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The world's childhood cancer experts

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A National Cancer Institute-supported member group of the National Clinical Trials Network

May 22, 2017

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Operations and Informatics Branch
Cancer Therapy Evaluation Program
Division of Cancer Treatment and Diagnosis
National Cancer Institute
Executive Plaza North Room 730
Bethesda, MD 20892

Dear Ms. Kruhm,

Enclosed please find Amendment #2 to protocol **AOST1421**, *A Phase 2 Study of Human-Mouse Chimeric Anti-Disialoganglioside Monoclonal Antibody ch14.18 (Dinutuximab, NSC# 764038, IND# 4308) in Combination with Sargramostim (GM-CSF) in Patients with Recurrent Osteosarcoma*.

This amendment updates the protocol by modifying treatment with dinutuximab from 2, 20-hour infusions on Days 4 and 5 of a cycle to 4, 10-hour infusions on Days 4-7 of a cycle. With the change in infusion schedule, the PK will now be mandatory for all patients. Unexpected death that is not related to disease and probably/likely related to treatment has been added to the list of unacceptable toxicities. The inclusion criteria have been updated to require a normal QTc interval per EKG and to only allow patients without a history of seizure disorder.

The CAEPR and risks associated with ch14.18 have been updated in response to an RRA from Dr. Jeffrey Moscow (jeffrey.moscow@nih.gov).

Several other administrative changes have been made; specific changes are detailed below. Minor administrative updates (such as the correction of typographical errors or updates to the numbers of referenced sections) are tracked in the protocol but not specified below.

Please let me know if we can offer further information.

Sincerely,

Jeannette Cassar, BA, Protocol Coordinator (for)
Pooja Hingorani, MD, AOST1421 Study Chair
Peter Adamson, MD, Children's Oncology Group Chair

SUMMARY OF CHANGES: PROTOCOL DOCUMENT

In accordance with the above discussion, the following specific revisions have been made to the protocol. Additions are in **boldfaced** font and deletions in ~~strikethrough~~ font.

#	Section	Page(s)	Change
1.	General	All	Updated the protocol version date in the footer.
2.	Title page	1	Updated Version Date and Amendment number.
3.	Contact Information	2	Updated the instructions for submission of site registration documents.
4.	Study Committee	8	Updated the protocol coordinator to Jeannette Cassar.
5.	Experimental Design Schema	10	Updated schema to reflect the change in Ch14.18 (dinutuximab) treatment from a 2-day to a 4-day infusion schedule and mandatory PK sampling for all patients enrolled after this amendment.
6.	1.2.1	11	Revised the aim "To characterize the pharmacokinetics of ch14.18 (dinutuximab) in patients with recurrent osteosarcoma ~~in the proposed administration schedule~~."
7.	2.1	12-13	• Updated text to reflect the change in Ch14.18 (dinutuximab) treatment to a 4-day infusion schedule. • Deleted text referring to dosing ch14.18 in an alternative (2-day) infusion schedule. The two recent SAEs on AOST1421 of sudden death and grade 4 toxicity may have been related to potentially higher peak plasma concentrations of dinutuximab from the experimental 2-day infusion schedule being used on AOST1421. Therefore, a decision was made by the study committee and COG leadership to change the dinutuximab infusion to the standard 4-day schedule as used in neuroblastoma patients as much more experience exists with treating patients on this schedule.
8.	2.5.1	15	Updated section to include the serious adverse events observed with the alternative (2-day) infusion schedule and modifications to the infusion schedule effective with the approval of this amendment.
9.	2.5.2	16	Updated section to provide justification for now requiring PK sampling for all patients enrolled after the approval of this amendment.
10.	3.1.2	19	Updated the instructions for submission of IRB/REB documents.
11.	3.2.4	23	Updated the link to the performance status scales scoring.
12.	3.2.7.4	24	Added inclusion criteria: "**Baseline EKG shows normal QTc interval of ≤ 470 ms.**" -This has been added in light of the recent SAE of sudden death the cause for which remains unexplained but could have been a cardiac event secondary to dinutuximab therapy. Addition of this inclusion criteria would potentially ensure that at baseline, a patient is not at increased risk for arrhythmias.
13.	3.2.7.6	24	Modified inclusion criteria to only allow patients with no known history of seizure disorder. -Patients with a known epilepsy disorder are at increased risk of "Sudden Death in Epilepsy Patients" (SUDEP), the cause for which remains unknown. Given the concern for increased neurotoxicity with dinutuximab patients, this risk may be potentiated in patients with

#	Section	Page(s)	Change
			underlying epilepsy. Therefore, for patient safety, we would like to exclude patients with underlying seizure disorder.
14.	4.1	26	• Updated section to reflect the change in Ch14.18 (dinutuximab) treatment to a 4-day infusion schedule and mandatory PK sampling for all patients enrolled after this amendment. • Added 24-hour patient observation: "**Patients will be observed in the hospital for an additional 24 hours after completion of the last day of dinutuximab infusion in each cycle.**" - This was added as the risk of SAEs seems to be highest during and shortly after infusion completion. Additional observation in the hospital will potentially mitigate the risk of these events happening outside of the hospital.
15.	4.1.1.4	27	Updated the link to the COG supportive care guidelines.
16.	4.2.1	28	Updated section to reflect the change in Ch14.18 (dinutuximab) treatment to a 4-day infusion schedule.
17.	4.2.2.i	29	Updated TDM and Required Observations to include mandatory PK testing for patients enrolled after the approval of this amendment.
18.	4.2.3	30-32	• Updated the administration instruction of sargramostim from days 4 and 5 to days 4-7. • Updated the administration of dinutuximab to the 10-hour x 4 day schedule. • For pain management, changed the dose of morphine from milligrams to micrograms to be consistent with other sections of the protocol. • Added specific dosing details for alternative pain management regimen.
19.	4.3.1	33	Updated section to reflect the change in Ch14.18 (dinutuximab) treatment to a 4-day infusion schedule.
20.	4.3.2.f	34	Updated TDM and Required Observations to include mandatory PK testing for patients enrolled after the approval of this amendment.
21.	4.3.3	35	• Updated the administration instruction of sargramostim from days 4 and 5 to days 4-7. • Updated the administration of dinutuximab to the 10-hour x 4 day schedule.
22.	5.2.1.1	40	Added the following text clarifying the procedures for restarting dinutixumab for patients who required pressors for hypotension: "**If the 50% rate is tolerated without further hypotension, rate can be increased to 100% as tolerated. If patient becomes hypotensive again at 100% rate, it should be decreased back to 50% after patient is clinically stable to receive the infusion again and remainder of the infusion should be completed at the decreased rate.**"

#	Section	Page(s)	Change
23.	6.1	46-52	• Updated the toxicity/ CAEPR information per the RRA received from CTEP. Added New Risk: • Also Reported on MoAb 14.18, chimeric Trials But With Insufficient Evidence for Attribution: Cardiac disorders - Other (gallop on exam); Cardiac disorders - Other (N-terminal BNP); General disorders and administration site conditions - Other (cold and clammy); Syncope Increase in Risk Attribution: • Changed to Less Likely from Also Reported on MoAb 14.18, chimeric Trials But With Insufficient Evidence for Attribution: Hypocalcemia • Changed to Rare but Serious from Also Reported on MoAb 14.18, chimeric Trials But With Insufficient Evidence for Attribution: Myelitis; Reversible posterior leukoencephalopathy syndrome; Sudden death NOS • Updated the preparation instructions to align with the new dosing amount and schedule and to be consistent with other COG protocols that use ch14.18. • Clarified supplier and indication to not use commercial supply. • Updated information regarding agent accountability. • Added information where to obtain the investigator's brochure. • Added a section for useful links.
24.	6.2	53-55	Updated information for Canadian sites.
25.	7.2	57	Updated footnote to reflect mandatory PK sampling for all patients enrolled after the approval of this amendment.
26.	8.1.b	58	Updated reference to Section 9.3.
27.	9.3	59-60	• Updated section to reflect mandatory PK sampling for all patients enrolled after the approval of this amendment. • Added "**Unexpected death**" as an Unacceptable Toxicity.
28.	15.1.4	77	Updated section to indicate that the BPC will forward tumor tissue for the GD2 assay to Cambridge Biomedical.
29.	15.2	78	Updated section to reflect mandatory PK sampling for all patients enrolled after the approval of this amendment.
30.	15.2.1	78	Updated timing of PK sampling to reflect the change in Ch14.18 (dinutuximab) infusion schedule.
31.	Appendix I	88-89	Updated the instructions for submitting regulatory documents.
32.	Appendix II: YIS age 13-17	91	Deleted text about the PKs being done in the first 10 patients because with this amendment, the PK tests will be mandatory for all patients.
33.	Appendix III	N/A	Removed Appendix III which contained examples of calculations for extended ch14.18 administration based on the 2-day infusion schedule.

SUMMARY OF CHANGES: CONSENT DOCUMENTS

In accordance with the above discussion, the following specific revisions have been made to the protocol. Additions are in **boldfaced** font and deletions in ~~strikethrough~~ font.

#	Section	Page(s)	Change
1.	General	All	Updated version date to match the version of the protocol.
2.	Summary of Study Treatment	3	Updated the days of ch14.18 treatment from Days 4-5 to Days 4-7.
3.	Diagram of Treatment	4	Updated the diagram of treatment to indicate the correct number of days of administration for ch14.18.
4.	Treatment for Subjects	5	• Updated the table to indicate the correct number of days of administration for ch14.18. • Added that patients will be required to stay in the hospital for an additional 24 hours after the end of the last infusion of ch.14.18 for observation.
5.	Ch14.18 PK Studies	6	• Added that the PK for ch14.18 is required. • Removed language regarding the PK being optional.
6.	Optional Research Study Tests	6	Deleted paragraph about optional PK testing.
7.	Risks of Study	8	Added paragraph describing serious side effects experienced by 2 patients.
8.	Possible Side Effects of ch14.18	9	**Increase in Risk Attribution:** • Changed to Rare from Also Reported on MoAb 14.18, chimeric Trials But With Insufficient Evidence for Attribution (i.e., added to the Risk Profile): Death; Swelling of the spinal cord; Brain damage which may cause headache, seizure, blindness (also known as Reversible Posterior Leukoencephalopathy Syndrome). **Provided Further Clarification:** • Anemia which may cause tiredness, or may require blood transfusion (under Occasional) and Anemia, kidney problems which may cause swelling, or may require dialysis (under Rare) are now reported as Anemia, kidney problems which may cause tiredness, or may require blood transfusion or dialysis (under Occasional). • High blood pressure which may cause headaches, dizziness, blurred vision (under Occasional) is now reported as High blood pressure which may cause dizziness, blurred vision (under Occasional).
9.	Where Can I get More Information?	14	Updated the URL for the patient and family handbook.
10.	Specimens for Optional Research Tests	16	Removed signature/choice option for the PK tests as they are now mandatory.

Activated: 11/30/15
Closed:

Version Date: 05/17/2017
Amendment: #2

CHILDREN'S ONCOLOGY GROUP

AOST1421

A Phase 2 Study of Human-Mouse Chimeric Anti-Disialoganglioside Monoclonal Antibody ch14.18 (Dinutuximab, NSC# 764038, IND# 4308) in Combination with Sargramostim (GM-CSF) in Patients with Recurrent Osteosarcoma

NCI Supplied Agent: ch14.18 (Dinutuximab, NSC# 764038, IND# 4308)
IND sponsor for ch14.18: DCTD, NCI

A COG Groupwide Phase 2 Study

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CONTACT INFORMATION		
To submit site registration documents:	**For patient enrollments:**	**Submit study data**
Regulatory documentation must be submitted to the CTSU via the Regulatory Submission Portal. Regulatory Submission Portal: (Sign in at www.ctsu.org, and select the Regulatory Submission sub-tab under the Regulatory tab.) Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 to receive further instruction and support. Contact the CTSU Regulatory Help Desk at 1-866-651-2878 for regulatory assistance.	Please refer to the patient enrollment section of the protocol for instructions on using the Oncology Patient Enrollment Network (OPEN) which can be accessed at https://www.ctsu.org/OPEN_SYSTEM/ or https://OPEN.ctsu.org. Contact the CTSU Help Desk with any OPEN-related questions at ctsucontact@westat.com.	Data collection for this study will be done exclusively through Medidata Rave. Please see the Data Submission Schedule in the CRF packet for further instructions.
The most current version of the **study protocol and all supporting documents** must be downloaded from the protocol-specific Web page of the CTSU Member Web site located at https://www.ctsu.org. Access to the CTSU members' website is managed through the Cancer Therapy and Evaluation Program - Identity and Access Management (CTEP-IAM) registration system and requires user log on with CTEP-IAM username and password. Permission to view and download this protocol and its supporting documents is restricted and is based on person and site roster assignment housed in the CTSU RSS.		
For clinical questions (i.e. patient eligibility or treatment-related) contact the Study PI of the Lead Protocol Organization.		
For non-clinical questions (i.e. unrelated to patient eligibility, treatment, or clinical data submission) contact the CTSU Help Desk by phone or e-mail: CTSU General Information Line – 1-888-823-5923, or ctsucontact@westat.com. All calls and correspondence will be triaged to the appropriate CTSU representative.		
The CTSU Website is located at https://www.ctsu.org.		

