,$CD56^{bright/dim}$,$NKp46^{pos}$	Basic NK cell identity, subsets
CD45RA	Early stage NK cells
KIR isoforms (CD158a-h,CD159a,NKB1, NKG2a)	Killer Immunoglobulin-like Receptors - MHC class I recognition & inhibition

Rationale: NK cells are associated with killing of cells lacking self MHC class I, or lacking MHC class I altogether, as is the case with neuroblastoma. As these cells are apparently short lived, it is expected that their numbers and types in circulation will coincide with treatment phases and associated MRD.

*Note: Experimental MRD phase deleted with Amendment 8.0

16.4.2 To measure T-lymphocyte subset number and function during Induction, Intensification/Expermimental MRD*, and MRD/Maintenance

Responsible Investigator: William Janssen
Responsible Biostatisticians: Deo Kumar Srivastava, Shenghua Mao
Estimated date for completion of data collection: 12/30/2020

T-lymphocyte and subset number and function will be measured using phenotypic markers, idenfitied with fluorochrome tagged monoclonal antibodies and enumerated using flow cytometry. The resultant data will be summarized by descriptive statistics.

Specific T-lymphocyte markers that will be examined are summarized in the following table:

Cell Surface Markers	Rationale
CD3,CD4,CD8	T lymphocytes, helper T and cytotoxic/suppressor T cells
CD4,CD25,CD127dim	Regulatory T cells
CD45RA,TCRalpha/beta,CD62L	Naïve T-lymphocytes
CD45RO,TCRgamma/delta	Memory T-lymphocytes

*Note: Experimental MRD phase deleted with Amendment 8.0

16.4.3 To genotype natural killer (NK) cell receptors and measure their expression at diagnosis and after the initial six weeks of therapy, and to associate these features with treatment outcome.*

Responsible Investigator: William Janssen
Responsible Biostatisticians: Deo Kumar Srivastava, Shenghua Mao

Estimated date for completion of data collection: 12/30/2020

For these explorative objectives, associations of genotypes (or gene expressions) with long-term treatment outcomes will be explored by failure time models; associations of the genotypes with categorical clinical outcomes (e.g., MRD status at the end of the first 6 weeks of treatment) will be analyzed by contingency tables and Chi-square tests; associations of gene expressions with categorical clinical outcomes will be analyzed by rank-based procedures such as the Wilcoxon test.
*Note: Experimental MRD phase deleted with Amendment 8.0

16.4.4 To describe the relative frequency of positive bone marrow and peripheral blood by sensitive MRD methods at diagnosis, after the initial six weeks of therapy, at the time of stem cell harvest, and at several time points following the completion of intensification. These results will be compared with timing and pattern of disease recurrence.

Responsible Investigators: Paul Mead, Wayne Furman, Sara Federico
Responsible Biostatisticians: Deo Kumar Srivastava, Shenghua Mao
Estimated date for completion of data collection: 12/30/2020

The relative frequency of positive bone marrow by sensitive MRD methods will be assessed at diagnosis, after six weeks, at the time of stem cell harvest, and at several time points after completion of intensification. The relationships of these results with timing and pattern of disease recurrence will be assessed using appropriate methods, including longitudinal methods.

16.4.5 To procure tumor samples for construction of tissue microarray blocks that will be utilized in further biologic characterization of these tumors.

This objective has no statistical issues.

16.4.6 To assess the feasibility of measuring plasma catecholamine metabolites in patients with known neuroblastoma and to obtain preliminary data on the possible utility of plasma catecholamine metabolite analyses in patients with neuroblastoma

The numbers of patients with elevated plasma catecholamine metabolites will be presented and the results will be reported descriptively.

16.5 Exploratory Diagnostic Imaging Objective

Responsible Investigators: Beth McCarville, Barry Shulkin, Wayne Furman,

Sara Federico
Responsible Biostatisticians: Deo Kumar Srivastava, Shenghua Mao
Estimated date for completion of data collection: 12/30/2020

To determine if there is a correlation between the number of Image Defined Risk Factors (as defined in the International Neuroblastoma Risk Group Staging System[1]) and outcome of patients with high risk neuroblastoma.

The correlation between the number of Image Defined Risk Factors and outcome will be investigated by regression model.

16.6 Exploratory Radiation Oncology Objective

Responsible Investigators: Matthew Krasin, John Lucas
Responsible Biostatisticians: Deo Kumar Srivastava, Shenghua Mao
Estimated date for completion of data collection: 12/30/2020

To perform a simple comparison of renal radiation doses and other available adjacent organs between photon IMRT techniques and proton beam radiation techniques.

We will compare by simple descriptive statistics doses received by the targets, adjacent kidney as well as other organs such as bowel and liver based to treatment technique (photon vs. proton).

16.7 Exploratory Pharmacokinetic Study Objective

Responsible Investigators: Allison Bragg, Ashok Srinivasan, Wayne Furman, Sara Federico
Responsible Biostatisticians: Deo Kumar Srivastava, Shenghua Mao
Estimated date for completion of data collection: 7/30/2022

To assess the ability to achieve the target systemic exposure of intravenous busulfan in patients with neuroblastoma undergoing stem cell transplant, and to explore possible associations between busulfan pharmacokinetic parameters and patient's outcome (e.g., response, event-free survival and overall survival) and specific covariates (e.g. age, sex, race, weight).

The association between busulfan pharmacokinetic parameters and event-free survival/overall survival will be studied by Cox regression model, and the association between busulfan pharmacokinetic parameters and patient's response and covariates will be explored by regression model.

17.0 OBTAINING INFORMED CONSENT

17.1 Consent/Assent at Enrollment

The process of informed consent for NB2012 will follow institutional policy. The informed consent process is an ongoing one that begins at the time of diagnosis and ends after the completion of therapy. Informed consent should be obtained by the attending physician or his/her designee, in the presence of at least one non-physician witness. Initially, informed consent will be sought for the institutional banking protocol (research study), and other procedures as necessary. After the diagnosis of neuroblastoma is established, we will invite the patient to participate in the NB2012 protocol. Informed consent will be obtained prior to any screening procedures/evaluations/tests are performed for eligibility or to meet pre-study requirements.

Throughout the entire treatment period, participants and their parents receive constant education from health professionals at St. Jude and are encouraged to ask questions regarding alternatives and therapy. All families have ready access to chaplains, psychologists, social workers, and the St. Jude ombudsperson for support, in addition to that provided by the primary physician and other clinicians involved in their care.

We will also obtain verbal assent from children 7 to 14 years old and written assent for all participants $\geq$14 years of age. Participants who reach the age of majority while on study will be re-consented for continued participation on NB2012, according to Cancer Center and institutional policy.

17.2 Consent at Age of Majority

The age of majority in the state of Tennessee is 18 years old. Research participants must be consented at the next clinic visit after their 18th birthday. If an affiliate is located in a country or state where a different age of majority applies, that location must consent the participants according to their local laws.

17.3 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.

18.0 REFERENCES

1. Monclair T, Brodeur GM, Ambros PF, et al. The International Neuroblastoma Risk Group (INRG) staging system: an INRG Task Force report. J Clin Oncol 2009;27:298–303.
2. Matthay KK, Villablanca JG, Seeger RC, et al. Treatment of high–risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13–cis–retinoic acid. Children's Cancer Group. N Engl J Med 1999;341:1165–73.
3. Yu AL, Gilman AL, Ozkaynak MF, et al. Anti–GD2 antibody with GM–CSF, interleukin–2, and isotretinoin for neuroblastoma. N Engl J Med 2010;363:1324–34.
4. Schulz G, Cheresh DA, Varki NM, Yu A, Staffileno LK, Reisfeld RA. Detection of ganglioside GD2 in tumor tissues and sera of neuroblastoma patients. Cancer Res 1984;44:5914–20.
5. Maris JM. The biologic basis for neuroblastoma heterogeneity and risk stratification. Curr Opin Pediatr 2005;17:7–13.
6. Nowak AK, Robinson BW, Lake RA. Synergy between chemotherapy and immunotherapy in the treatment of established murine solid tumors. Cancer Res 2003;63:4490–6.
7. Lake RA, Robinson BW. Immunotherapy and chemotherapy––a practical partnership. Nat Rev Cancer 2005;5:397–405.
8. Buhtoiarov IN, Sondel PM, Wigginton JM, et al. Anti–tumour synergy of cytotoxic chemotherapy and anti–CD40 plus CpG–ODN immunotherapy through repolarization of tumour–associated macrophages. Immunology 2011;132:226–39.
9. Holden SA, Lan Y, Pardo AM, Wesolowski JS, Gillies SD. Augmentation of antitumor activity of an antibody–interleukin 2 immunocytokine with chemotherapeutic agents. Clin Cancer Res 2001;7:2862–9.
10. Ghiringhelli F, Larmonier N, Schmitt E, et al. CD4+CD25+ regulatory T cells suppress tumor immunity but are sensitive to cyclophosphamide which allows immunotherapy of established tumors to be curative. European journal of immunology 2004;34:336–44.
11. Menard C, Martin F, Apetoh L, Bouyer F, Ghiringhelli F. Cancer chemotherapy: not only a direct cytotoxic effect, but also an adjuvant for antitumor immunity. Cancer immunology, immunotherapy : CII 2008;57:1579–87.
12. Zitvogel L, Apetoh L, Ghiringhelli F, Kroemer G. Immunological aspects of cancer chemotherapy. Nature reviews Immunology 2008;8:59–73.
13. Barker E, Mueller BM, Handgretinger R, Herter M, Yu AL, Reisfeld RA. Effect of a Chimeric Anti–Ganglioside GD2 Antibody on Cell–mediated Lysis of Human Neuroblastoma Cells. Cancer Res 1991;51:144–9.
14. Raffaghello L, Marimpietri D, Pagnan G, et al. Anti–GD2 monoclonal antibody immunotherapy: a promising strategy in the prevention of neuroblastoma relapse. Cancer Lett 2003;197:205–9.