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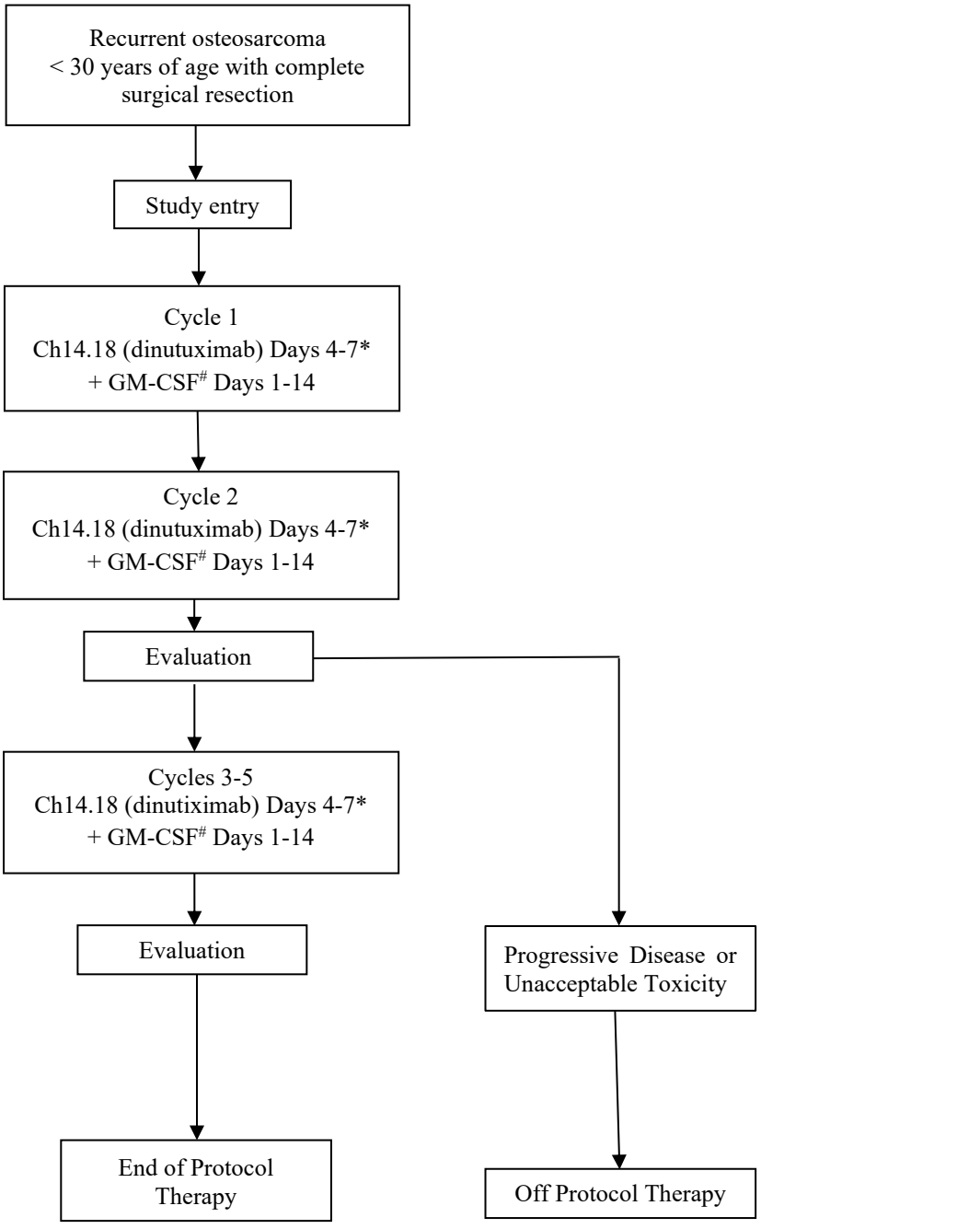
AGENT	NSC#
Dinutuximab	764038
Sargramostim	613795

SEE SECTION 15 FOR SPECIMEN SHIPPING ADDRESSES

ABSTRACT

Patients with recurrent osteosarcoma have a dismal prognosis with an overall survival of less than 30% at 5 years. Limited treatment options exist for these patients, with surgery remaining the mainstay of therapy at recurrence. A subset of patients with recurrent osteosarcoma is able to achieve a second or subsequent complete surgical remission and this subset holds a better prognosis than those who do not achieve a complete surgical remission. Nonetheless, the majority of patients who achieve a second or subsequent complete surgical remission will recur. GD2 is a cell surface disialoganglioside that is ubiquitously expressed on osteosarcoma tumor cells and therefore is a rational therapeutic target. Historical experience from neuroblastoma (NB) trials shows that anti-GD2 based therapy works best in the setting of minimal residual disease. Therefore, we propose to evaluate the effects of ch14.18 (dinutuximab), an anti-GD2 antibody, in combination with sargramostim (GM-CSF) in patients with pulmonary recurrence of osteosarcoma that are able to achieve a complete surgical resection. The main objective of the study is to determine the disease control rate in patients with recurrent osteosarcoma treated with ch14.18 (dinutuximab) in combination with sargramostim. Secondary objectives include analysis of pharmacokinetics and toxicity of ch14.18 (dinutuximab) in this patient population.

EXPERIMENTAL DESIGN SCHEMA



*Ch14.18 (dinutuximab) infusion will be given over 4 days.
#GM-CSF = sargramostim
Pharmacokinetic sampling will be mandatory for all patients enrolled after protocol amendment #2.

1.0 GOALS AND OBJECTIVES (SCIENTIFIC AIMS)

1.1 Primary Aims

1.1.1 To determine the disease control rate in patients with completely resected recurrent osteosarcoma treated with ch14.18 (dinutuximab) in combination with sargramostim (GM-CSF) as compared to historical COG experience.

1.2 Secondary Aims

1.2.1 To characterize the pharmacokinetics of ch14.18 (dinutuximab) in patients with recurrent osteosarcoma.

1.2.2 To determine the occurrence of unacceptable toxicity (UT) in patients with recurrent osteosarcoma treated with ch14.18 (dinutuximab) in combination with sargramostim.

1.2.3 To assess the relationship between probability of disease control and tumor GD2 expression.

1.3 Exploratory Aims

1.3.1 To assess the relationship between probability of disease control and tumor GD2 expression.

1.3.2 To assess KIR and FcγR genotypes, NKp30 isoforms and its circulating ligand, B7-H6, and their relationships to the probability of disease control.

1.3.3 To attempt banking of tumor samples for future research studies from patients enrolled on study who undergo biopsy or resection of suspected metastatic disease recurrence while on protocol therapy or during the evaluation period.

1.3.4 To determine a descriptive profile of human anti-chimeric antibody (HACA) during immunotherapy.

1.3.5 To bank serial plasma samples for future studies of circulating tumor DNA (ctDNA) detection as a marker of disease progression and response.

2.0 BACKGROUND

2.1 Introduction/Rationale for Development

Treatment of patients with recurrent osteosarcoma remains challenging with very few effective therapeutic options. A subset of patients with relapsed disease is able to achieve subsequent surgical remission, but the majority of patients will subsequently relapse. Hence these patients continue to have a dismal prognosis.

The main predictors of survival after osteosarcoma recurrence include the time to first recurrence, disease burden, and ability to achieve complete surgical remission after recurrence. Solitary pulmonary nodule and greater than 24 months to first recurrence are

favorable prognostic factors.[1] Data from the Cooperative Osteosarcoma Study Group (COSS) as well as for other studies show that for patients with first osteosarcoma relapse that the median time to first relapse is 15-18 months from the time of original diagnosis.[2,1] The median time to second relapse from first relapse is around 8 months and all subsequent relapses occur within approximately 6 months.[3] Although the median time to first relapse seems longer than subsequent relapses, a large duration of this interval includes administration of upfront therapy for osteosarcoma (an average of 9 months). Therefore, the median time to first relapse from the end of upfront therapy is not significantly different compared to median time to second relapse.

Five-year overall survival rates of 39% are reported for patients with first relapse who are able to achieve a second surgical remission, and 32% for patients who are able to achieve a third surgical remission (COSS data).[2,3] Data from the Rizzoli Institute show reported 5-year event-free actuarial survival of 38% after first metastasectomy and 32% after second metastasectomy, suggesting that patients who had the second metastasectomy had the same probability of survival as patients after the first metastasectomy.[4] The disease-free interval was an important prognostic factor in this study with longer interval portending a favorable outcome within the first 5 years but this difference was annulled after 5 years. Together, these data indicate that the most important factor for survival after pulmonary relapse is complete surgical resection and that long term survival is not significantly different in patients with one or more subsequent relapses.

The Bone Tumor Committee has performed a detailed review of the past COG studies, which included recurrent osteosarcoma patients. A review of seven single-agent Phase 2 studies conducted by COG over the past 15 years identified 96 eligible patients with relapsed osteosarcoma who had measurable disease at the time of enrollment. The estimated 4-month progression-free survival probability was 12% with a 95% confidence interval of 6%-19%. AOST0221, a Phase 2 study of inhaled sargramostim (GM-CSF) in patients with first pulmonary recurrence of osteosarcoma who achieved a complete resection, is the only study available to estimate EFS in a cohort with complete surgical remission. Of the 43 eligible patients who received inhaled sargramostim, 41 were evaluated for disease outcome. The 12-month EFS was 20% with a 95% confidence interval of 10-34% (Mark Krailo, personal communication). Based on the above data for the fully resected cohort, disease control (DC) rate, defined as the probability of patients who do not have progressive disease or death due to progressive disease 12 months after start of protocol therapy, will be the primary outcome measure for this study.

Limited progress has been made in identifying novel targets that may be therapeutic in osteosarcoma and there remains an urgent need for development of new agents that are effective in improving survival. GD2, a cell surface disialoganglioside that is ubiquitously expressed in malignancies such as melanoma and neuroblastoma, has been successfully used in NB to improve overall and event-free survival. GD2 has also been shown to be expressed in more than 95% of osteosarcoma samples, as discussed below, thus making it a potential therapeutic target for osteosarcoma.

This study is a Phase 2 trial to evaluate disease control rate as compared to historical experience in patients with recurrent pulmonary osteosarcoma who achieve a complete surgical resection following treatment with ch14.18 (dinutuximab) plus cytokine therapy. Based on data from NB clinical trials, it is well established that anti-GD2 therapy is most beneficial in the setting of minimal residual disease and hence in this trial, only patients

with recurrent osteosarcoma who achieve a complete surgical remission are eligible. Enrolled patients will receive ch14.18 (dinutuximab) as a 10-hour infusion per day for 4 days in each cycle. This updated schedule has been modified from the prior experimental schedule of 20-hour infusion over 2 days. Therapy will be delivered every 28 days for a total of 5 cycles or until disease progression or recurrence. If ch14.18 (dinutuximab) plus cytokine therapy is found to be tolerable and effective the COG Bone Tumor Committee will consider incorporating this agent into the therapeutic regimen for patients with osteosarcoma in first remission.

2.2 Preclinical Studies

2.2.1 Rationale for anti-GD2 Therapy in Osteosarcoma

Strong immunoreactivity for the GD2 antigen was detected in 15 of 17 osteosarcomas, including both primary and metastatic tumors.[5] Moreover, each of 24 patient-derived osteosarcoma cell lines showed strong expression of GD2 and only 4.3% of osteosarcoma patient samples (n=44) were negative for GD2 by immunohistochemical analysis using 14.2G-2a antibody.[6] These data suggest that GD2, which is expressed in most osteosarcomas, may be a rational immunotherapeutic target for this disease.

2.2.2 Rationale for Using Cytokines in Combination with Ch14.18

Preclinical data with murine monoclonal antibodies (MoAb) 3F8 and 14G2a demonstrated that the *in vitro* and *in vivo* activity against tumor cells was mediated by activation of complement and antibody dependent cell mediated toxicity (CDC and ADCC respectively) by effector cells such as lymphocytes, NK cells, neutrophils and monocytes.[7,8] Cytokines such as sargramostim and IL-2 augmented the ADCC effect of anti-GD2 antibodies.[9,10] This was also confirmed with ch14.18 which is 50-100 times more efficient than the murine antibody in mediating tumor ADCC in vitro.[11] Therefore, clinical trials have used anti-GD2 antibody in conjunction with cytokines for potentially enhanced efficacy of the antibody. However, for the purpose of AOST1421, IL-2 will not be used for the following reasons: a) more recent trials conducted by SIOP have shown that IL-2 may not have any additive benefit to GD2 antibody therapy and b) IL-2 causes significant treatment related toxicity and this may be intolerable in the adolescent and young adult osteosarcoma patient population. GM-CSF and ch14.18 cycles on the other hand are much better tolerated in the NB protocols. The dose and schedule for GM-CSF is identical to what is used in the current COG NB protocols.

2.3 Adult Studies

Both the murine and the chimeric anti-GD2 antibodies have undergone Phase 1-3 clinical trials over the past two decades, primarily in patients with NB and melanoma. At least four clinical trials have been completed using ch14.18 (dinutuximab) either alone or in combination with cytokines in adults with melanoma or sarcoma. In a Phase 1 trial of ch14.18 (dinutuximab) in patients with malignant melanoma, patients received ch14.18 (dinutuximab) as a single dose of 5-100 mg. The major toxicity was infusion-related abdominal-pelvic pain syndrome requiring intravenous morphine. In a subsequent Phase Ia/Ib trial of ch14.18 (dinutuximab) and sargramostim in patients with malignant melanoma, patients received ch14.18 (dinutuximab) in doses between 15-60 mg/m^2 intravenously for 4 hours on Day 1. Dose limiting toxicities were observed at 60 mg/m^2. Significant side effects included abdominal or extremity pain, headache, nausea, diarrhea,

myalgias, blood pressure changes and weakness. No antitumor activity was seen. In a Phase Ib trial of ch14.18 (dinutuximab) with interleukin-2 (IL2) in melanoma patients, the ch14.18 (dinutuximab) maximum tolerated dose (MTD) was 7.5 mg/m^2/day x 5 days. Major adverse events included abdominal, chest or extremity pain, severe allergic reaction, weakness and pericardial effusion. Antitumor activity included one complete response, one partial response, stable disease in eight patients, and one patient with >50% decrease in hepatic metastases. More recently, a Phase 1 trial of ch14.18 (dinutuximab) along with R24 monoclonal antibodies and IL2 was completed in patients with melanoma (23 patients) and sarcoma (4 patients). The MTD of ch14.18 (dinutuximab) was 5 mg/m^2/day x 5 days, lower than the 7.5 mg/m^2/day determined with IL2 alone. Two melanoma patients had partial responses, four patients had stable disease (one patient had gastrointestinal stromal sarcoma) and one patient remained without evidence of disease. Anecdotal evidence of anti-GD2 therapy exists in osteosarcoma from prior Phase 1 trials that included patients with osteosarcoma. In a Phase 1/1B trial of the murine antibody (14.2G2a) plus IL2, 2 of 33 patients had osteosarcoma. One patient had multiple bone metastases and received one cycle of therapy. Two months later, repeat scans showed a complete response. This patient remained disease free for 8 months but subsequently relapsed.[12] In a Phase 1 trial of ch14.18 (dinutuximab) in patients with refractory NB and osteosarcoma, 1 of 11 treated patients had metastatic osteosarcoma to the bones and lungs. The patient had improvement in bone pain but progression of the lung lesions after one cycle.

2.4 Pediatric Studies

2.4.1

A Phase 1 trial of ch14.18 (dinutuximab) in children with refractory NB and osteosarcoma established the safety of antibody administration, although no MTD was reached. Pain was the most common side effect during treatment. Clinical responses (1 partial response and 4 mixed responses) were observed in 5 of 10 NB patients. Subsequently a Phase 1 trial of ch14.18 (dinutuximab) in combination with sargramostim and aldesleukin (interleukin-2, IL2) in patients with NB immediately following autologous bone marrow transplant was performed. The MTD of ch14.18 (dinutuximab) was determined to be 25 mg/m^2/d x 4 days every 28 days (NCI product); IL2 was given during Cycles 2 and 4 at a dose of 3 MU/m^2/d for 4 days one week before the antibody and at a dose of 4.5 MU/m^2/d for 4 days concurrently with the antibody; sargramostim was administered in Cycles 1, 3 and 5 at 250 mcg/m^2/d subcutaneously starting 3 days prior to ch14.18 (dinutuximab) and continued for 3 days after the antibody therapy. Common toxicities including neuropathic pain, fever, hypersensitivity reactions and capillary leak syndrome were manageable with aggressive supportive care. No patient developed human anti-chimeric antibody (HACA). The study demonstrated feasibility of incorporating sargramostim and IL2 with dinutuximab. In another Phase 2 trial of ch14.18 (dinutuximab) (50 mg/m^2/day over 5 hours x 4 days) plus sargramostim (10 mcg/kg/d x 14 days) pain, fever and tachycardia were the main adverse events. Of the 27 patients evaluable for response, there was one complete response, three partial responses, one mixed response and two stable disease. These early trials were followed by a Phase III randomized trial in patients with newly diagnosed NB who achieved complete or a partial response after induction chemotherapy.[13] Interim analysis revealed a significant increases in 2-year OS (86% versus 75%; P=0.02) and EFS (66% versus 46%; P=0.01) for patients randomized to the ch14.18 (dinutuximab) plus cytokine arm along with

cis-retinoic acid compared to the cis-retinoic acid alone.[14] This trial established the efficacy of this regimen in patients with minimal residual disease.

2.4.2 Rationale for Anti-GD2 Strategy in Minimal Residual Disease Setting in Osteosarcoma

Objective radiographic responses are rare in osteosarcoma, even with active neoadjuvant chemotherapy regimens in newly diagnosed patients. This is likely due to the presence of calcified bone matrix within these tumors. Based on the data from the NB trials in the setting of minimal residual disease detailed above, the committee believes that if active in osteosarcoma, the greatest potential for benefit would similarly be in following complete surgical resection of metastases, i.e. in a setting of minimal residual disease.

2.5 Dosing Rationale

2.5.1 Alternative Dosing Schedule of Ch14.18 (Dinutuximab)

In the COG NB trials, the MTD of ch14.18 (dinutuximab) was determined to be 25 mg/m^2/day (based upon NCI product which was used in these trials; equivalent to 17.5 mg/m^2/day of the United Therapeutics product to be used in the AOST1421 study) infused over 10 hours per day x 4 days every 28 days; IL2 was given in Cycles 2 and 4 at a dose of 3 MU/m^2/day for 4 days one week before the antibody and at a dose of 4.5 MU/m^2/day for 4 days concurrently with the antibody; sargramostim was administered in Cycles 1, 3 and 5 at 250 mcg/m^2/day subcutaneously starting 3 days prior to ch14.18 (dinutuximab) and continued for 7 days after antibody therapy. Common toxicities were neuropathic pain, fever, hypersensitivity reactions and capillary leak syndrome, likely related to ch14.18 (dinutuximab) infusion alone and in combination with cytokines. Data from Dr. Frank Balis' lab based on simulations of different antibody administration schedules with the same total dose per cycle (daily for 10 hours x 4 days; continuous 48 hour infusion; continuous 96 hour infusion; and continuous 120 hour infusion) in 14 NB patients reveal no differences in the overall area under the curve (AUC) and drug exposure of ch14.18 (dinutuximab) (personal communication, Dr. Balis) and hence we hypothesized that the schedule of administration should not affect the efficacy of dinutuximab. Therefore, patients enrolled on this study were given dinutuximab as a 20-hour infusion each day for a total of 2 days (35 mg/m^2/day for a total of 70 mg/m^2/cycle). The infusion was allowed to be extended for an additional 2 days, if needed, for managing adverse effects.

However, during the conduct of this trial, there were two serious adverse events experienced by 2 separate patients who were treated on protocol therapy. One patient experienced sudden unexplained death and the other experienced Grade 4 neurotoxicity (depressed level of consciousness and respiratory depression). While the exact cause of these events remains unclear, it is possible that these events may have been related to the experimental infusion schedule of dinutuximab, possibly leading to higher peak concentrations of the drug. In light of these events, the infusion schedule of dinutuximab has been modified for the remainder of the study to a 10-hour infusion each day for a total of 4 days. This revised schedule is identical to the schedule used in COG NB trials.

The total dose of ch14.18 (dinutuximab) delivered per cycle will be 70 mg/m^2 (United Therapeutics compound), which is equivalent to 100 mg/m^2 of the NCI compound used historically in NB studies. We will evaluate the median time to infusion completion in our study patients.

Recent data from SIOPEN presented at the 2014 Advances in Neuroblastoma Research meeting showed that ch14.18 (dinutuximab) + IL2 is more toxic than ch14.18 (dinutuximab) alone, and no more effective.[15] IL2 will therefore not be used in this study due to lack of conclusive data that it adds to the efficacy of ch14.18 (dinutuximab) therapy and significant concerns regarding toxicity of IL2, such as capillary leak syndrome in the adolescent patient population.

2.5.2 Pharmacokinetics

In Phase 1 trials of dinutuximab, the plasma clearance of ch14.18 (dinutuximab) followed a biphasic pattern. In patients with NB, the $t_{½}\alpha$ was 3.4±3.1 hours and the $t_{½}\beta$ was 66.6±27.4 hours.[16] In adult patients with melanoma and one patient with osteosarcoma, the $t_{½}\alpha$ and $t_{½}\beta$ were significantly longer than in younger patients with NB.[17] However, more recent data at the current dosing schedule in young NB patients show comparable $t_{½}$ and AUCs to adults,[18] but PK data are lacking for the adolescent population. Although the simulation data from Dr. Balis' lab suggest that the AUC should remain similar with varying schedules of ch14.18 (dinutuximab) infusion, it remains to be confirmed in actual patient samples. Therefore, PK analysis at defined time points was mandatory for a minimum of 10 patients who received the experimental infusion schedule in this study.

Further, in order to obtain the most data possible to identify any changes in PK parameters such as Cmax and serum half-life, we will require all patients who enroll subsequent to the approval of this amendment to consent to pharmacokinetic sampling as outlined in the amended protocol.

2.6 Correlative studies

2.6.1 To Assess the Relationship Between Probability of Disease Control and Tumor GD2 Expression

No data currently exist on the correlation between GD2 expression level in tumors and clinical response. We hypothesize that patients with increased expression of GD2 antigen will have an increased probability of response. Formalin-fixed, paraffin-embedded (FFPE) tissue blocks or cut sections of the tumor tissue (from the most recent surgery showing metastatic osteosarcoma) will be required from institutions for patients enrolled on this study.