15. Nowak AK, Lake RA, Marzo AL, et al. Induction of tumor cell apoptosis in vivo increases tumor antigen cross–presentation, cross–priming rather than cross–tolerizing host tumor–specific CD8 T cells. J Immunol 2003;170:4905–13.
16. Hiddemann W, Kneba M, Dreyling M, et al. Frontline therapy with rituximab added to the combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) significantly improves the outcome for patients with advanced–stage follicular lymphoma compared with therapy with CHOP alone: results of a prospective randomized study of the German Low–Grade Lymphoma Study Group. Blood 2005;106:3725–32.
17. Coiffier B, Lepage E, Briere J, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large–B–cell lymphoma. NEnglJ Med 2002;346:235–42.
18. Slamon DJ, Leyland–Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. NEnglJ Med 2001;344:783–92.
19. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N EnglJ Med 2004;350:2335–42.
20. Dillman RO. Cancer immunotherapy. Cancer biotherapy & radiopharmaceuticals 2011;26:1–64.
21. Robert C, Thomas L, Bondarenko I, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med 2011;364:2517–26.
22. Yoshida S, Kawaguchi H, Sato S, Ueda R, Furukawa K. An anti–GD2 monoclonal antibody enhances apoptotic effects of anti–cancer drugs against small cell lung cancer cells via JNK (c–Jun terminal kinase) activation. JpnJ Cancer Res 2002;93:816–24.
23. Kowalczyk A, Gil M, Horwacik I, Odrowaz Z, Kozbor D, Rokita H. The GD2–specific 14G2a monoclonal antibody induces apoptosis and enhances cytotoxicity of chemotherapeutic drugs in IMR–32 human neuroblastoma cells. Cancer Lett 2009;281:171–82.
24. Nakamura K, Hanibuchi M, Yano S, et al. Apoptosis induction of human lung cancer cell line in multicellular heterospheroids with humanized antiganglioside GM2 monoclonal antibody. Cancer Res 1999;59:5323–30.
25. Aixinjueluo W, Furukawa K, Zhang Q, et al. Mechanisms for the apoptosis of small cell lung cancer cells induced by anti–GD2 monoclonal antibodies: roles of anoikis. J Biol Chem 2005;280:29828–36.
26. Katano M, Irie RF. Human monoclonal antibody to tumor–associated ganglioside GD2: suppressed growth of human melanoma in nude mice. Immunology letters 1984;8:169–74.
27. Katano M, Jien M, Irie RF. Human monoclonal antibody to ganglioside GD2–inhibited human melanoma xenograft. European journal of cancer & clinical oncology 1984;20:1053–9.
28. Carter P. Improving the efficacy of antibody–based cancer therapies. Nat

Rev Cancer 2001;1:118–29.
29. Yoshida S, Fukumoto S, Kawaguchi H, Sato S, Ueda R, Furukawa K. Ganglioside G(D2) in small cell lung cancer cell lines: enhancement of cell proliferation and mediation of apoptosis. Cancer Res 2001;61:4244–52.
30. Navid F, Santana VM, Barfield RC. Anti–GD2 antibody therapy for GD2–expressing tumors. Curr Cancer Drug Targets 2010;10:200–9.
31. Navid F BR, Handgretinger R, Sondel PM, Shulkin BL, Kaufman R, Billups C, Wu J, Furman WL, McGregor LM, Otto M, Gillies S, Santana VM. A novel anti–GD2 monoclonal antibody (mAb), hu14.18K322A, in children with refractory or recurrent neuroblastoma: Early–phase evaluation. J Clin Oncol 2011;29:a9523.
32. Modak S, Cheung NK. Disialoganglioside directed immunotherapy of neuroblastoma. Cancer Invest 2007;25:67–77.
33. Park JR, Scott JR, Stewart CF, et al. Pilot Induction Regimen Incorporating Pharmacokinetically Guided Topotecan for Treatment of Newly Diagnosed High–Risk Neuroblastoma: A Children's Oncology Group Study. Journal of Clinical Oncology 2011;29:4351–57.
34. McWilliams NB, Hayes FA, Green AA, et al. Cyclophosphamide/doxorubicin vs. cisplatin/teniposide in the treatment of children older than 12 months of age with disseminated neuroblastoma: a Pediatric Oncology Group Randomized Phase II study. Med Pediatr Oncol 1995;24:176–80.
35. Pearson AD, Pinkerton CR, Lewis IJ, Imeson J, Ellershaw C, Machin D. High–dose rapid and standard induction chemotherapy for patients aged over 1 year with stage 4 neuroblastoma: a randomised trial. Lancet Oncol 2008;9:247–56.
36. Pinkerton CR, Blanc Vincent MP, Bergeron C, Fervers B, Philip T. Induction chemotherapy in metastatic neuroblastoma––does dose influence response? A critical review of published data standards, options and recommendations (SOR) project of the National Federation of French Cancer Centres (FNCLCC). Eur J Cancer 2000;36:1808–15.
37. Matthay KK, Reynolds CP, Seeger RC, et al. Long–term results for children with high–risk neuroblastoma treated on a randomized trial of myeloablative therapy followed by 13–cis–retinoic acid: a children's oncology group study. J Clin Oncol 2009;27:1007–13.
38. Zage PE, Kletzel M, Murray K, et al. Outcomes of the POG 9340/9341/9342 trials for children with high–risk neuroblastoma: a report from the Children's Oncology Group. Pediatr Blood Cancer 2008;51:747–53.
39. Grupp SA, Stern JW, Bunin N, et al. Tandem High–Dose Therapy in Rapid Sequence for Children With High–Risk Neuroblastoma. Journal of Clinical Oncology 2000;18:2567–75.
40. Kreissman SGV, J.G.;Diller,L;London,W.B.Maris,J.M.;Park,J.R.;Reynolds,C.P.;von Allmen, D.;Cohn,S.L.;Matthay,K.K. Response and toxicity to a dose–intensive multi–agent chemotherapy induction regimen for high risk neuroblastoma (HR–NB): A Children's Oncology Group (COG A3973) study. Journal of Clinical

Oncology2007:a9505.
41. Kushner BH, Kramer K, Meyers PA, Wollner N, Cheung NK. Pilot study of topotecan and high–dose cyclophosphamide for resistant pediatric solid tumors. MedPediatrOncol 2000;35:468–74.
42. Santana VM, Furman WL, Billups CA, et al. Improved response in high–risk neuroblastoma with protracted topotecan administration using a pharmacokinetically guided dosing approach. Journal of Clinical Oncology 2005;23:4039–47.
43. Furman WL, McGregor LM, McCarville MB, et al. A single–arm pilot phase II study of gefitinib and irinotecan in children with newly diagnosed high–risk neuroblastoma. Invest New Drugs 2011;30:1660–70.
44. Kretschmar CS, Kletzel M, Murray K, et al. Response to paclitaxel, topotecan, and topotecan–cyclophosphamide in children with untreated disseminated neuroblastoma treated in an upfront phase II investigational window: a pediatric oncology group study. J Clin Oncol 2004;22:4119–26.
45. Castleberry RP, Cantor AB, Green AA, et al. Phase II investigational window using carboplatin, iproplatin, ifosfamide, and epirubicin in children with untreated disseminated neuroblastoma: a Pediatric Oncology Group study. J Clin Oncol 1994;12:1616–20.
46. Kushner BH, LaQuaglia MP, Bonilla MA, et al. Highly effective induction therapy for stage 4 neuroblastoma in children over 1 year of age. J Clin Oncol 1994;12:2607–13.
47. Brodeur GM, Pritchard J, Berthold F, et al. Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. JClinOncol 1993;11:1466–77.
48. Brodeur GM, Seeger RC, Barrett A, et al. International criteria for diagnosis, staging, and response to treatment in patients with neuroblastoma. J Clin Oncol 1988;6:1874–81.
49. Furman WL, Fairclough DL, Huhn RD, et al. Therapeutic effects and pharmacokinetics of recombinant human granulocyte–macrophage colony–stimulating factor in childhood cancer patients receiving myelosuppressive chemotherapy. J Clin Oncol 1991;9:1022–8.
50. Wagner LM, Furman WL. Haemopoietic growth factors in paediatric oncology: a review of the literature. Paediatr Drugs 2001;3:195–217.
51. Furman WL, Luo X, Marina N, Garrison L, Pratt CB, Meyer WH. Comparison of cytokines in children with recurrent solid tumors treated with intensive chemotherapy. J Pediatr Hematol Oncol 1998;20:62–8.
52. Furman WL, Fairclough D, Cain AM, et al. Use of GM–CSF in children after high–dose chemotherapy. Medical and pediatric oncology Supplement 1992;2:26–30.
53. Waller EK. The role of sargramostim (rhGM–CSF) as immunotherapy. Oncologist 2007;12 Suppl 2:22–6.
54. Wittman B, Horan J, Lyman GH. Prophylactic colony–stimulating factors in children receiving myelosuppressive chemotherapy: a meta–analysis of randomized controlled trials. Cancer Treat Rev 2006;32:289–303.