2.6.2 Rationale for Genotyping FcR, KIR and KIR Ligand

In general, the anti-tumor activities of unconjugated mAbs require the contribution of either complement- or Fcγ-receptor (FcγR)-expressing effector cells to achieve tumor cell killing. However, because most tumor cells express increased amounts of complement-inhibiting proteins, which protect against complement-mediated lysis, antibody-dependent cell cytotoxicity (ADCC) is considered the key antitumor mechanism of therapeutic antibodies *in vivo*. Natural killer (NK) cells, T-lymphocytes, monocytes and granulocytes are capable of mediating ADCC

against antibody-coated targets via their expression of FcγR for IgG. The FcγR genes display polymorphisms that greatly influence the affinity of IgG for the Fcγ receptor. NK cells bearing the FcγRIIIa 158V/V allele mediate ADCC more effectively than those with F/F allele. Similarly, for FcγRIIA, the high-affinity H allele at position 131 results in greater affinity of FcγRIIa for IgG, whereas the low-affinity R allele is associated with decreased binding. The FCGR3A and FCGR2A gene polymorphism was reported to influence the response of lymphoma to rituximab (anti-CD20). As ch14.18 (dinutuximab) is very effective in mediating ADCC, its efficacy in osteosarcoma may be associated with FCGR3 and/or FCGR2 genotype.

Killer-Immunoglobulin-like Receptors (KIR) recognize specific HLA molecules, regulate function of human natural killer (NK) cells and control self-tolerance. The interactions between KIR on donor NK cells and KIR ligands (KIR-L) on recipient tissues influence anti-tumor efficacy of allogeneic hematopoietic stem cell transplantation (HSCT).[19] Furthermore, inherited KIR and KIR-L alleles influence the antitumor effects of autologous HSCT. Since the genes encoding for KIR and KIR-L are inherited independently, it is possible for an individual to be KIR-receptor ligand mismatched with oneself. In a COG Phase 2 study of 38 relapsed/refractory NB receiving humanized anti-GD2 antibody linked to IL2, 7 of 24 mismatched patients experienced either complete response or improvement of their disease after immunocytokine therapy, while no responses or comparable improvements of disease were observed in 14 patients who were KIR and KIR-L matched ($p = 0.03$).[20] This data suggests that patients with KIR receptor-ligand mismatch may show better clinical responses to immunotherapy with anti-GD2 antibody.

2.6.3 Rationale for NKp30 Isoform and B7-H6 Ligand Levels Determination

The NK cell receptor NKp30 is selectively expressed by all human NK cells and plays an important role in triggering NK-mediated cytotoxicity. NKp30 is also involved in the cross-talk between NK and dendritic cells. Three NKp30 splice variants have been shown to be of prognostic significance in gastrointestinal stromal tumor sarcoma (GIST), which expresses NKp30 ligands and that is treated with NK-stimulatory KIT tyrosine kinase inhibitors. Healthy individuals and those with GIST show different NKp30 isoform expression patterns. In GIST patients, predominant expression of the immunosuppressive NKp30*c* isoform (compared to the immunostimulatory NKp30a and NKp30b isoforms) was associated with reduced survival, decreased NKp30-dependent tumor necrosis factor-α (TNF-α), IL10 and CD107a release, and defective secretions of interferon-γ (IFN-γ) and interleukin 12 (IL12) involved in NK-DC cross-talk. Preferential NKp30c expression resulted partly from a single-nucleotide polymorphism at position 3790 in the 3' untranslated region of the gene encoding NKp30. [21] In a recent French study, high levels of the immunosuppressive Nkp30*c*-isoform were associated with worse prognosis (PFS, P= 0.01) in patients with high risk NB.[22] No impact of the Nkp30*a*-isoform was noted. B7H6 is a tumor cell ligand for the NK cell receptor NKp30,[23] which is shed from tumor cells into circulation.[24] Soluble B7-H6 accumulated in the serum of high risk NB patients, correlating with NKp30 down regulation on peripheral blood NK cells, disease dissemination and resistance to chemotherapy.[25] Since NK cytotoxicity plays a major role in tumor immunity in general, and in anti GD2-mediated tumor cytotoxicity in particular, the impact of

NKp30 isoforms and circulating B7-H6 levels may be more pronounced in patients receiving ch14.18 (dinutuximab) therapy.

2.6.4 Banking Metastatic Osteosarcoma Tumor Samples for Future Research Studies

While a significant number of primary tumor samples have been banked as part of the osteosarcoma biology study AOST06B1, a striking paucity exists in the number of banked relapsed or metastatic samples. The majority of osteosarcoma patients who succumb to disease die of progressive metastatic or recurrent disease. In several cancers, metastatic or recurrent tumors have been shown to have a distinct molecular phenotype compared to the primary tumor. Therefore, it is imperative to analyze the metastatic and relapse samples of osteosarcoma, in addition to the primary tumor, to be able to identify novel therapeutic targets in recurrent disease. This study will attempt to increase the number of available metastatic and relapsed osteosarcoma samples to aid in future research studies in this specific cohort.

Any available tumor specimen (either formalin-fixed or frozen) obtained from biopsy or resection of a suspected disease recurrence site will be requested for banking at the COG tumor bank for potential future research studies that may require analysis of metastatic or relapsed samples. Left over specimens from the GD2 tumor expression analysis will be banked for future research studies.

2.6.5 To Determine a Descriptive Profile of Human Anti-Chimeric Antibody (HACA) During Immunotherapy.

While no significant development of HACA has been observed in recent trials in neuroblastoma with using ch14.18 (dinutuximab) in comparable doses as this study, the immune profile of neuroblastoma patients is likely significantly different due to majority of patients having undergone an autologous stem cell transplant. The recurrent osteosarcoma patient population will likely be more immunocompetent and may have a higher potential of developing HACA or neutralizing antibodies to dinutuximab. Therefore, these will be assessed in peripheral blood of all patients receiving ch14.18 (dinutuximab) during the course of therapy.

2.6.6 Circulating Tumor DNA (ctDNA)

There are few biomarkers proven to predict outcome in osteosarcoma. Percent tumor necrosis has been a useful measure of treatment response in this disease although its value remains questionable across studies. In addition, histologic necrosis is not helpful to predict response at diagnosis or any sooner than definitive surgery usually around Week 11 of therapy. Further, no biomarker currently exists to predict early relapse in these patients. Thus, a new approach to measuring disease response would aid in developing risk-stratification approaches for adding new therapies to standard three-drug chemotherapy treatment for this disease. One appealing alternative approach includes minimal residual disease testing by detection, quantification and serial tracking of ctDNA, also known as liquid biopsy studies, obtained from peripheral blood draws. Liquid biopsy technologies have demonstrated superior detection and quantification rates compared to assays for circulating tumor cells.[26] Furthermore, ctDNA can be reliably extracted from plasma using commercially available kits making it feasible for any clinical or

research laboratory to draw and ship appropriate samples for ctDNA studies. The integrity of ctDNA can be further enhanced by the use of specialized blood tubes, such as Streck Cell-Free DNA BCT tubes, widely employed for circulating fetal DNA studies and increasingly utilized in ctDNA assays. Many reports have now demonstrated the clinical utility of detecting and quantifying circulating tumor DNA from patients with solid tumors.[26,27] These assays have been used to assess disease burden, track disease response to therapy, identify clonal evolution, and predict relapse after remission. However, the application of cell free DNA technologies has yet to be applied systematically in pediatric osteosarcoma. In this study, we propose to bank serial plasma samples from patients on therapy to aid in future studies to develop ctDNA strategies in osteosarcoma.

3.0 STUDY ENROLLMENT PROCEDURES AND PATIENT ELIGIBILITY

3.1 Study Enrollment

3.1.1 Patient Registration

Prior to enrollment on this study, patients must be assigned a COG patient ID number.

This number is obtained via the COG Registry system once authorization for the release of protected health information (PHI) has been obtained. The COG patient ID number is used to identify the patient in all future interactions with COG. If you have problems with the registration, please refer to the online help.

A Biopathology Center (BPC) number will be assigned as part of the registration process. Each patient will be assigned only one BPC number per COG Patient ID. For additional information about the labeling of specimens please refer to the Pathology and/or Biology Guidelines in this protocol.

Please see Appendix I for detailed CTEP Registration Procedures for Investigators and Associates, and Cancer Trials Support Unit (CTSU) Registration Procedures including: how to download site registration documents; requirements for site registration, submission of regulatory documents and how to check your site's registration status.

3.1.2 IRB Approval

Sites must obtain IRB/REB approval for this protocol and submit IRB/REB approval and supporting documentation to the CTSU Regulatory Office before they can be approved to enroll patients. Allow 3 business days for processing. The submission must include a fax coversheet (or optional CTSU IRB Transmittal Sheet) and the IRB approval document(s). The CTSU IRB Certification Form may be submitted in lieu of the signed IRB approval letter. All CTSU forms can be located on the CTSU web page (https://www.ctsu.org). Any other regulatory documents needed for access to the study enrollment screens will be listed for the study on the CTSU Member's Website under the RSS Tab.

IRB/REB approval documents may be submitted to the CTSU Regulatory Office via the Regulatory Submission Portal, where they will be entered and tracked in the CTSU RSS.

Regulatory Submission Portal: www.ctsu.org (members' area) → Regulatory Tab →Regulatory Submission

Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 in order to receive further instruction and support. For general (non-regulatory) questions call the CTSU General Helpdesk at: 1-888-823-5923.

Study centers can check the status of their registration packets by querying the Regulatory Support System (RSS) site registration status page of the CTSU members' web site by entering credentials at https://www.ctsu.org. For sites under the CIRB initiative, IRB data will automatically load to RSS.

Note: Sites participating on the NCI CIRB initiative and accepting CIRB approval for the study are not required to submit separate IRB approval documentation to the CTSU Regulatory Office for initial, continuing or amendment review. This information will be provided to the CTSU Regulatory Office from the CIRB at the time the site's Signatory Institution accepts the CIRB approval. The Signatory site may be contacted by the CTSU Regulatory Office or asked to complete information verifying the participating institutions on the study. Other site registration requirements (ie, laboratory certifications, protocol-specific training certifications, or modality credentialing) must be submitted to the CTSU Regulatory Office or compliance communicated per protocol instructions.

3.1.3 Reservation Requirements

Prior to obtaining informed consent and enrolling a patient, a reservation must be made following the steps below. Reservations may be obtained 24 hours a day through the Oncology Patient Enrollment Network (OPEN) system.

Patient enrollment for this study will be facilitated using the Slot-Reservation System in conjunction with the Registration system in OPEN. Prior to discussing protocol entry with the patient, site staff must use the CTSU OPEN Slot Reservation System to ensure that a slot on the protocol is available for the patient. Once a slot-reservation confirmation is obtained, site staff may then proceed to enroll the patient to this study.

If the study is active, a reservation can be made by following the steps below:

1) Log in to https://open.ctsu.org/open/ using your CTEP IAM user name and password.
2) In order to make a reservation, the patient must have an OPEN patient number. Click on the 'Slot Reservation' tab to create an OPEN patient number, under 'Patients'.
3) Using the OPEN patient number '**RESERVE'** a slot for that patient.

4) On the 'Create Slot Reservation' page, select the Protocol Number, enter the COG Patient ID, and choose the required stratum (if applicable) in order to obtain a reservation.

   Refer to the 'SITE – Slot Reservation Quick Reference' guide posted under the 'Help' tab in OPEN for detailed instructions:
   https://www.ctsu.org/readfile.aspx?fname=OPEN/OPEN_SlotReservation_QuickReference_SiteUserGuide_102612.pdf&ftype=PDF

3.1.4 Study Enrollment

Patient enrollment will be facilitated using the Oncology Patient Enrollment Network (OPEN). OPEN is a web-based registration system available on a 24/7 basis. To access OPEN, the site user must have an active CTEP-IAM account (check at < https://eapps-ctep.nci.nih.gov/iam/index.jsp >) and a 'Registrar' role on either the lead protocol organization (LPO) or participating organization roster.

All site staff will use OPEN to enroll patients to this study. It is integrated with the CTSU Enterprise System for regulatory and roster data and, upon enrollment, initializes the patient position in the Rave database. OPEN can be accessed at https://open.ctsu.org or from the OPEN tab on the CTSU members' side of the website at https://www.ctsu.org.

Prior to accessing OPEN, site staff should verify the following:

- All eligibility criteria have been met within the protocol stated timeframes.
- All patients have signed an appropriate consent form and HIPAA authorization form (if applicable).

Note: The OPEN system will provide the site with a printable confirmation of registration and treatment information. Please print this confirmation for your records.

Further instructional information is provided on the CTSU members' web site OPEN tab or within the OPEN URL (https://open.ctsu.org). For any additional questions contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

3.1.5 Timing

Patients must be enrolled before treatment begins. The date protocol therapy is projected to start must be no later than **fourteen (14)** calendar days after the date of study enrollment. **Patients who are started on protocol therapy on a Phase 2 study prior to study enrollment will be considered ineligible**.