55. Sung L, Nathan PC, Lange B, Beyene J, Buchanan GR. Prophylactic granulocyte colony–stimulating factor and granulocyte–macrophage colony–stimulating factor decrease febrile neutropenia after chemotherapy in children with cancer: a meta–analysis of randomized controlled trials. J Clin Oncol 2004;22:3350–6.
56. Armitage JO. Emerging applications of recombinant human granulocyte–macrophage colony–stimulating factor. Blood 1998;92:4491–508.
57. Ragnhammar P, Frodin JE, Trotta PP, Mellstedt H. Cytotoxicity of white blood cells activated by granulocyte–colony–stimulating factor, granulocyte/macrophage–colony–stimulating factor and macrophage–colony–stimulating factor against tumor cells in the presence of various monoclonal antibodies. Cancer immunology, immunotherapy : CII 1994;39:254–62.
58. Barker E, Reisfeld RA. A mechanism for neutrophil–mediated lysis of human neuroblastoma cells. Cancer Res 1993;53:362–7.
59. Kushner BH, Cheung NK. GM–CSF enhances 3F8 monoclonal antibody–dependent cellular cytotoxicity against human melanoma and neuroblastoma. Blood 1989;73:1936–41.
60. Cheung IY, Hsu K, Cheung NK. Activation of Peripheral–Blood Granulocytes Is Strongly Correlated With Patient Outcome After Immunotherapy With Anti–GD2 Monoclonal Antibody and Granulocyte–Macrophage Colony–Stimulating Factor. J Clin Oncol 2011.
61. Lindemann A, Brossart P, Hoffken K, et al. Immunomodulatory effects of ultra–low–dose interleukin–2 in cancer patients: a phase–IB study. Cancer immunology, immunotherapy : CII 1993;37:307–15.
62. Smith KA. Lowest dose interleukin–2 immunotherapy. Blood 1993;81:1414–23.
63. Caligiuri MA, Murray C, Soiffer RJ, et al. Extended continuous infusion low–dose recombinant interleukin–2 in advanced cancer: prolonged immunomodulation without significant toxicity. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 1991;9:2110–9.
64. Goldstein D, Sosman JA, Hank JA, et al. Repetitive weekly cycles of interleukin 2: effect of outpatient treatment with a lower dose of interleukin 2 on non–major histocompatibility complex–restricted killer activity. Cancer research 1989;49:6832–9.
65. Lissoni P. Effects of low–dose recombinant interleukin–2 in human malignancies. The cancer journal from Scientific American 1997;3 Suppl 1:S115–20.
66. Meropol NJ, Porter M, Blumenson LE, et al. Daily subcutaneous injection of low–dose interleukin 2 expands natural killer cells in vivo without significant toxicity. Clinical cancer research : an official journal of the American Association for Cancer Research 1996;2:669–77.
67. Hanninen EL, Korfer A, Hadam M, et al. Biological monitoring of low–dose interleukin 2 in humans: soluble interleukin 2 receptors, cytokines, and cell

surface phenotypes. Cancer research 1991;51:6312–6.
68. Munn DH, Cheung NK. Interleukin–2 enhancement of monoclonal antibody–mediated cellular cytotoxicity against human melanoma. Cancer research 1987;47:6600–5.
69. La Quaglia MP, Kushner BH, Heller G, Bonilla MA, Lindsley KL, Cheung NK. Stage 4 neuroblastoma diagnosed at more than 1 year of age: gross total resection and clinical outcome. JPediatrSurg 1994;29:1162–5.
70. Haase GM, O'Leary MC, Ramsay NK, et al. Aggressive surgery combined with intensive chemotherapy improves survival in poor–risk neuroblastoma. JPediatrSurg 1991;26:1119–23.
71. Tsuchida Y, Yokoyama J, Kaneko M, et al. Therapeutic significance of surgery in advanced neuroblastoma: a report from the study group of Japan. JPediatrSurg 1992;27:616–22.
72. Shorter NA, Davidoff AM, Evans AE, Ross AJ, III, Zeigler MM, O'Neill JA, Jr. The role of surgery in the management of stage IV neuroblastoma: a single institution study. MedPediatrOncol 1995;24:287–91.
73. Medary I, Aronson D, Cheung NK, Ghavimi F, Gerald W, La Quaglia MP. Kinetics of primary tumor regression with chemotherapy: implications for the timing of surgery. AnnSurgOncol 1996;3:521–5.
74. Davidoff AM, Corey BL, Hoffer FA, Santana VM, Furman WL, Shochat SJ. Radiographic assessment of resectability of locoregional disease in children with high–risk neuroblastoma during neoadjuvant chemotherapy. PediatrBlood Cancer 2004.
75. Bowman LC, Hancock ML, Santana VM, et al. Impact of intensified therapy on clinical outcome in infants and children with neuroblastoma: the St Jude Children's Research Hospital experience, 1962 to 1988. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 1991;9:1599–608.
76. Cheung NV, Heller G. Chemotherapy dose intensity correlates strongly with response, median survival, and median progression–free survival in metastatic neuroblastoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 1991;9:1050–8.
77. Matthay KK, O'Leary MC, Ramsay NK, et al. Role of myeloablative therapy in improved outcome for high risk neuroblastoma: review of recent Children's Cancer Group results. EurJ Cancer 1995;31A:572–5.
78. Pritchard J. "Megatherapy" for advanced neuroblastoma––rationale and role. European journal of cancer 1995;31A:134–6.
79. Stram DO, Matthay KK, O'Leary M, et al. Consolidation chemoradiotherapy and autologous bone marrow transplantation versus continued chemotherapy for metastatic neuroblastoma: a report of two concurrent Children's Cancer Group studies. JClinOncol 1996;14:2417–26.
80. Ladenstein R, Potschger U, Hartman O, et al. 28 years of high–dose therapy and SCT for neuroblastoma in Europe: lessons from more than 4000 procedures. Bone marrow transplantation 2008;41 Suppl 2:S118–27.
81. Flandin I, Hartmann O, Michon J, et al. Impact of TBI on late effects in

children treated by megatherapy for Stage IV neuroblastoma. A study of the French Society of Pediatric oncology. International journal of radiation oncology, biology, physics 2006;64:1424–31.
82. Yalcin B, Kremer LC, Caron HN, van Dalen EC. High–dose chemotherapy and autologous haematopoietic stem cell rescue for children with high–risk neuroblastoma. The Cochrane database of systematic reviews 2010:CD006301.
83. Berthold F, Boos J, Burdach S, et al. Myeloablative megatherapy with autologous stem–cell rescue versus oral maintenance chemotherapy as consolidation treatment in patients with high–risk neuroblastoma: a randomised controlled trial. The Lancet Oncology 2005;6:649–58.
84. Matthay KK, Reynolds CP, Seeger RC, et al. Long–term results for children with high–risk neuroblastoma treated on a randomized trial of myeloablative therapy followed by 13–cis–retinoic acid: a children's oncology group study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2009;27:1007–13.
85. Molina B, Alonso L, Gonzalez–Vicent M, et al. High–dose busulfan and melphalan as conditioning regimen for autologous peripheral blood progenitor cell transplantation in high–risk neuroblastoma patients. Pediatric hematology and oncology 2011;28:115–23.
86. Valteau–Couanet D, Benhamou E, Vassal G, et al. Consolidation with a busulfan–containing regimen followed by stem cell transplantation in infants with poor prognosis stage 4 neuroblastoma. Bone marrow transplantation 2000;25:937–42.
87. Leung W, Chen AR, Klann RC, et al. Frequent detection of tumor cells in hematopoietic grafts in neuroblastoma and Ewing's sarcoma. Bone marrow transplantation 1998;22:971–9.
88. Rill DR, Santana VM, Roberts WM, et al. Direct demonstration that autologous bone marrow transplantation for solid tumors can return a multiplicity of tumorigenic cells. Blood 1994;84:380–3.
89. Handgretinger R, Greil J, Schurmann U, et al. Positive selection and transplantation of peripheral CD34+ progenitor cells: feasibility and purging efficacy in pediatric patients with neuroblastoma. Journal of hematotherapy 1997;6:235–42.
90. Takaue Y, Watanabe T, Kawano Y, et al. Isolation and storage of peripheral blood hematopoietic stem cells for autotransplantation into children with cancer. Blood 1989;74:1245–51.
91. Lasky LC, Fox SB, Smith J, Bostrom B. Collection and use of peripheral blood stem cells in very small children. Bone marrow transplantation 1991;7:281–4.
92. Cho HJ, Jung HK, Sung KW, Ku HH, Lee SH, Kim DW. Autologous peripheral blood stem cell collections in children weighing less than 10 Kg with solid tumors: experience of a single center. Journal of clinical apheresis 2005;20:65–71.
93. Dominici C, Deb G, Angioni A, et al. Peripheral blood stem cells in children