All clinical and laboratory studies to determine eligibility must be performed within 7 days prior to enrollment unless otherwise indicated in the eligibility section below.

Protocol therapy will begin no sooner than 14 days after the last major surgical procedure. The study enrollment must be within 4 weeks from the last surgical procedure to achieve complete remission.

3.1.6 Participation in Biology Studies

During the informed consent process, patients/guardians must be made aware that this study requires submission of research samples for pharmacokinetic and biology studies, including the fact that a number of non-standard blood samples will be required.

3.2 Patient Eligibility Criteria

Important note: The eligibility criteria listed below are interpreted literally and cannot be waived. All clinical and laboratory data required for determining eligibility of a patient enrolled on this trial must be available in the patient's medical/research record which will serve as the source document for verification at the time of audit.

All clinical and laboratory studies to determine eligibility must be performed within 7 days prior to enrollment unless otherwise indicated. Laboratory values used to assess eligibility must be no older than seven (7) days at the start of therapy. Laboratory tests need not be repeated if therapy starts within seven (7) days of obtaining labs to assess eligibility. If a post-enrollment lab value is outside the limits of eligibility, or laboratory values are > 7 days old, then the following laboratory evaluations must be re-checked within 48 hours prior to initiating therapy: CBC with differential, bilirubin, ALT (SGPT), AST, electrolytes and serum creatinine. If the recheck is outside the limits of eligibility, the patient may not receive protocol therapy and will be considered off protocol therapy.

Required imaging studies must be completed prior to enrollment as outlined in Table 16.1.1. A repeat post-operative CT chest must be obtained within 7 days prior to enrollment to provide a post-operative baseline for comparison with future scans.

3.2.1 Age

Patients must be less than 30 years of age at enrollment.

3.2.2 Diagnosis

3.2.2.1 Patients must have histologic diagnosis of osteosarcoma at original diagnosis.

3.2.2.2 Patients must have had at least one episode of disease recurrence in the lungs without limitation on number of episodes of recurrence as long as they meet the following criteria:

- Surgical resection of all possible sites of suspected pulmonary metastases in order to achieve a complete remission within 4 weeks prior to study enrollment.*
- Pathologic confirmation of metastases from at least one of the resected sites.

* For patients with bilateral pulmonary metastases, resection must be performed from both lungs and the study enrollment must be within 4 weeks from date of the last lung surgery.

Note: If surgery related changes such as atelectasis are seen on the post-operative CT scan, patients will remain eligible to enroll as long as the operating surgeon believes that all sites of metastases were resected. Patients with positive microscopic margins will be eligible to enroll.

3.2.3 Specimen Submission

Patient must have adequate tumor specimen available for submission (see Section 15.1).

3.2.4 Performance Level

Patients must have a performance status corresponding to ECOG scores of 0, 1 or 2. Use Karnofsky for patients > 16 years of age and Lansky for patients ≤ 16 years of age. See https://www.cogmembers.org/_files/protocol/Standard/PerformanceStatusScalesScoring.pdf.

3.2.5 Prior Therapy

Patients must have fully recovered from the acute toxic effects of all prior chemotherapy, immunotherapy, or radiotherapy prior to entering this study.

a. Myelosuppressive anti-cancer therapy: Must not have been received within 2 weeks of study entry (4 weeks if prior nitrosourea).
b. Biologic (anti-neoplastic agent): At least 7 days since the completion of therapy with a biologic agent.
c. Radiation therapy (RT): ≥ 2 weeks for local palliative RT (small port); ≥ 6 weeks must have elapsed if prior craniospinal RT or if ≥ 50% radiation of pelvis; ≥ 6 weeks must have elapsed if other substantial BM radiation.
d. Surgery: ≥ 2 weeks from last major surgery, including pulmonary metastasectomy, with the exclusion of a central line placement and core needle or small open biopsies.

3.2.6 Concomitant Medications Restrictions

a. Patient must not have received pegfilgrastim within 14 days of enrollment.
b. Patient must not have received filgrastim (G-CSF, Neupogen) within 7 days of enrollment.
c. Patient must not have received immune suppressants: corticosteroids (for other than allergic reactions and anaphylaxis), cyclosporine or tacrolimus within 7 days of enrollment.
   Note: The use of topical and/or inhalational steroids is allowed.

Please see Section 4.1.1 for the concomitant therapy restrictions for patients during treatment.

3.2.7 Organ Function Requirements

3.2.7.1 Hematological:

- Total absolute phagocyte count [APC = (%neutrophils + %monocytes) x WBC] is at least 1000/μL
- Platelet count ≥ 50,000/μL

3.2.7.2 Adequate Renal Function Defined As:

- Creatinine clearance or radioisotope GFR ≥ 70 mL/min/1.73 m^2 or
- A serum creatinine based on age/gender as follows:

Age	Maximum Serum Creatinine (mg/dL)	
	Male	Female
1 month to < 6 months	0.4	0.4
6 months to < 1 year	0.5	0.5
1 to < 2 years	0.6	0.6
2 to < 6 years	0.8	0.8
6 to < 10 years	1	1
10 to < 13 years	1.2	1.2
13 to < 16 years	1.5	1.4
≥ 16 years	1.7	1.4

The threshold creatinine values in this Table were derived from the Schwartz formula for estimating GFR.[28]

3.2.7.3 Adequate Liver Function Defined As:

- Total bilirubin ≤ 1.5 x upper limit of normal (ULN) for age.
- SGPT (ALT) ≤ 110 U/L (for the purpose of this study, the ULN for SGPT is 45 U/L).
- Serum albumin ≥ 2 g/dL.

3.2.7.4 Adequate Cardiac Function Defined As:

- Baseline EKG shows normal QTc interval of ≤ 470 ms.
- Shortening fraction of ≥ 27% by echocardiogram, or
- Ejection fraction of ≥ 50% by radionuclide angiogram or echocardiogram.

3.2.7.5 Adequate Pulmonary Function Defined As:

- No evidence of dyspnea at rest, no history of exercise intolerance, and a pulse oximetry > 94%.

3.2.7.6 Central Nervous System Function Defined As:

- Patient has no known history of seizure disorder.
- CNS toxicity including peripheral neuropathy ≤ Grade 2.

3.2.8 Exclusion Criteria

3.2.8.1 Patients with distant bone metastases at original diagnosis or relapse

(patients with only skip lesions will be eligible).

3.2.8.2 Patients with concurrent local and pulmonary recurrence at the time of enrollment. Note: patients who had local recurrence previously that has been treated and now present with an isolated pulmonary recurrence and meet the surgical resection criteria stated above will be eligible (see Section 3.2.2.2).

3.2.8.3 Patients with primary refractory disease with progression of the primary tumor on initial therapy.

3.2.8.4 Patients with CNS disease or other sites of extra-pulmonary metastases at the time of most recent episode of disease recurrence preceding enrollment.

3.2.8.5 Patients with a prior hypersensitivity reaction to sargramostim.

3.2.8.6 Patients who have received prior anti-GD2 therapy, including CAR T cells directed against GD2 antigen.

3.2.8.7 Pregnancy and Breast Feeding

3.2.8.7.1 Female patients who are pregnant are ineligible.

3.2.8.7.2 Lactating females are not eligible unless they have agreed not to breastfeed their infants.

3.2.8.7.3 Female patients of childbearing potential are not eligible unless a negative pregnancy test result has been obtained.

3.2.8.7.4 Sexually active patients of reproductive potential are not eligible unless they have agreed to use an effective contraceptive method for the duration of their study participation. Patients should maintain adequate contraception for a minimum of 2 months after the last dose of ch14.18 (dinutuximab).

3.2.9 Informed Consent/Assent

The investigational nature and objectives of the trial, the procedures and treatments involved and their attendant risks and discomforts, and potential alternative therapies will be carefully explained to the patient or the patient's parents or guardian if the patient is a child, and a signed informed consent and assent will be obtained according to institutional guidelines. All institutional, FDA, and NCI requirements for human studies must be met.

4.0 TREATMENT PROGRAM

Timing of protocol therapy administration, response assessment studies, and surgical interventions are based on schedules derived from the experimental design or on established standards of care. Minor unavoidable departures (up to 72 hours) from protocol directed therapy and/or disease evaluations (and up to 1 week for surgery) for valid clinical, patient and family logistical, or facility, procedure and/or anesthesia scheduling issues are acceptable (except where explicitly prohibited within the protocol).

4.1 Overview of Treatment Plan

In this single arm trial, eligible patients will receive 70 mg/m^2/cycle of ch14.18 (dinutuximab). Ch14.18 (dinutuximab) will be delivered as 17.5 mg/m^2/day for 4 days. The minimum infusion duration will be 10 hours per day with the option to increase up to 20 hours per day if the infusion needs to be slowed down for managing adverse events. Further rate escalation or reduction will be as guided by the dose modifications listed in Section 5. The maximum duration of infusion each day is 20 hours from the start time of ch14.18. Patients will be observed in the hospital for an additional 24 hours after completion of the last day of dinutuximab infusion in each cycle.

Sargramostim (GM-CSF), 250 mcg/m^2/day will be administered subcutaneously once daily for 14 days/cycle starting 3 days prior to the start of ch14.18 (dinutuximab) infusion in each treatment cycle. The maximum dose for sargramostim is 500 micrograms/dose. It is strongly encouraged that Day 1 of all 5 cycles be on a Friday and the start of ch14.18 (dinutuximab) infusion be the subsequent Monday. If Monday is a holiday, the ch14.18 (dinutuximab) infusion can start on Tuesday for that particular cycle.

A baseline disease evaluation will be performed prior to study enrollment (Please see the table in Section 16.1.1 for details). Imaging studies can be repeated for clinical care at any time per institutional preference or as clinically indicated.

Subsequent disease evaluations will be performed after Cycles 2 and 5 of treatment, at months 8 and 12 from the start of therapy, and at relapse if it occurs during therapy or the subsequent evaluation period. Disease evaluations may include a CT scan of the chest, nuclear medicine imaging to evaluate for bone metastases (bone scan or PET scan, per institutional standard for osteosarcoma) and an MRI of the original primary tumor site (substituting plain radiograph if metallic hardware impedes MRI scan).

Pharmacokinetic (PK) sampling during the first and second cycles of treatment will be required for the first 10 patients enrolled on this study and for all patients enrolled after protocol amendment #2. HACA samples are required of all patients.

4.1.1 Concomitant Medications and Supportive Care

4.1.1.1 Pneumocystis Prophylaxis

In the neuroblastoma protocols using dinutuximab, pneumocystis prophylaxis is routinely recommended. On this study, the use of pneumocystis prophylaxis will be left to the discretion of the treating physician.

4.1.1.2 Other Anti-Cancer Therapy

No other systemic anti-cancer therapy will be permitted. Radiotherapy is also not permitted during protocol therapy.

4.1.1.3 Corticosteroid Therapy

Pharmacologic doses of systemic corticosteroids should be used ONLY for life-threatening conditions (i.e., life-threatening allergic reactions and anaphylaxis such as bronchospasm, stridor) unresponsive to other measures. The use of dexamethasone as an anti-emetic is not permitted. Corticosteroid therapy can be used as a premedication for transfusion in patients known to have a history of transfusion reactions or for treatment of an unexpected transfusion reaction (hydrocortisone 2 mg/kg or less or an equivalent dose of an alternative corticosteroid). **The use of steroids during protocol therapy requires clear justification and documentation.**

4.1.1.4 Other Supportive Care

Appropriate antibiotics, blood products, anti-emetics, fluids, electrolytes and general supportive care are to be used as necessary. Note: the use of gabapentin as an adjunct for pain management should be considered.

For COG Supportive Care Guidelines see: https://childrensoncologygroup.org/index.php/cog-supportive-care-guidelines under Standard Sections for Protocols.

4.1.2 Criteria to Start All Cycles

a. No evidence of serious infection, or infection under control with no active disease and negative blood culture.
b. Serum creatinine less than or equal to the upper limit of normal based on age (see Section 3.2.8.2).
c. Platelet count ≥ 50,000/µL (untransfused).
d. Absolute phagocyte count of ≥ 1000/µL.
e. Total bilirubin ≤ 1.5 x upper limit of normal (ULN) for age

Note: Cycle-specific requirements for ALT (Cycle 1 vs Cycles 2-5) and skin toxicity (Cycles 2-5) are outlined on the first page of each therapy delivery map.

4.2 Cycle 1

4.2.1 Therapy Delivery Map – Cycle 1 This cycle lasts 28 days. This TDM is on 2 pages.	_____ Patient COG ID number _____ DOB

Criteria to start this cycle: ALT ≤ 110 U/L; no evidence of serious infection, or infection under control with negative blood culture; serum creatinine ≤ ULN based on age (see Section 3.2.8.2); total bilirubin ≤ 1.5x ULN for age; platelet count ≥ 50,000/µL; absolute phagocyte count of ≥ 1000/µL.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES
Sargramostim (GM-CSF)	SubQ	250 micrograms/m^2/dose	1-14	See administration guidelines in Section 4.2.3. Start on Friday whenever possible. The maximum dose is 500 micrograms/dose.
ch14.18 (Dinutuximab)	IV for 4 days	17.5 mg/m^2/dose over 10 hours/day[$]	4-7	[$]The daily ch14.18 infusion may be extended up to a total of 20 hours per day for anticipated toxicities. The max. ch14.18 dose for one cycle is 70 mg/m^2. See Section 4.2.3 for detailed administration guidelines, including premedications and monitoring during the infusion. Start on Monday whenever possible.

Ht _____cm Wt _____kg BSA _____m^2

Date Due	Date Given	Day	Sargramostim ____ mcg	Ch14.18 (dinutuximab) _____mg					Studies
			Enter calculated dose for total cycle above and actual dose administered below						
				Start Date/Time ____/____	Stop Date/Time ____/____	Infusion Interrupted? Yes/No	Dose Delivered ____mg	Infusion Rate Start/ End	
		1	____mcg						a-h, j-n
		2	____mcg						
		3	____mcg						
		4	____mcg	____/____	____/____	**Yes/No**	____mg*	____/____	a, b, c, d, i
		5	____mcg	____/____	____/____	**Yes/No**	____mg*	____/____	a, b
		6	____mcg	____/____	____/____	**Yes/No**	____mg*	____/____	a, b
		7	____mcg	____/____	____/____	**Yes/No**	____mg*	____/____	a, b, i
		8	____mcg						
		9	____mcg						
		10	____mcg						
		11	____mcg						a, c, d
		12	____mcg						
		13	____mcg						
		14	____mcg						
		28	Begin next cycle on Day 29 or when criteria to begin cycle are met, whichever occurs later. See Section 4.2.3.						

See Section 5.0 for Dose Modifications for Toxicities and the COG Member website for Supportive Care Guidelines.

* Please record actual dinutuximab dose delivered each day.

4.2.2 Required Observations in Cycle 1

All baseline studies must be performed prior to starting protocol therapy unless otherwise indicated below. Observations a-h, m can be performed up to 7 days before the start of therapy.

a. PE, weight: daily during ch14.18 (dinutuximab) infusion; once between Day 11-14. Note: height is only required at the beginning of this cycle.
b. Electrolytes, creatinine, BUN, Ca^{++}, PO_4, Mg^{++}. Note: perform daily during ch14.18 (dinutuximab) infusion;
c. CBC/diff/platelets. Note: perform once between Day 11-14.
d. Bilirubin, AST, ALT, albumin. Note: perform once between Day 11-14.
e. Performance status.
f. EKG.
g. MUGA or ECHO.
h. GFR or creatinine clearance (if serum creatinine abnormal for age).
i. Pharmacokinetics. Mandatory for patients enrolled after amendment #2. See Section 15.2 for extensive details, including data reporting requirements.
j. Imaging evaluation (MRI/X-ray of primary site, CT chest, bone scan or whole body PET/CT). See Section 3.2 and Section 16.
k. Pregnancy test. A negative pregnancy test is required in females of childbearing potential prior to starting treatment; sexually active patients must use an acceptable method of birth control.
l. Tumor GD2 expression. See Section 15.1 for details.
m. Correlative biology studies: KIR/ FCγR/ NKp30/ B7-H6 and ctDNA (consenting patients only). Samples can be obtained up to 7 days before the start of therapy. See Section 15.3 for details.
n. HACA sampling: once, anytime between Day 1 and Day 4. See Section 15.2 for details.

This listing only includes evaluations necessary to answer the primary and secondary aims. OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD CLINICAL CARE.

Comments
(Include any held doses, or dose modifications)

Cycle 1

4.2.3 Treatment Details for Cycle 1

Each 28-day cycle consists of ch14.18 (dinutuximab) and sargramostim (GM-CSF).

NOTE: It is suggested that Day 1 of Cycle 1 be on a Friday.

Drug doses should be adjusted based on the BSA calculated from **height and actual weight obtained within one week prior** to the beginning of each cycle.

Sargramostim (GM-CSF): Subcutaneous
Dose: 250 micrograms/m²/dose (maximum 500 mcg/dose) subcutaneously daily.
Days: 1 through 14.

NOTE: On Days 4 to 7 of ch14.18 (dinutuximab) infusion, sargramostim should be administered prior to start of dinutuximab, around 8:00 - 9:00 AM. Follow sargramostim with 0.9% sodium chloride (NS) bolus (10 mL/kg) over 1 hour on each day of antibody infusion.

Sargramostim dose will be held if the total white cell count is > 50,000/µL. This is not a toxicity of sargramostim but rather a possible outcome related to its use. Sargramostim will be held until the total white cell count is < 20,000/µL and then will be resumed at 50% dose for the remainder of the cycle. Full dose sargramostim will be used for subsequent cycles.

If criteria for holding sargramostim are met (e.g., see Sections 5.2.1.8 and 5.2.2), then ch14.18 (dinutuximab) should be continued without sargramostim.

Dinutuximab: IV infusion over 10 hours per day* for 4 days
Dose: 70 mg/m²/cycle to be given as 17.5 mg/m²/dose once daily times four days on Days 4-7 of the cycle.

Ch14.18 (dinutuximab) infusion may be extended up to 20 hours per day for anticipated toxicities that are not responsive to supportive measures. Infusion must be stopped after 20 hours from the start time.

NOTE: Begin antibody infusion one hour after sargramostim injection on Day 4 (ie, immediately following the IV bolus of normal saline). Each daily dose should start at 0.88 mg/m²/hour x 0.5 hour and then increased gradually to 1.75 mg/m²/hour for the remainder of the infusion as tolerated. Further rate escalation or reduction should be guided by the dose modifications listed in Section 5.

The rate and the duration of infusion should be recorded carefully each day so the total daily dose can be calculated.

- Recommended premedications include:
  - Hydroxyzine (0.5-1 mg/kg; max dose 50 mg) PO or diphenhydramine (0.5-1 mg/kg; maximum dose 50 mg) PO/IV over 10 minutes to start approximately 20 minutes prior to ch14.18 (dinutuximab) infusion; may be

Cycle 1

repeated every 6 hours as needed during ch14.18 (dinutuximab) infusion. Note: intravenous hydroxyzine is NOT recommended.

- Acetaminophen (10-15 mg/kg; max dose 650 mg) PO given approximately 20 minutes prior to ch14.18 (dinutuximab) infusion; in patients not able to tolerate oral medications, acetaminophen 15 mg/kg (max dose 650 mg) IV over 15 minutes can be used; may be repeated every 4-6 hours as needed for fever.

- Recommended pain management*:
  - Morphine sulfate loading dose immediately prior to ch14.18 (dinutuximab) administration. A dose of 50 micrograms/kg is recommended, though this may be adjusted based on a given patient's pain history.
  - Continue with morphine sulfate infusion titrated to effect. The recommended dose range for the continuous morphine infusion is 20-50 micrograms/kg/hour to continue for 2 hours after completion of the ch14.18 (dinutuximab) infusion.
  - Other opiates such as hydromorphone or fentanyl can be used instead of morphine as clinically indicated.
  - Gabapentin may be used in conjunction with other pain medications per institutional practice.
  - The use of additional pain medications (lidocaine, ketamine) in extenuating circumstances should be undertaken in consultation with pediatric pain management specialists.
    - For example, a lidocaine infusion may be used in conjunction with IV bolus of morphine on prn basis. Suggested administration guidelines for a lidocaine infusion are shown below:
      - Give lidocaine IV bolus at 2 mg/kg in 50 mL NS over 30 minutes prior to the start of ch14.18 (dinutuximab) infusion.
      - At the beginning of ch14.18 (dinutuximab) infusion, start IV lidocaine infusion at 1 mg/kg/hour and continue until two hours after the completion of ch14.18 (dinutuximab) infusion.
      - May give morphine IV bolus 20-50 micrograms/kg every 2 hours prn pain.
      - Patient should be monitored closely for sedation scale, EKG (rate and rhythm), HR, BP, RR, oxygen saturation (pulse oximeter) and pain score. If patient develops dizziness, perioral numbness, tinnitus attributable to lidocaine, then stop lidocaine infusion.
  - Another alternative pain regimen that may be used in subsequent cycles if the above regimen is not well tolerated or inadequate to control pain is a combination of hydromorphone and dexmedetomidine as described by Gorges et al.[29] and outlined in Section 4.3.3. This should be undertaken in consultation with pediatric pain management specialists and institutional guidelines followed for monitoring.
    1. Start both hydromorphone and dexmedetomidine infusions 1 hour prior to commencement of ch14.18 (dinutuximab) infusion.
    2. Start hydromorphone at 2 micrograms/kg/hour and titrate up to a maximum of 8 micrograms/kg/hour
    3. Start dexmedetomidine at 0.1 micrograms/kg/hour and titrate up to a maximum of 0.6 micrograms/kg/hour to achieve adequate analgesia
    4. Alternate increases in hydromorphone and dexmedetomidine

Cycle 1

every 30 minutes as needed.

5. Patients should be monitored closely for hypotension and respiratory depression.
6. Gabapentin can be used in conjunction with this regimen as detailed above.

* Please note that all of the above pain management guidelines are suggestions and can be varied based on institutional/ patient preference as long as they meet safety standards.

- Monitoring during ch14.18 (dinutuximab) infusion:
  - Vital signs should be assessed every 15 minutes for the first hour of the infusion, then hourly during the remainder of the infusion if stable after the first hour. More frequent assessment may be required based on the patient's clinical condition.
  - Strict observation of intake and output is required on the days of ch14.18 (dinutuximab) administration.
  - Daily physical examination including a careful eye exam for pupillary reflexes and extraocular movements.
  - Patients should be weighed daily on the days of ch14.18 (dinutuximab) administration.

- Have immediately available during the ch14.18 (dinutuximab) infusion:
  - Epinephrine (1:10,000) 0.01 mg/kg or 0.1 mL/kg: IVP (max 5 mL) every 3-5 minutes.
  - Hydrocortisone 2 mg/kg (max 100 mg) IVP. Only use it for life-threatening reactions (hypotension, bronchospasm, angioedema involving the airway) not responsive to other measures.

See Section 5.0 for Dose Modifications based on Toxicities. Information on management of toxicities including anaphylaxis and hypertension is also included in Section 5.0. It is recommended that these be reviewed, printed and available on the inpatient unit to facilitate treatment decisions.

Following completion of Cycle 1, Cycle 2 starts on Day 29 or when starting criteria are met, (whichever occurs later). If the starting criteria are not met within 4 weeks, then the patient will go off protocol therapy.

4.3 Cycles 2-5

4.3.1 Therapy Delivery Map – Cycles 2-5 Each cycle lasts 28 days. This TDM is on 2 pages. Use a copy of this page once for each cycle. Please note cycle number below.	_____ Patient COG ID number _____ DOB

Criteria to start a cycle: ALT < 5x ULN (45 U/L) provided that the usual causes of transaminitis such as infections, tumor progression, or drug toxicity are excluded by appropriate blood and imaging studies AND the transaminitis is stable if not improving; skin toxicity ≤ Grade 1; no evidence of serious infection, or infection under control with no active disease and negative blood culture; serum creatinine ≤ ULN based on age as stated in Section 3.2.7.2; platelet count ≥ 50,000/µL; absolute phagocyte count of ≥ 1000/µL; total bilirubin ≤ 1.5 x ULN for age.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES
Sargramostim (GM-CSF)	SubQ	250 micrograms/m^2/dose	1-14	See administration guidelines in Section 4.3.3. Start on Friday whenever possible. The maximum dose is 500 micrograms/dose.
ch14.18 (Dinutuximab)	IV for 4 days	17.5 mg/m^2/dose over 10 hours/day[$]	4-7	[$]The daily ch14.18 infusion may be extended up to a total of 20 hours per day for anticipated toxicities. The max. ch14.18 dose for each cycle is 70 mg/m^2. See Section 4.3.3 for detailed administration guidelines, including premedications and monitoring during the infusion. Start on Monday whenever possible.