with solid tumors. Part II. Immunocytologic detection of tumor cells in bone marrow and peripheral blood stem cell harvests. Anticancer research 1993;13:2573–5.
94. Bensimhon P, Villablanca JG, Sender LS, et al. Peripheral blood stem cell support for multiple cycles of dose intensive induction therapy is feasible with little risk of tumor contamination in advanced stage neuroblastoma: a report from the Childrens Oncology Group. Pediatric blood & cancer 2010;54:596–602.
95. Shen V, Woodbury C, Killen R, Van de Ven C, Sender L, Cairo MS. Collection and use of peripheral blood stem cells in young children with refractory solid tumors. Bone marrow transplantation 1997;19:197–204.
96. Moss TJ, Sanders DG, Lasky LC, Bostrom B. Contamination of peripheral blood stem cell harvests by circulating neuroblastoma cells. Blood 1990;76:1879–83.
97. Neal ZC, Yang JC, Rakhmilevich AL, et al. Enhanced activity of hu14.18–IL2 immunocytokine against murine NXS2 neuroblastoma when combined with interleukin 2 therapy. Clin Cancer Res 2004;10:4839–47.
98. Smyth MJ, Hayakawa Y, Takeda K, Yagita H. New aspects of natural–killer–cell surveillance and therapy of cancer. Nature reviews Cancer 2002;2:850–61.
99. Caligiuri MA. Human natural killer cells. Blood 2008;112:461–9.
100. Vivier E, Raulet DH, Moretta A, et al. Innate or adaptive immunity? The example of natural killer cells. Science 2011;331:44–9.
101. Nair MP, Schwartz SA. Suppression of human natural and antibody–dependent cytotoxicity by soluble factors from unstimulated normal lymphocytes. Journal of immunology 1982;129:2511–8.
102. Beum PV, Lindorfer MA, Taylor RP. Within peripheral blood mononuclear cells, antibody–dependent cellular cytotoxicity of rituximab–opsonized Daudi cells is promoted by NK cells and inhibited by monocytes due to shaving. Journal of immunology 2008;181:2916–24.
103. Preithner S, Elm S, Lippold S, et al. High concentrations of therapeutic IgG1 antibodies are needed to compensate for inhibition of antibody–dependent cellular cytotoxicity by excess endogenous immunoglobulin G. Molecular immunology 2006;43:1183–93.
104. Wang SY, Racila E, Taylor RP, Weiner GJ. NK–cell activation and antibody–dependent cellular cytotoxicity induced by rituximab–coated target cells is inhibited by the C3b component of complement. Blood 2008;111:1456–63.
105. Gersuk GM, Chang WC, Pattengale PK. Inhibition of human natural killer cell activity by platelet–derived growth factor. II. Membrane binding studies, effects of recombinant IFN–alpha and IL–2, and lack of effect on T cell and antibody–dependent cellular cytotoxicity. Journal of immunology 1988;141:4031–8.
106. Gersuk GM, Carmel R, Pattamakom S, Challita PM, Rabinowitz AP, Pattengale PK. Quantitative and functional studies of impaired natural killer (NK) cells in patients with myelofibrosis, essential thrombocythemia, and

polycythemia vera. I. A potential role for platelet–derived growth factor in defective NK cytotoxicity. Natural immunity 1993;12:136–51.
107. Iyengar R, Handgretinger R, Babarin–Dorner A, et al. Purification of human natural killer cells using a clinical–scale immunomagnetic method. Cytotherapy 2003;5:479–84.
108. Leung W, Iyengar R, Leimig T, Holladay MS, Houston J, Handgretinger R. Phenotype and function of human natural killer cells purified by using a clinical–scale immunomagnetic method. Cancer ImmunolImmunother 2005;54:389–94.
109. Rubnitz JE, Inaba H, Ribeiro RC, et al. NKAML: a pilot study to determine the safety and feasibility of haploidentical natural killer cell transplantation in childhood acute myeloid leukemia. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2010;28:955–9.
110. Sreedharan A, Bowyer S, Wallace CA, et al. Macrophage activation syndrome and other systemic inflammatory conditions after BMT. Bone Marrow Transplant 2006;37:629–34.
111. Ostronoff M, Ostronoff F, Coutinho M, et al. Hemophagocytic syndrome after autologous peripheral blood stem cell transplantation for multiple myeloma; successful treatment with high–dose intravenous immunoglobulin. Bone Marrow Transplant 2006;37:797–8.
112. Abe Y, Choi I, Hara K, et al. Hemophagocytic syndrome: a rare complication of allogeneic nonmyeloablative hematopoietic stem cell transplantation. Bone Marrow Transplant 2002;29:799–801.
113. Asano T, Kogawa K, Morimoto A, et al. Hemophagocytic lymphohistiocytosis after hematopoietic stem cell transplantation in children: a nationwide survey in Japan. Pediatr Blood Cancer 2012;59:110–4.
114. Takagi S, Masuoka K, Uchida N, et al. High incidence of haemophagocytic syndrome following umbilical cord blood transplantation for adults. Br J Haematol 2009;147:543–53.
115. Takagi S, Miyakoshi S, Masuoka K, et al. Haemophagocytic syndrome; the most frequent cause of engraftment failure following reduced–intensity umbilical cord blood transplantation. Bone Marrow Transplant 2006;37:S246–S7.
116. Abdelkefi A, Ben Jamil W, Torjman L, et al. Hemophagocytic syndrome after hematopoietic stem cell transplantation: a prospective observational study. International journal of hematology 2009;89:368–73.
117. Morimoto A, Nakazawa Y, Ishii E. Hemophagocytic lymphohistiocytosis: Pathogenesis, diagnosis, and management. Pediatrics international : official journal of the Japan Pediatric Society 2016;58:817–25.
118. Filipovich A, McClain K, Grom A. Histiocytic disorders: recent insights into pathophysiology and practical guidelines. Biol Blood Marrow Transplant 2010;16:S82–9.
119. Arico M, Danesino C, Pende D, Moretta L. Pathogenesis of haemophagocytic lymphohistiocytosis. Br J Haematol 2001;114:761–9.

120. Al-Samkari H, Berliner N. Hemophagocytic Lymphohistiocytosis. Annual review of pathology 2017.
121. Fujiwara F, Hibi S, Imashuku S. Hypercytokinemia in hemophagocytic syndrome. Am J Pediatr Hematol Oncol 1993;15:92-8.
122. Wang S, Degar BA, Zieske A, Shafi NQ, Rose MG. Hemophagocytosis exacerbated by G-CSF/GM-CSF treatment in a patient with myelodysplasia. Am J Hematol 2004;77:391-6.
123. Padhi S, Varghese RG, Ramdas A, Phansalkar MD, Sarangi R. Hemophagocytic lymphohistiocytosis: critical reappraisal of a potentially under-recognized condition. Frontiers of medicine 2013;7:492-8.
124. Navid F, Sondel PM, Barfield R, et al. Phase I Trial of a Novel Anti-GD2 Monoclonal Antibody, Hu14.18K322A, Designed to Decrease Toxicity in Children With Refractory or Recurrent Neuroblastoma. J Clin Oncol 2014.
125. Ladenstein RP, U;Luksch, R.;Brock, P.; Castel,V.; Yaniv,I.; Tikva,P.; Papadakis,V.; Laureys,G.; Pearson,A. Final Results from the HR-NBL1/SIOPEN Trial favour Busulphan-Melphalan as Superior Myeloablative Therapy (MAT) for High Risk Neuroblastoma. Advances in Neuroblastoma Research Conference 2012; 2012; Toronto, Canada; 2012. p. 80.
126. Bradfield SM DJ, Hawkins DS, et al. Fractionated low-dose radiotherapy after myeloablative stem cell transplantation for local control in patients with high-risk neuroblastoma. Cancer 2004;100:1268-75.
127. Kushner BH WS, LaQuaglia MP, et al. Hyperfractionated low-dose radiotherapy for high-risk neuroblastoma after intensive chemotherapy and surgery. J Clin Oncol 2001;19:2821-8.
128. Pai Panandiker AS, McGregor L, Krasin MJ, Wu S, Xiong X, Merchant TE. Locoregional tumor progression after radiation therapy influences overall survival in pediatric patients with neuroblastoma. International journal of radiation oncology, biology, physics 2010;76:1161-5.
129. Kushner BH ORR, Mandell LR, et al. Myeloablative combination chemotherapy without total body irradiation for neuroblastoma. J Clin Oncol 1991;9:274-9.
130. Matthay KK, Villablanca JG, Seeger RC, et al. Treatment of High-Risk Neuroblastoma with Intensive Chemotherapy, Radiotherapy, Autologous Bone Marrow Transplantation, and 13-cis-Retinoic Acid. N Engl J Med 1999;341:1165-73.
131. National Cancer Institute, National Institutes of Health: Phase III Randomized Trial of Single vs. Tandem Myeloablative Consolidation Therapy for High-Risk Neuroblastoma.
132. Haas-Kogan DA, Swift PS, Selch M, et al. Impact of radiotherapy for high-risk neuroblastoma: a Children's Cancer Group study. International Journal of Radiation Oncology*Biology*Physics 2003;56:28-39.
133. Kremens B KT, Herrmann F, Bender-Gotze C, Burdach S, Ebell W, Friedrich W, Koscielniak E, Schmid H, Siegert W. High-dose consolidation with local radiation and bone marrow rescue in patients with advanced neuroblastoma. Med Pediatr Oncol 1994;23:470-5.

134. Wolden SL, Gollamudi SV, Kushner BH, et al. Local control with multimodality therapy for stage 4 neuroblastoma. International journal of radiation oncology, biology, physics 2000;46:969–74.
135. George RE, Li S, Medeiros–Nancarrow C, et al. High–Risk Neuroblastoma Treated With Tandem Autologous Peripheral–Blood Stem Cell–Supported Transplantation: Long–Term Survival Update. J Clin Oncol 2006;24:2891–6.
136. Matthay KK, Atkinson JB, Stram DO, Selch M, Reynolds CP, Seeger RC. Patterns of relapse after autologous purged bone marrow transplantation for neuroblastoma: a Childrens Cancer Group pilot study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 1993;11:2226–33.
137. Imai K, Matsuyama S, Miyake S, Suga K, Nakachi K. Natural cytotoxic activity of peripheral–blood lymphocytes and cancer incidence: an 11–year follow–up study of a general population. Lancet 2000;356:1795–9.
138. Raulet DH, Vance RE. Self–tolerance of natural killer cells. Nature reviews Immunology 2006;6:520–31.
139. Waldhauer I, Steinle A. NK cells and cancer immunosurveillance. Oncogene 2008;27:5932–43.
140. Ruggeri L, Capanni M, Casucci M, et al. Role of natural killer cell alloreactivity in HLA–mismatched hematopoietic stem cell transplantation. Blood 1999;94:333–9.
141. Hsu KC, Keever–Taylor CA, Wilton A, et al. Improved outcome in HLA–identical sibling hematopoietic stem–cell transplantation for acute myelogenous leukemia predicted by KIR and HLA genotypes. Blood 2005;105:4878–84.
142. Wolfl M, Jungbluth AA, Garrido F, et al. Expression of MHC class I, MHC class II, and cancer germline antigens in neuroblastoma. Cancer immunology, immunotherapy : CII 2005;54:400–6.
143. Venstrom JM, Zheng J, Noor N, et al. KIR and HLA genotypes are associated with disease progression and survival following autologous hematopoietic stem cell transplantation for high–risk neuroblastoma. Clinical cancer research : an official journal of the American Association for Cancer Research 2009;15:7330–4.
144. Forlenza CJ, Boudreau JE, Zheng J, et al. KIR3DL1 Allelic Polymorphism and HLA–B Epitopes Modulate Response to Anti–GD2 Monoclonal Antibody in Patients With Neuroblastoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2016;34:2443–51.
145. Grzywacz B, Kataria N, Sikora M, et al. Coordinated acquisition of inhibitory and activating receptors and functional properties by developing human natural killer cells. Blood 2006;108:3824–33.
146. Knorr DA, Ni Z, Hermanson D, et al. Clinical–scale derivation of natural killer cells from human pluripotent stem cells for cancer therapy. Stem Cells Transl Med 2013;2:274–83.
147. Oostendorp RA, Harvey, K., Dzierzak, E.A. Generation of Murine Stromal Cell Lines: Models for the Microenvironment of the Embryonic Mouse

Aorta–Gonads–Mesonephros Region. Basic Cell Culture Protocols. New Jersy: Humana Press; 2004:163–72.
148. Dezell SA, Ahn YO, Spanholtz J, et al. Natural killer cell differentiation from hematopoietic stem cells: a comparative analysis of heparin- and stromal cell–supported methods. Biol Blood Marrow Transplant 2012;18:536–45.
149. Svane IM, Verdegaal EM. Achievements and challenges of adoptive T cell therapy with tumor–infiltrating or blood–derived lymphocytes for metastatic melanoma: what is needed to achieve standard of care? Cancer immunology, immunotherapy : CII 2014;63:1081–91.
150. Balermpas P, Michel Y, Wagenblast J, et al. Tumour–infiltrating lymphocytes predict response to definitive chemoradiotherapy in head and neck cancer. British journal of cancer 2014;110:501–9.
151. Wolf GT, Chepeha DB, Bellile E, et al. Tumor infiltrating lymphocytes (TIL) and prognosis in oral cavity squamous carcinoma: a preliminary study. Oral oncology 2015;51:90–5.
152. Geiger J, Hutchinson R, Hohenkirk L, McKenna E, Chang A, Mule J. Treatment of solid tumours in children with tumour–lysate–pulsed dendritic cells. Lancet 2000;356:1163–5.
153. Geiger JD, Hutchinson RJ, Hohenkirk LF, et al. Vaccination of pediatric solid tumor patients with tumor lysate–pulsed dendritic cells can expand specific T cells and mediate tumor regression. Cancer research 2001;61:8513–9.
154. Coustan–Smith E, Sancho J, Hancock ML, et al. Clinical importance of minimal residual disease in childhood acute lymphoblastic leukemia. Blood 2000;96:2691–6.
155. Seeger RC, Reynolds CP, Gallego R, Stram DO, Gerbing RB, Matthay KK. Quantitative tumor cell content of bone marrow and blood as a predictor of outcome in stage IV neuroblastoma: a Children's Cancer Group Study. J Clin Oncol 2000;18:4067–76.
156. Cheung NK, Von Hoff DD, Strandjord SE, Coccia PF. Detection of neuroblastoma cells in bone marrow using GD2 specific monoclonal antibodies. JClinOncol 1986;4:363–9.
157. Beiske K, Burchill SA, Cheung IY, et al. Consensus criteria for sensitive detection of minimal neuroblastoma cells in bone marrow, blood and stem cell preparations by immunocytology and QRT–PCR: recommendations by the International Neuroblastoma Risk Group Task Force. BrJCancer 2009;100:1627–37.
158. Eisenhofer G, Keiser H, Friberg P, et al. Plasma metanephrines are markers of pheochromocytoma produced by catechol–O–methyltransferase within tumors. J Clin Endocrinol Metab 1998;83:2175–85.
159. Eisenhofer G, Huynh TT, Hiroi M, Pacak K. Understanding catecholamine metabolism as a guide to the biochemical diagnosis of pheochromocytoma. Rev Endocr Metab Disord 2001;2:297–311.
160. Lenders JW, Pacak K, Walther MM, et al. Biochemical diagnosis of

pheochromocytoma: which test is best? Jama 2002;287:1427–34.
161. Eisenhofer G, Lenders JW, Linehan WM, Walther MM, Goldstein DS, Keiser HR. Plasma normetanephrine and metanephrine for detecting pheochromocytoma in von Hippel–Lindau disease and multiple endocrine neoplasia type 2. N Engl J Med 1999;340:1872–9.
162. Eisenhofer G, Walther MM, Huynh TT, et al. Pheochromocytomas in von Hippel–Lindau syndrome and multiple endocrine neoplasia type 2 display distinct biochemical and clinical phenotypes. J Clin Endocrinol Metab 2001;86:1999–2008.
163. LaBrosse EH, Comoy E, Bohuon C, Zucker JM, Schweisguth O. Catecholamine metabolism in neuroblastoma. J Natl Cancer Inst 1976;57:633–8.
164. Berthold F, Hunneman DH, Harms D, Kaser H, Zieschang J. Serum vanillylmandelic acid/homovanillic acid contributes to prognosis estimation in patients with localised but not with metastatic neuroblastoma. Eur J Cancer 1992;28A:1950–4.
165. Nishi M, Miyake H, Takeda T, et al. The relationship between homovanillic / vanillylmandelic acid ratios and prognosis in neuroblastoma. Oncol Rep 1998;5:631–3.
166. Schulz G, Cheresh DA, Varki NM, Yu A, Staffileno LK, Reisfeld RA. Detection of ganglioside GD2 in tumor tissues and sera of neuroblastoma patients. Cancer research 1984;44:5914–20.
167. Vujanovic L, Stahl EC, Pardee AD, et al. Tumor–Derived alpha–Fetoprotein Directly Drives Human Natural Killer–Cell Activation and Subsequent Cell Death. Cancer Immunol Res 2017;5:493–502.
168. Huang Q, Zhou X, Liu D, et al. A new liquid chromatography/tandem mass spectrometry method for quantification of gangliosides in human plasma. Anal Biochem 2014;455:26–34.
169. Kushner BH, LaQuaglia MP, Kramer K, Cheung NK. Radically different treatment recommendations for newly diagnosed neuroblastoma: pitfalls in assessment of risk. J Pediatr Hematol Oncol 2004;26:35–9.
170. Cohn SL, Pearson AD, London WB, et al. The International Neuroblastoma Risk Group (INRG) classification system: an INRG Task Force report. J Clin Oncol 2009;27:289–97.
171. Cecchetto G, Mosseri V, De Bernardi B, et al. Surgical risk factors in primary surgery for localized neuroblastoma: the LNESG1 study of the European International Society of Pediatric Oncology Neuroblastoma Group. J Clin Oncol 2005;23:8483–9.
172. De Bernardi B, Mosseri V, Rubie H, et al. Treatment of localised resectable neuroblastoma. Results of the LNESG1 study by the SIOP Europe Neuroblastoma Group. Br J Cancer 2008;99:1027–33.
173. Rosenberg SA. Cell transfer immunotherapy for metastatic solid cancer--what clinicians need to know. Nature reviews Clinical oncology 2011;8:577–85.
174. Veal GJ, Nguyen L, Paci A, et al. Busulfan pharmacokinetics following

intravenous and oral dosing regimens in children receiving high–dose myeloablative chemotherapy for high–risk neuroblastoma as part of the HR–NBL–1/SIOPEN trial. European journal of cancer 2012;48:3063–72.
175. Kilmartin JV, Wright B, Milstein C. Rat monoclonal antitubulin antibodies derived by using a new nonsecreting rat cell line. The Journal of cell biology 1982;93:576–82.
176. Lachmann PJ, Oldroyd RG, Milstein C, Wright BW. Three rat monoclonal antibodies to human C3. Immunology 1980;41:503–15.
177. Shinkawa T, Nakamura K, Yamane N, et al. The absence of fucose but not the presence of galactose or bisecting N–acetylglucosamine of human IgG1 complex–type oligosaccharides shows the critical role of enhancing antibody–dependent cellular cytotoxicity. The Journal of biological chemistry 2003;278:3466–73.
178. Albertini MR, Gan J, Jaeger P, et al. Systemic interleukin–2 modulates the anti–idiotypic response to chimeric anti–GD2 antibody in patients with melanoma. J ImmunotherEmphasisTumor Immunol 1996;19:278–95.
179. Ady N, Zucker JM, Asselain B, et al. A new 123I–MIBG whole body scan scoring method––application to the prediction of the response of metastases to induction chemotherapy in stage IV neuroblastoma. European journal of cancer 1995;31A:256–61.
180. Messina JA, Cheng SC, Franc BL, et al. Evaluation of semi–quantitative scoring system for metaiodobenzylguanidine (mIBG) scans in patients with relapsed neuroblastoma. Pediatric blood & cancer 2006;47:865–74.
181. Xiong X. A class of sequential conditional probability ratio tests. Journal of American Statistical Association 1995;90:1463–73.
182. Cytel East version 6.