Enter Cycle #: _____ Ht _____cm Wt _____kg BSA _____m^2

Date Due	Date Given	Day	Sargramostim ____ mcg	Ch14.18 (dinutuximab) _____mg					Studies
			Enter calculated dose for total cycle above and actual dose administered below						
				Start Date/Time /	Stop Date/Time /	Infusion Interrupted? Yes/No	Dose Delivered ____mg	Infusion Rate Start/ End	
		1	____mcg						a, e, f, h, i, j
		2	____mcg						
		3	____mcg						
		4	____mcg	____/____	____/____	**Yes/No**	____mg*	____/____	a, b, c, d
		5	____mcg	____/____	____/____	**Yes/No**	____mg*	____/____	a, b
		6	____mcg	____/____	____/____	**Yes/No**	____mg*	____/____	a, b
		7	____mcg	____/____	____/____	**Yes/No**	____mg*	____/____	a, b
		8	____mcg						
		9	____mcg						
		10	____mcg						
		11	____mcg						a, c, d
		12	____mcg						
		13	____mcg						
		14	____mcg						
		28	Begin next cycle on Day 29 or when criteria to begin cycle are met (whichever occurs later). See Section 4.3.3. Treatment should continue for a total of 5 cycles, in the absence of disease progression or unacceptable toxicity. If disease progression or unacceptable toxicity occurs, the patient will be taken off protocol therapy. See Section 7.1 for End of Therapy evaluations including required imaging.						g, j

* Please record actual dose delivered each day. See Section 5.0 for Dose Modifications for Toxicities and the COG Member website for Supportive Care Guidelines.

Cycles 2-5

Cycles 2-5

4.3.2 Required Observations During Cycles 2-5

a. Height: Day 1 only. PE, weight: perform on Day 1, daily during ch14.18 (dinutuximab) infusion and once between Day 11-14.
b. Electrolytes, creatinine, BUN, Ca^{++}, PO_4, Mg^{++}. Note: perform daily during ch14.18 (dinutuximab) infusion.
c. CBC/diff/platelets. Note: perform once between Day 11-14.
d. Bilirubin, AST, ALT, albumin. Note: perform once between Day 11-14.
e. Performance status.
f. Pharmacokinetics. Perform once before the start of Cycle 2 only. Mandatory for patients enrolled after protocol amendment #2. See Section 15.2 for extensive details, including data reporting requirements.
g. Imaging evaluation. CT of the chest must be obtained between Day 24 to 28 of Cycle 2 and also during a 7-day window around the last day of Cycle 5. Primary tumor site imaging and bone scan/ PET-CT scan should be obtained during a 7 day window around the last day of Cycle 5. See Section 16 for details.
h. Correlative biology study. B7-H6 ligand (consenting patients only). Obtain this sample before the start of Cycle 2 only. See Section 15.3 for details.
i. HACA sampling: once, anytime between Day 1 and Day 4. See Section 15.2 for details.
j. Correlative biology study. ctDNA (consenting patients only). Obtain sample prior to ch14.18 infusion start and at end of Cycle 2 and 5 along with tumor evaluations. See Section 15.4 for details.

This listing only includes evaluations necessary to answer the primary and secondary aims. OBTAIN OTHER STUDIES AS REQUIRED FOR GOOD CLINICAL CARE.

Comments
(Include any held doses, or dose modifications)

4.3.3 Treatment Details for Cycles 2-5

Each cycle consists of ch14.18 (dinutuximab) and sargramostim, and lasts 28 days.

NOTE. It is suggested that Day 1 of each cycle be on a Friday.

Drug doses should be adjusted based on the BSA calculated from **height and weight obtained within one week prior** to the beginning of each cycle.

Sargramostim (GM-CSF): Subcutaneous
Dose: 250 micrograms/m²/dose (maximum 500 mcg/dose) subcutaneously daily.
Days: 1 through 14.

NOTE: On Days 4 to 7 of ch14.18 (dinutuximab) infusion, sargramostim should be administered prior to start of dinutuximab, around 8:00 - 9:00 AM. Follow sargramostim with 0.9% sodium chloride (NS) bolus (10 mL/kg) over 1 hour on each day of ch14.18 (dinutuximab) infusion.

Sargramostim dose will be held if the total white cell count is > 50,000/µL. This is not a toxicity of sargramostim but rather a possible outcome related to its use. Sargramostim will be held until the total white cell count is less than 20,000/µL and then will be resumed at 50% dose for the remainder of that cycle. Full dose sargramostim will be used for subsequent cycles.

If criteria for holding sargramostim are met (e.g., see Sections 5.2.1.8 and 5.2.2), then ch14.18 (dinutuximab) should be continued without sargramostim.

Dinutuximab: IV infusion over 10 hours per day* for 4 days
Dose: 70 mg/m²/cycle to be given as 17.5 mg/m²/dose once daily times four days on Days 4- 7 of the cycle.

Ch14.18 (dinutuximab) infusion may be extended up to a total 20 hours per day for anticipated toxicities that are not responsive to supportive measures. Infusion must be stopped after 20 hours from the start time.

NOTE: Begin antibody infusion one hour after sargramostim injection on Day 4 (ie, immediately following the IV bolus of normal saline). Each daily dose should start at 0.88 mg/m²/hour x 0.5 hour and then increase infusion gradually to 1.75 mg/m²/hour for the remainder of the infusion as tolerated. Further rate escalation or reduction should be guided by the dose modifications listed in Section 5.

The rate and duration of the infusion should be recorded carefully each day so the total daily dose can be calculated.

- Recommended premedications include:
  - Hydroxyzine (0.5-1 mg/kg; max dose 50 mg) PO or diphenhydramine (0.5-1 mg/kg; max dose 50 mg) IV over 10 minutes to start approximately 20 minutes prior to ch14.18 (dinutuximab) infusion; may be repeated

every 6 hours as needed during ch14.18 (dinutuximab) infusion. Note: intravenous hydroxyzine is NOT recommended.
  - Acetaminophen (10 mg/kg; max dose 650 mg) PO given approximately 20 minutes prior to ch14.18 (dinutuximab) infusion; in patients not able to tolerate oral medications, acetaminophen 15 mg/kg (max dose 650 mg) IV over 15 minutes can be used; may be repeated every 4-6 hours as needed for fever.

- Recommended pain management*:
  - Morphine sulfate loading dose immediately prior to ch14.18 (dinutuximab) administration. A dose of 50 micrograms/kg is recommended, though this may be adjusted based on a given patient's pain history.
  - Continue with morphine sulfate drip titrated to effect. The recommended dose range for the continuous infusion morphine is 20-50 micrograms/kg/hour to continue for 2 hours after completion of the ch14.18 (dinutuximab) infusion.
  - Other narcotics such as hydromorphone or fentanyl can be used.
  - Gabapentin may be used in conjunction with other pain medications per institutional practice.
  - The use of additional pain medications (lidocaine, ketamine) in extenuating circumstances should be undertaken in consultation with pediatric pain management specialists.
    - For example, a lidocaine infusion may be used in conjunction with IV bolus of morphine on prn basis. Suggested administration guidelines for a lidocaine infusion are shown below:
      - Give lidocaine IV bolus at 2 mg/kg in 50 mL NS over 30 minutes prior to the start of ch14.18 (dinutuximab) infusion.
      - At the beginning of ch14.18 (dinutuximab) infusion, start IV lidocaine infusion at 1 mg/kg/hr and continue until two hours after the completion of ch14.18 (dinutuximab) infusion.
      - May give morphine IV bolus 20-50 microgram/kg every 2 hours prn pain.
      - Patient should be monitored closely for sedation scale, EKG (rate and rhythm), HR, BP, RR, oxygen saturation (pulse oximeter) and pain score. If patient develops dizziness, perioral numbness, tinnitus attributable to lidocaine, then stop lidocaine infusion.
  - Another alternative pain regimen that may be used in subsequent cycles if the above regimen is not well tolerated or adequate to control pain is a combination of hydromorphone and dexmedetomidine as described by Gorges et al.[29] This should be undertaken in consultation with pediatric pain management specialists.
    1. Start both hydromorphone and dexmedetomidine infusions 1 hour prior to commencement of ch14.18 (dinutuximab) infusion.
    2. Start hydromorphone at 2 micrograms/kg/hour and titrate up to a maximum of 8 micrograms/kg/hour
    3. Start dexmedetomidine at 0.1 micrograms/kg/hour and titrate up

to a maximum of 0.6 micrograms/kg/hour to achieve adequate analgesia
4. Alternate increases in hydromorphone and dexmedetomidine every 30 minutes as needed.
5. Patients should be monitored closely for hypotension and respiratory depression.
6. Gabapentin can be used in conjunction with this regimen as detailed above.

* Please note that all of the above pain management guidelines are suggestions and can be varied based on institutional/ patient preference as long as they meet safety standards.

- Monitoring during ch14.18 (dinutuximab) infusion:
  - Vital signs should be assessed every 15 minutes for the first hour of the infusion, then hourly during the remainder of the infusion if stable after the first hour. More frequent assessment may be required based on the patient's clinical condition. Between antibody doses, vitals should be assessed every 4 hours.
  - Strict observation of intake and output is required on the days of ch14.18 (dinutuximab) administration.
  - Daily physical examination including a careful eye exam for pupillary reflexes and extraocular movements.
  - Patients should be weighed daily on the days of ch14.18 (dinutuximab) administration.

- Have immediately available at the bedside during the ch14.18 (dinutuximab) infusion:
  - Epinephrine (1:10,000) 0.01 mg/kg or 0.1 mL/kg: IVP (max 5 mL) every 3-5 minutes.
  - Hydrocortisone 2 mg/kg (max 100 mg) IVP. Only use it for life-threatening reactions (hypotension, bronchospasm, angioedema involving the airway) not responsive to other measures.

See Section 5.0 for Dose Modifications based on Toxicities. Information on management of toxicities including anaphylaxis and hypertension is also included in Section 5.0. It is recommended that these be reviewed, printed and available on the inpatient unit to facilitate treatment decisions.

Following completion of a cycle, the next cycle starts on Day 29 or when starting criteria are met, (whichever occurs later). If the starting criteria are not met within 4 weeks, then the patient will go off protocol therapy.

5.0 DOSE MODIFICATIONS FOR TOXICITIES

In addition to dose modifications, this section provides information regarding management of toxicities known to be associated with protocol therapy.

5.1 Dose Modifications for Hematologic Toxicity

5.1.1

If there is a delay of ≥ 28 days in recovery of APC to ≥ 1000/µL and/ or platelets to ≥ 50,000/µL to start the next treatment cycle, the patient will be removed from protocol therapy.

5.1.2

Patients who develop atypical hemolytic uremic syndrome (which is one of the extremely rare side effects of ch14.18) should discontinue all further protocol therapy.

5.2 Dose Modifications for Non-Hematologic Toxicity

An elevation in ALT that causes a delay of ≥ 14 days between treatment cycles will require a 25% reduction in the dose of ch14.18 (dinutuximab) for subsequent cycles (i.e., ch14.18 (dinutuximab) dose: 52.5 mg/m^2/cycle). If a delay of ≥ 14 days between treatment cycles recurs due to elevation in ALT despite the dose reduction, the patient will be removed from protocol therapy.