APPENDIX I: GUIDELINES FOR COLLECTION AND DELIVERY OF TISSUE SAMPLES FOR PATHOLOGY

Specimen priority:

1. Formalin fixed, paraffin embedded tumor at diagnosis, at time of definitive surgery, and at second look surgery (or any other time tumor may be available) for diagnosis and Shimada classification.
2. Formalin fixed, paraffin embedded tumor for in N-myc amplification by fluorescence *in situ* hybridization – may use same paraffin blocks as used for diagnosis and classification.
3. Formalin fixed, paraffin embedded tumor for research immunohistochemistry (EGFR, pEGFR and BCRP) – may use same paraffin blocks as used for diagnosis and classification.
4. Snap frozen tumor tissue for research testing.

Priority for primary tumor tissue

Specimen	Amount (minimum)	Tissue Preparation	Studies and priority use of available tissue	Timing
Tumor	10 mm^3 in separate blocks no more than 4 mm thick	Formalin fixed, paraffin embedded	1. Diagnosis & Shimada classification. 2. FISH for *MYCN.* 3. IHC: synaptophysin, chromogranin A, NB84, if required for diagnostic confirmation of undifferentiated NB, T-cell, myeloid cell, and PD-1 pathway expression	Diagnosis, second look

IHC-immunohistochemistry; FISH- fluorescence *in situ* hybridization.

Pathology central review of tumor diagnosis and classification, research immunohistochemistry, and fluorescence *in situ* hybridization on tumor tissue will be done in the laboratory of:

Department of Pathology
St. Jude Children's Research Hospital
262 Danny Thomas Place

Memphis, Tennessee 38105-3678
Phone: 901-595-3520, Fax: 901-595-3100

All of the paraffin blocks and a copy of the like numbered surgical pathology reports should be shipped via FedEx to the pathology laboratory as soon as possible after the surgical procedure. The paraffin blocks and a copy of the review diagnosis will be returned to the contributing pathologist as soon as possible.

APPENDIX II: INTERNATIONAL NEUROBLASTOMA STAGING[47,48]

Stage 1: Localized tumor with complete gross excision, with or without microscopic residual disease; representative ipsilateral lymph nodes negative microscopically (nodes attached to and removed with the primary tumor may be positive).

Stage 2A: Localized tumor with incomplete gross excision; representative ipsilateral non-adherent lymph nodes negative for tumor microscopically.

Stage 2B: Localized tumor with or without complete gross excision; with ipsilateral non-adherent lymph nodes positive for tumor. Enlarged contralateral lymph nodes must be negative microscopically.

Stage 3: Unresectable unilateral tumor infiltrating across the midline* with or without regional lymph node involvement; or, localized unilateral tumor with contralateral regional lymph node involvement; or, midline tumor with bilateral extension by infiltration (unresectable) or by lymph node involvement. The midline is defined as the vertebral column. Tumors originating on one side and crossing the midline must infiltrate to or beyond the opposite side of the vertebral column.

Stage 4: Any primary tumor with dissemination to distant lymph nodes, bone, bone marrow, liver, skin and/or other organs (except as defined for Stage 4S).

Stage 4S: Localized primary tumor (as defined for Stage 1 or 2A or 2B), with dissemination limited to liver, skin, and/or bone marrow** (limited to infants < 1 year of age). Marrow involvement should be < 10% of total nucleated cells identified as malignant on bone marrow biopsy or on marrow aspirate. MIBG scan (if performed) should be negative in the marrow.

*The midline is defined as the vertebral column. Tumors originating on one side and crossing the midline must infiltrate to or beyond the opposite side of the vertebral column.

**Marrow involvement is stage 4S should be minimal, i.e., < 10% of total nucleated cells identified as malignant on bone marrow biopsy or on marrow aspirate. More extensive marrow involvement would be considered to be stage 4. The MIBG scan (if performed) should be negative in the marrow.

APPENDIX III: THE INTERNATIONAL NEUROBLASTOMA RISK GROUP STAGING SYSTEM (INRGSS)[1] AND IMAGE DEFINED RISK FACTORS[170]

Table 2: INRGSS

Stage	Description
L1	Localized tumor not involving vital structures as defined by the list of Image Defined Risk Factors and confined to one body compartment
L2	Locoregional tumor with presence of one or more Image Defined Risk Factors
M	Distant metastatic disease (except Stage MS)
MS	Metastatic disease in children < 18 months with metastases confined to skin, liver and/or bone marrow

Complete stage definitions:

Stage L1: Localized tumors that do not involve vital structures as defined by the list of IDRF. The tumor must be confined within one body compartment, neck, chest, abdomen, or pelvis. The isolated finding of intraspinal tumor extension that does not fulfill the criteria for an IDRF is consistent with stage L1.

Stage L2: Locoregional tumors with one or more IDRF. The tumor may be ipsilaterally continuous within body compartments (i.e., a left-sided abdominal tumor with left-sided chest involvement should be considered stage L2). However, a clearly left-sided abdominal tumor with right-sided chest (or vice versa) involvement is defined as metastatic disease.

Stage M: Distant metastatic disease (i.e., not contiguous with the primary tumor) except as defined for MS. Non-regional (distant) lymph node involvement is metastatic disease. However, an upper abdominal tumor with enlarged lower mediastinal nodes or a pelvic tumor with inguinal lymph node involvement is considered locoregional disease. Ascites and a pleural effusion, even with malignant cells, do not constitute metastatic disease unless they are remote from the body compartment of the primary tumor.

Stage MS: Metastatic disease in patients younger than 18 months (547 days) with metastases confined to skin, liver, and/or bone marrow. Bone marrow involvement should be limited to less than10% of total nucleated cells on smears or biopsy. MIBG scintigraphy must be negative in bone and bone marrow. Provided there is mIBG uptake in the primary tumor, bone scans are not required. The primary tumor can be with or without IDRF (L2 or L1) and there is no restriction regarding crossing or infiltration of the midline.

Table 3: IDRFs[170]

Region	IDRF Description
Multiple body compartments	Ipsilateral tumor extension within two body compartments (e.g., neck–chest, chest–abdomen, or abdomen–pelvis)
Neck	Tumor encasing carotid and/or vertebral artery and/or internal jugular vein Tumor extending to base of skull Tumor compressing the trachea
Cervico–thoracic junction	Tumor encasing brachial plexus roots Tumor encasing subclavian vessels and/or vertebral and/or carotid artery Tumor compressing the trachea
Thorax	Tumor encasing the aorta and/or major branches Tumor compressing the trachea and/or principal bronchi Lower mediastinal tumor, infiltrating the costo–vertebral junction between T9 and T12
Thoraco–abdominal junction	Tumor encasing the aorta and/or vena cava
Abdomen/pelvis	Tumor infiltrating the porta hepatis and/or the hepatoduodenal ligament Tumor encasing branches of the superior mesenteric artery at the mesenteric root Tumor encasing the origin of the coeliac axis, and/or of the superior mesenteric artery Tumor invading one or both renal pedicles Tumor encasing the aorta and/or vena cava Tumor encasing the iliac vessels Pelvic tumor crossing the sciatic notch
Intraspinal tumor extension	Intraspinal tumor extension (whatever the location) provided that: More than 1/3 of the spinal canal in the axial plane is invaded, or the perimedullary leptomeningeal spaces are not visible, or the spinal cord signal is abnormal
Infiltration of adjacent organs/structures	Pericardium, diaphragm, kidney, liver, duodeno–pancreatic block and mesentery
Other conditions considered equivalent to the above listed IDRFs*	Condition to be specified

Conditions to be recorded, but NOT considered IDRFs: Multifocal primary tumors Pleural effusion, with or without malignant cells Ascites, with or without malignant cells

APPENDIX IV: NEUROBLASTOMA RESPONSE CRITERIA (INTERNATIONAL RECOMMENDATIONS[47])

SITE	TEST	COMPLETE RESPONSE	VERY GOOD PARTIAL RESPONSE	PARTIAL RESPONSE	MIXED RESPONSE	STABLE DISEASE	PROGRESSIVE DISEASE
Primary	3 dimensional CT or MRI imaging (determine volume from product of three dimensions physical exam and/or surgical measurement	no tumor	> 90% reduction in 3-dimensional tumor volume	50-90% reduction in 3-dimensional tumor volume			
Metastases	Bone marrow [1] (aspirate x 2 and biopsy x 2)	no tumor	no tumor	No or only one sample with tumor[2]	50-90% reduction of any measurable lesion (primary or metastases); no new lesions; < 25% increase in any existing lesions, exclude bone marrow evaluation	No new lesions; <25% increase in any lesion; exclude bone marrow evaluation	Any new lesions; increase of any measurable lesion by > 25%; previous negative bone marrow positive
	Bone x-rays and scintigraphy (^{99}Tc Bone Scan and/or MIBG[3])	no lesions	all lesions improved, no new lesions	all lesions improved, no new lesions[4]			
	Liver imaging (ultrasound, CT, or MRI)	no tumor	no tumor	50-90 % reduction			
	Chest x-ray, chest CT scans if x-ray abnormal	no tumor	no tumor	50-90% reduction			
	Physical exam[5]	no tumor	no tumor	50-90% reduction			
Tumor Marker	Urine catecholamine metabolites (HVA & VMA)	normal	Normal or both decreased >90%	Both decreased 50-90%			

Response must be evaluated before and after surgery for the primary site. If complete response, very good partial response, or partial response is achieved surgically, indicate such when reporting the response. The total response can be no better than the worst response in any subcategory (e.g. if primary = complete response, metastases = partial response, and tumor marker = very good partial response, the total response = partial response).

1) Immunocytology results are not used to determine response.
2) One sample may be positive only if there is a reduction in the number of sites originally positive for tumor at diagnosis.
3) ^{99}Tc bone scan may show residual abnormalities but the MIBG scan (if performed) must be negative.
4) ^{99}Tc bone scan and/or MIBG scan (if performed) must show improvement, but residual abnormalities may be present on either scan.
5) Measure palpable lymph nodes in 3 dimensions and calculate tumor volume.

APPENDIX V: CHECKLISTS TO BE FILLED OUT FOR EACH PATIENT BY CONSENSUS OF STUDY TEAM.

This checklist is intended to facilitate proper and consistent staging according to INRGSS. It can be used as a standardized report form, and should accompany images if sent for central review. Since the list is not intended to substitute for the ordinary radiology reports, information on the investigation modalities and dates are not asked for. The list contains the minimum requirements for proper INRGSS staging. It is designed for possible scientific use by the cooperative groups, and it is therefore important that not only the "Yes"-boxes, but also the "No"-boxes are checked. A specific "No"-information is necessary to document the absence of an IDRF.

Checklist to ensure correct INRGSS Staging of Patients with Neuroblastic Tumors

Section A and B to be completed by the radiologist(s). Section C (if used) to be completed by the multidisciplinary treatment team. Every row of the form should be completed with either: "Yes", "No" or "N.A." (Not Assessed or Not Assessable) INRG = Image Defined Risk Factors

Patient name: _____ Patient ID:_____

(Optional in case name cannot be used)

Date of birth: _____

Section A

EXTENT OF PRIMARY TUMOR – IDRF	Yes	No	N/A
A Ipsilateral tumor extension within two body compartments			
A.1 Neck–chest			
A.2 Chest–abdomen			
A.3 Abdomen–pelvis			
B Neck			
B.1 Tumor encasing carotid and/or vertebral artery and/or internal jugular vein			
B.2 Tumor extending to base of skull			
B.3 Tumor compressing the trachea			
C Cervico–thoracic junction			
C.1 Tumor encasing brachial plexus roots			
C.2 Tumor encasing subclavian vessels and/or vertebral and/or carotid artery			
C.3 Tumor compressing the trachea			
D Thorax			
D.1 Tumor encasing the aorta and/or major branches			
D.2 Tumor compressing the trachea and/or principal bronchi			
D.3 Lower mediastinal tumor, infiltrating the costo–vertebral junction between T9 and T12			
E Thoraco–abdominal			
E.1 Tumor encasing the aorta and/or vena cava			
F Abdomen/pelvis			
F.1 Tumor infiltrating the porta hepatis (liver hilum) and/or hepatoduodenal ligament			
F.2 Tumor encasing branches of the superior mesenteric artery at the mesenteric root			
F.3 Tumor encasing the origin of the celiac axis and/or ofthe superior mesenteric artery			
F.4 Tumor invading one or both renal pedicles			
F.5 Tumor encasing the aorta and/or vena cava			
F.6 Tumor encasing the iliac vessels			
F.7 Tumor crossing the sciatic notch			

Checklist to ensure correct INRGSS Staging of Patients with Neuroblastic Tumors (cont.)

Patient name: _____ Patient ID:_____

(Optional in case name cannot be used)

EXTENT OF PRIMARY TUMOR – IDRF (cont.)	Yes	No	N/A
G Intraspinal tumor extension whatever location provided that:			
G.1 More than one third of the spinal canal in the axial plane is invaded and/or the perimedullary leptomeningeal spaces are not visible and/or the spinal cord signal is abnormal			
H Infiltration of adjacent organs/structures			
H.1 Pericardium			
H.2 Diaphragm			
H.3 Kidney			
H.4 Liver			
H.5 Duodeno-pancreatic block			
H.6 Mesentery			
H.7 Other organ (H.8) considered to be of similar significance			
H.8 Organ (H.7) infiltrated:			
I Other conditions considered equivalent to the above listed IDRFs:			
I.1 Condition (specify):			

IDRF status of <u>the primary tumor</u> at diagnosis:

IDRF Negative – All rows were checked "No" or "N.A.": ☐

IDRF Positive – One or more rows were checked "Yes" ☐

Section B

J Conditions to be recorded but NOT considered IDRFs	Yes	No	N/A
J.1 Multifocal primary tumors			
J.2 Rightsided pleural effusion			
J.3 Leftsided pleural effusion			
J.4 Ascites			

_____ _____
Date Radiologist 1

Radiologist 2 (Optional)

Checklist to ensure correct INRGSS Staging of Patients with Neuroblastic Tumors (cont.)

Patient name: _____ Patient ID:_____

(Optional in case name cannot be used)
Date of birth: _____

Section C

If the patient does not have metastases, check "No" for all M-rows

METASTASES	Yes	No	N/A
M Metastases present			
Location of metastases			
M.1 Malignant cells present in bone marrow on smears or biopsy			
M.2 Malignant cells present in bone marrow on smears or biopsy but limited to less than 10% of total nucleated cells			
M.3 Malignant cells present in bone marrow on smears or biopsy and constituting more than 10% of total nucleated cells			
M.4 Skin			
M.5 Liver			
M.6 Cortical bone			
M.7 Distant lymph nodes (see INRGSS staging definitions)			
M.8 Other metastases (specify):			

AGE	Yes
N Age of patient	
N.1 <12 months (<365 days)	
N.2 12 - <18 months (365 - <547 days)	

N.3 ≥ 18 months (≥ 547 days)	

Checklist to ensure correct INRGSS Staging of Patients with Neuroblastic Tumors (cont.)

Patient name: _____ Patient ID:_____

(Optional in case name cannot be used)
Date of birth: _____

Patient INRGSS stage at diagnosis:

L1 Localized tumor IDRF Negative:
(All rows in section A and C are checked "No" or "N.A.") ☐

L2 Locoregional tumor IDRF Positive:
(One or more rows in section A are checked "Yes", and all rows in section C are checked "No" or "N.A.") ☐

M Any metastatic disease except stage MS: ☐

MS Metastases confined to skin, liver and/or bone marrow in children younger than 18 months:
(Rows M.3, M.6, M.7, M.8 and N.3 can NOT be checked "Yes") ☐

_____ _____ _____
Date Sign.

The patient's INRGSS stage is decided once and for all at the time of diagnosis.

Section A and B can be used for reassessments of the patient's IDRF status during treatment.

APPENDIX VI: PREVENTION AND MANAGEMENT OF HYPERSENSITIVITY REACTIONS TO HU14.18K322A

I. Primary prophylaxis:

Administer the following medications approximately 30 minutes prior to hu14.18K322A infusion:

1. Acetaminophen 10–15 mg/kg PO
2. Diphenhydramine 1 mg/kg PO/IV (maximum 50 mg/dose) or hydroxyzine 0.5 mg/kg PO (maximum 25 mg/dose)

Anaphylactic precautions should be undertaken. Dexamethasone and epinephrine must be available, along with equipment for assisted ventilation. A free-flowing intravenous line must be established at all times.

II. Grading of hypersensitivity reactions

Hypersensitivity graded according to CTCAE Version 4.0 as follows:

Grade 1: Transient rash or flushing, fever < 38°C
Grade 2: Rash; flushing; urticaria; fever ≥ 38°C; asymptomatic bronchospasm
Grade 3: Symptomatic bronchospasm, with or without urticaria; parenteral medications indicated; allergy related edema/angioedema; hypotension
Grade 4: Anaphylaxis

III. Management of suspected hypersensitivity reaction

Recommended treatment of hypersensitivity reactions is based on the severity of reaction:

Grade 1 reaction: Decrease rate of antibody infusion to 50% until recovery from symptoms, remain at beside and monitor patient. Diphenhydramine may be administered every 4–6 hours at the discretion of the treating physician.

Grade 2 reaction: Interrupt antibody infusion. If symptoms resolve, restart infusion at the decreased rate of 50%. If symptoms do not abate, administer ranitidine 1 mg/kg IV (maximum dose 50 mg), an additional dose of diphenhydramine 1 mg/kg PO/IV (maximum 50 mg/dose) or hydroxyzine 0.5 mg/kg PO (maximum 25 mg/dose) and monitor patient closely. Diphenhydramine (1 mg/kg every 6 hours PO/IV, max 50 mg/dose) or hydroxyzine (0.5 mg/kg every 6 hours PO, max 25 mg/dose) and ranitidine (PO: 4–5 mg/kg/day divided every 8–12 hours, max 300 mg/day; IV: 2–4 mg/kg/day divided every 6–8 hours, max 400 mg/day) may be administered at the discretion of the treating physician. Further retreatment should be delayed 24 hours and administered only if all symptoms have resolved and at a decreased rate of 50%. For patients with grade 2 hypersensitivity reactions, consider premedication and scheduled dosing of diphenhydramine or hydroxyzine, acetaminophen and ranitidine during subsequent infusions of

study drug.

Grade 3 or 4 reactions: Immediately discontinue antibody infusion. Give epinephrine, diphenydramine, ranitidine, and corticosteroids, bronchodilators or other medical measures as needed. Patients should be monitored for at least 24 hours in the hospital and until all symptoms have resolved. Patients with grade 3 or 4 hypersensitivity reactions which resolve rapidly the hu14.18K322A infusion may be restarted at 50% rate (section 9.3.1).

Grade 1 or 2* hypersensitivity reactions: mild symptoms (e.g., localized urticaria, rigors, etc.)

- Decrease rate of antibody by 50% until symptoms resolve. May complete infusion at planned rate when symptoms resolve. If symptoms do not resolve, lower infusion rate to 25%. Further treatment should be delayed 24 hrs. and given only after symptoms have resolved and at a decreased rate of 50%.
- Give diphenhydramine or hydroxyzine and ranitidine at discretion of treating physician.

Grade 3 or 4* hypersensitivity reactions: severe symptoms (e.g., bronchospasm, angioedema, anaphylactic shock)

- Immediately stop antibody/IL-2/GM-CSF
- Assess airway, breathing and circulation
- Place in Trendelenburg position if hypotensive

Depending on severity and physician's discretion, may treat with some or all of the following:

- Epinephrine (1:1000-0.01 mg/kg IM/SQ every 15-20 minutes prn
- Diphenhydramine or hydroxyzine
- Oxygen
- Hydrocortisone 1-2 mg/kg IV
- Albuterol prn wheeze
- Atropine 0.01-0.02 mg/kg prn bradycardia

For angioedema that does not affect the airway or mild bronchospasm without other symptoms, if symptoms resolve quickly after above measures, may resume antibody at 50% of rate. If

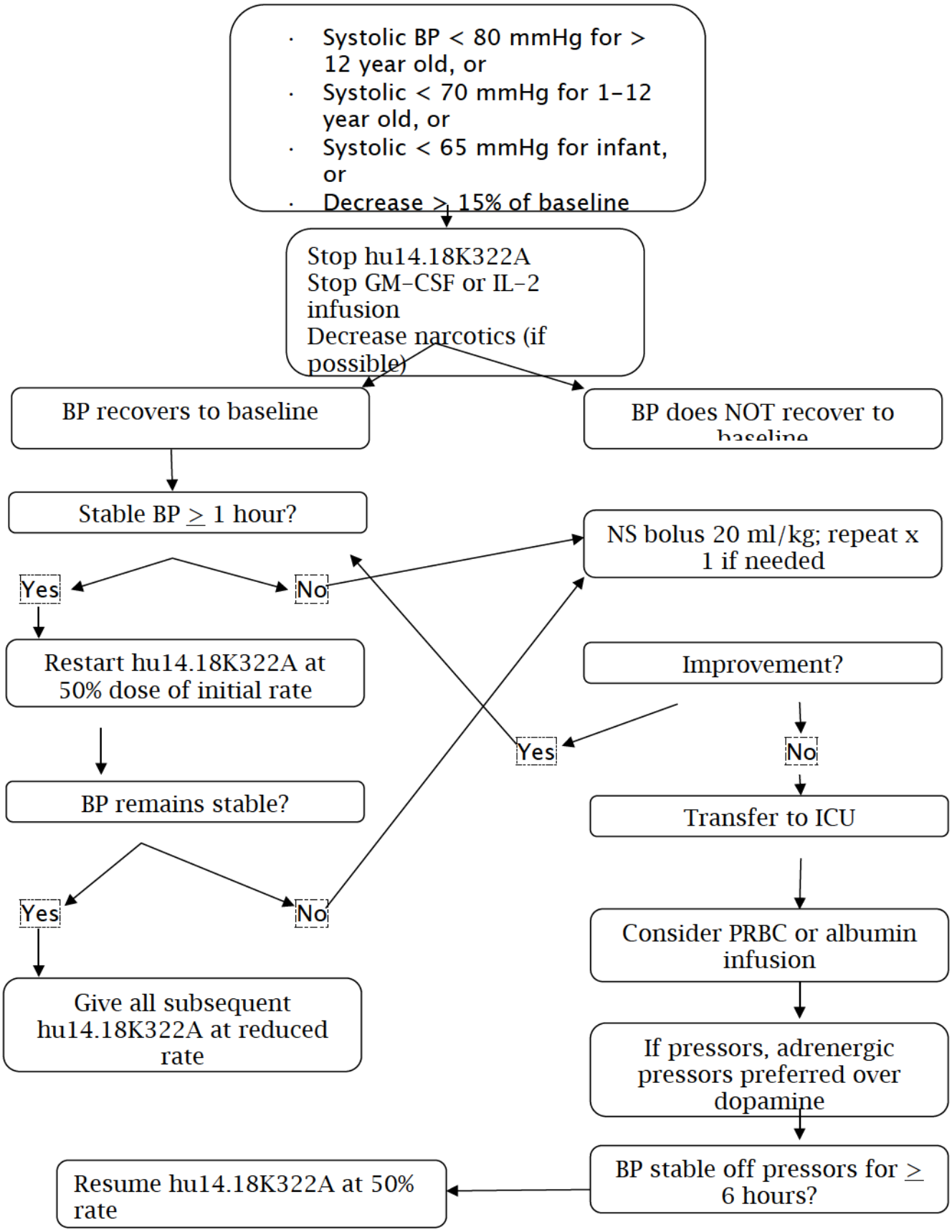
If antibody tolerated when restarted:

- For courses 1,3 and 5 of MRD Treatment Phase (see Section 4.9.3), give GM-CSF at 50% dose starting next day through last day of

If symptomatic angioedema or bronchospasm recurs, stop antibody therapy and resume next day with premedication of hydrocortisone 1 mg/kg IV

If hypotensive, see Appendix VIII

APPENDIX VII: HYPOTENSION MANAGEMENT WITH HU14.18K322A



APPENDIX VIII: CRITERIA FOR ACUTE & CHRONIC GRAFT VS. HOST DISEASE

Clinical staging of acute GVHD according to organ involvement

Stage	Skin	Liver	Intestinal Tract
0	No rash	Bilirubin <2.0 mg/dL	Diarrhea ≤500 ml/day or <280 ml/m2/day
+	Maculopapular rash <25% of body surface	Bilirubin 2.0 – 3.0 mg/dL	Diarrhea >500 but ≤ 1000 nl/day or 280–555 ml/m2/day
++	Maculopapular rash 25–50% of body surface	Bilirubin 3.1 – 6.0 mg/dL	Diarrhea > 1000 but ≤ 1500 ml/day or 556–833 ml/m2/day
+++	Generalized erythroderma	Bilirubin 6.1 – 15.0 mg/dL	Diarrhea > 1500 ml/day or >833 ml/m2/day
++++	Generalized erythroderma with bullous formation and desquamation	Bilirubin > 15.0 mg/dL	Severe abdominal pain with or without ileus

Overall clinical grading of severity of acute graft-versus-host disease

Grade	Degree of Organ Involvement
I	+ to ++ skin rash: [and] no gut involvement; [and] no liver involvement
II	+ to +++ skin rash; [or] + gut involvement [and/or] + liver involvement
III	++ to +++ skin rash; [and/or] ++ to +++ gut involvement [and/or] ++ to ++++ liver involvement
IV	Similar to Grade III with ++ to ++++ organ involvement [and] extreme decrease in clinical performance

Notes:

- Liver GVHD is downgraded one stage if additional cause(s) of increased bilirubin is documented
- Gut GVHD is downgraded one stage if additional cause(s) of increased loose stool volume is documented

- Specified stool volumes noted for gut GVHD applies to adult range body weights. For pediatric patients, the volume of stool should be based on body surface area
- Stool/urine mixture cannot be considered total stool volume. Stool volume needs to be unmixed stool without urine volume factored into volume total
- Chronic graft-vs-host disease (cGVHD) typically occurs after the approximate day +100 time point (without boosts or DLIs). The diagnosis is based on clinical assessment and may be supported by pathology findings.

Staging of chronic GVHD:

- Limited- localized skin and/or hepatic dysfunction

- Extensive- one or more of the following (as clinically judged by a physician and deemed as chronic GVHD by the principal investigator):

    - Generalized skin involvement
    - Liver histology showing chronic aggressive hepatitis, bridging necrosis or cirrhosis.
    - Eye dryness with Schirmer's test <5 mm wetting
    - Oral: involvement of salivary glands or oral mucosa.
    - Other: another target organ involvement.

APPENDIX IX: STANDARD OF CARE AND RESEARCH TESTS/EVALUATIONS/INTERVENTIONS

Standard of care/routine

- Chemotherapy agents (cyclophosphamide, topotecan, etoposide, etopophos, cisplatin, doxorubicin, mesna, busulfan, melphalan) and all supportive care medications, interventions, evaluations
- Immunotherapy/cytokines/growth factors (IL-2, GM-CSF, G-CSF)
- PBSC mobilization and collection and re-infusion
- Surgical consult and surgical resection
- Radiation oncology consult and radiation therapy
- History and physical exams (including weights, height)
- CBC, diff, platelets
- All serum chemistries
- Serum ferritin
- Neuron specific enolase
- Urinalysis
- Urine HVA, VMA
- Bilateral bone marrow biopsy and aspirate procedures
- CT chest, abdomen, pelvis (and/or whole body MRI)
- MIBG scan or bone scan
- ECHO/EKG
- Tumor pathology/histology (MYCN and Shimada)
- Dental evaluation
- Audiogram or BAER
- Renal scintigraphy
- Ophthalmology testing

Research items/procedures/devices

Neuroblastoma participant (NK cell recipient)

- Pharmacogenetic studies (per protocol PGEN5, section 11.1)
- Bone marrow MRD (section 11.1)
- Peripheral blood MRD (section 11.1)
- HLA typing
- BMTCT NK cell phenotype and functional studies (section 11.2)
- Biology research studies on resected tumor tissue (pre-trial and material taken during surgical resection – see section 11.4 & Appendix I).
- Human anti-human antibodies (HAHA) – send out to Dr. Sondel (see section 11.5.1)
- hu14.18K322A serum levels – send out to Dr. Sondel (see section 11.5.2)
- Peripheral blood for catechol metabolites (specimen drawn while participant in Nuclear Med for MIBG scan) – send out to Prof Eisenhofer (see section 11.6)

- Investigational agent, hu14.18K322A