5.2.1 Dinutuximab/Sargramostim Specific Dose Modifications and Toxicity Management Recommendations

5.2.1.1 Treatment of ch14.18 (dinutuximab) induced hypotension (without evidence of allergic reaction)

- If hypotension is severe and accompanied by poor perfusion, end organ dysfunction, or acidemia – Pediatric Advanced Life Support (PALS) or Advanced Cardiovascular Life Support (ACLS) guidelines should be followed and ch14.18 (dinutuximab) infusion should be discontinued for this cycle.
- In the absence of poor perfusion, end organ dysfunction or acidemia, moderate hypotension is defined as:
  - Symptomatic decreases in blood pressure and/or
  - Systolic blood pressure (SBP) < 80 mmHg for age > 12 years
  - SBP < 70 mmHg for age 1-12 years
  - SBP < 65 mmHg for age < 1 year OR
  - SBP or DBP decreased by > 20 % below baseline
- If moderate hypotension is observed:
  - Immediately hold dinutuximab
  - Give normal saline bolus (20 mL/kg as rapidly as possible)
  - Stop or adjust doses of narcotics and sedating H1 blockers
  - Consider use of Trendelenberg position
- If hypotension persists after the above measures have been taken:
  - Reassess perfusion and end organ function; follow PALS or ACLS algorithm if needed

- Repeat NS bolus
- Consider use of albumin if albumin < 3 gm/dL
- Consider use of PRBCs if Hb < 10 gm/dL
- Consider transfer to intensive care setting

- If hypotension persists following 2 volume boluses, give an additional bolus and prepare to administer pressors
  - Adrenergic pressors, norepinephrine or epinephrine, are preferred over dopamine.

- Resumption of ch14.18 (dinutuximab)
  - For patients whose hypotension resolves promptly and completely with limited volume resuscitation (≤ 40 mL/kg) and without requirement for pressor support, ch14.18 (dinutuximab) may be restarted at 50% of the previous infusion rate. The ch14.18 (dinutuximab) may be restarted once the blood pressure has normalized and remains stable for a minimum of 2 hours after volume resuscitation is completed. If blood pressures are stable for 2 hours after the restart of ch14.18 (dinutuximab) at 50% rate, the infusion may be given at full rate for that day and subsequent days. If the patient again experiences hypotension requiring multiple volume boluses (≥ 40 mL/kg) when ch14.18 (dinutuximab) is given at full rate but tolerates the 50% infusion rate, the remaining days' infusion of ch14.18 (dinutuximab) should be given at the tolerated 50% rate of infusion once blood pressures are stable again. The infusion may be started at the full rate at the start of subsequent cycles.
  - For patients who require multiple volume boluses (> 40 mL/kg) for hemodynamic stabilization, ch14.18 (dinutuximab) should be resumed the following day at 50% of the initial infusion rate.
    - If blood pressures are stable for 2 hours after resumption of ch14.18 (dinutuximab) at the reduced rate, the remainder of the antibody infusion may be given at the full rate. If hypotension recurs at full rate, but patient tolerates the 50% rate, then continue ch14.18 (dinutuximab) infusion at 50% rate for the remaining duration of infusion once blood pressures are stable again.
    - If hypotension recurs at the reduced rate, the measures above should again be taken and once blood pressure is stable for 2 hours post volume resuscitation, ch14.18 (dinutuximab) can be resumed at a further 50% decrease in rate. If the patient's blood pressures are stable at the second 50% reduction in rate the remaining days' infusion of ch14.18 (dinutuximab) should be given at that rate until 4 days from the start

of ch14.18 are completed.

- If hypotension recurs at the second rate reduction, discontinue ch14.18 (dinutuximab) for that day. The antibody infusion may be restarted the following day after ensuring the patient is volume replete at the rate that was reached with second 50% reduction. If blood pressure remains stable, continue daily 20-hour infusions at this rate until 4 days from the start of the ch14.18 (dinutuximab) infusion are completed. The infusion may be started at the full rate at the start of subsequent cycles.

- For patients who require pressors for treatment of hypotension, if blood pressure is stable off pressors for at least 6 hours, administration of ch14.18 (dinutuximab) may be resumed at 50% of the initial infusion rate on the day following the hypotensive episode as long as it is within the 4 day window from the start of ch14.18 (dinutuximab). If the 50% rate is tolerated without further hypotension, rate can be increased to 100% as tolerated. If patient becomes hypotensive again at 100% rate, it should be decreased back to 50% after patient is clinically stable to receive the infusion again and remainder of the infusion should be completed at the decreased rate. Care should be taken to ensure that the patient is volume replete. Ch14.18 (dinutuximab) should not be given to patients who continue to require pressor support. Patients who require pressor support for ≥ 24 hours due to treatment-related hypotension despite appropriate volume resuscitation should discontinue protocol therapy. Patients who again require pressor support when ch14.18 (dinutuximab) is resumed should discontinue protocol therapy.

5.2.1.2 Treatment of Allergic Reactions/Infusion Reactions

5.2.1.2.1 Mild allergic reactions/infusion reactions to ch14.18 (dinutuximab) infusion

- A mild allergic reaction is limited to rash, flushing, urticaria, mild dyspnea – Grade 1 or 2
- The following recommendations do NOT apply to Grade 3 or 4 allergic reactions, including anaphylaxis
- Management
  - Decrease rate of ch14.18 (dinutuximab) to 50% of full rate
  - Perform serial exams at bedside
  - Administer H1 blocker (diphenhydramine, cetirizine recommended)
  - Administer H2 blocker

- When symptoms resolve, resume original infusion rate
- If symptoms recur when original rate is resumed, decrease to 50% rate again
- Infusion must be stopped after 4th day infusion is completed (whether the full dose of ch14.18 (dinutuximab) has been administered or not); document total amount of drug given in the 4 day period

5.2.1.2.2 Moderate to severe allergic reactions/infusion reactions to ch14.18 (dinutuximab) infusion

- Moderate to severe reactions include any of the following: symptomatic bronchospasm, allergy-related edema/angioedema, hypotension, or anaphylaxis – Grade 3 or 4
- The following recommendations do NOT apply to Grade 1 or 2 allergic reactions
- Management
  - **Immediately hold ch14.18 (dinutuximab)**
  - Assess airway, breathing and circulation
  - Follow institutional guidelines for rapid response team notification if clinically indicated
  - For airway concerns
    - Administer oxygen and albuterol immediately for bronchospasm
    - Administer IV diphenhydramine
    - Administer epinephrine (1:1000 IM recommended) immediately if upper airway involved or if airway issues are accompanied by cardiovascular collapse
    - Administer IV hydrocortisone (1-2 mg/kg) if the patient has frank anaphylaxis with cardiorespiratory collapse OR if ≥ 2 doses of epinephrine are required OR if moderate to severe symptoms recur upon rechallenge with dinutuximab
  - For hypotension in the setting of allergic reaction
    - Give normal saline bolus (20 mL/kg as rapidly as possible)
    - Stop or adjust doses of narcotics and sedating H1 blockers
    - Consider use of Trendelenberg position
    - See previous section for management of persistent hypotension
  - For patients with mild bronchospasm or angioedema

that does not impact breathing, completely resolves without the use of epinephrine and hydrocortisone and for patients whose hypotension resolves following volume bolus, ch14.18 (dinutuximab) may be resumed at 50% of the previous rate of infusion on the same day as the reaction occurred. If symptomatic angioedema or asymptomatic bronchospasm recurs when the ch14.18 (dinutuximab) is restarted, discontinue immunotherapy for that day and if symptoms/signs resolve completely that day, resume the next day with additional pre-medication of hydrocortisone 1-2 mg/kg IV. For this re-challenge, the infusion should be given in an ICU setting.

- For patients whose bronchospasm or angioedema requires the use of systemic epinephrine, protocol therapy should be discontinued.
- For patients with bronchospasm or angioedema that does not require systemic epinephrine but whose hypotension requires more extensive volume resuscitation, guidance in Section 5.2.1.1 should be followed.

5.2.1.3 Management of Capillary Leak Syndrome (≥ Grade 3)

- Hold ch14.18 (dinutuximab) infusion
- Provide oxygen, fluids as needed
- Diuretics should be used with caution and hypotension avoided
- **See Section 5.2.1.1 for management of hypotension**
- Do NOT resume ch14.18 (dinutuximab) therapy if symptoms of severe capillary leak syndrome persist on the same day or subsequent days of a given cycle. Only resume ch14.18 (dinutuximab) therapy when the capillary leak syndrome resolves or requires less significant intervention (Grade 2 or less).
- If capillary leak resolves, may resume ch14.18 (dinutuximab) infusion at 50% rate the same day and for subsequent days during a given cycle. The infusion may be given at the full rate at the start of subsequent cycles
- If mechanical ventilation (any duration) or pressor support for ≥ 24 hours is required due to therapy-related capillary leak syndrome, the patient should discontinue protocol therapy.

5.2.1.4 Management of Renal Insufficiency (unrelated to hypotension)

- Consider the possibility of renal hypoperfusion in the context of borderline hypotension; administer volume if appropriate
- If the patient's creatinine is elevated to ≥ 2 x the upper limit of normal for age/gender (see table in Section 3.2.7.2) and elevation persists despite optimized fluid management, hold dinutuximab

- Modify dosing of concomitant medications that may contribute to or be affected by renal insufficiency
- When urine output returns to normal and creatinine returns to < 2 x upper limit of normal for age/gender, resume ch14.18 (dinutuximab) at 50% rate. If renal function normalizes by the following day, ch14.18 (dinutuximab) may be administered at full rate. If renal function is not sufficiently improved (urine output normal and creatinine < 2 x ULN for age/gender) by Day 7 of the cycle, no further ch14.18 (dinutuximab) should be given during that cycle of therapy. If renal function has normalized by the planned start date for the next cycle, retreatment with ch14.18 (dinutuximab) is permitted starting at full rate

5.2.1.5 Management of Hyponatremia (≥ Grade 3; Na < 130 mEq/L)

- Change hypotonic fluids to isotonic fluids as compatibilities permit
- Avoid administration of oral free water
- Correct fluid losses due to diarrhea
- 3% sodium chloride may be used to correct hyponatremia as per institutional discretion
- If Grade ≥ 3 hyponatremia persists despite optimal fluid management, discontinue ch14.18 (dinutuximab) for the remainder of the cycle. Sodium should be monitored closely during the next cycle of therapy. Empiric dose reduction is not required at the start of the next cycle of therapy, though ch14.18 (dinutuximab) would again be discontinued if Grade 4 hyponatremia were to persist despite optimal fluid management. In such cases, no additional cycle of therapy would be given.

5.2.1.6 Management of Fever in the Absence of Hypotension

- Administer antipyretics
- Adjust fluids to account for insensible losses if fever is persistent
- Obtain blood culture
- Administer empiric antibiotics if suggested by institutional policy

5.2.1.7 Management of Treatment-Related Pain*

- No further ch14.18 (dinutuximab) therapy should be given to patients who experience treatment related pain that cannot be controlled despite intensive pain management during a given cycle. Treatment with gabapentin or similar agent should be initiated if not already being administered. If pain that is not controlled with opiates (along with either lidocaine or dexmedetomidine) recurs during a subsequent cycle, the patient should discontinue protocol therapy
- For patients with treatment-related pain requiring intravenous narcotics for ≥ 48 hours following completion of ch14.18 (dinutuximab) therapy, gabapentin or similar agent should be initiated if not already being administered. If pain requiring

prolonged intravenous narcotics (≥ 96 hours following completion of ch14.18 (dinutuximab) therapy) recurs during a subsequent cycle despite this intervention, the patient should discontinue protocol therapy.

*Note: For patients who have previously undergone amputation of an extremity for local tumor control and experience phantom pain, there is a possibility of worsening of phantom pain with ch14.18 (dinutuximab) therapy. This should be managed similar to guidelines for pain management listed in Sections 4.2.3 and 4.3.3. In addition, tricyclic anti-depressants such as amitriptyline can be considered for these patients as it has shown to be of benefit in patients with phantom pain. Protocol therapy should be discontinued if phantom pain is uncontrolled despite aggressive pain management.

5.2.1.8 Management of Visual Changes

- Ch14.18 (dinutuximab) should be discontinued for the cycle for patients who develop dilated pupils with sluggish light reflexes with or without photophobia during administration of the antibody. Sargramostim should be completed for that cycle. If pupillary abnormalities remain stable or improve before the next immunotherapy cycle is due, the patient should receive ch14.18 (dinutuximab) at a dose that is 50% reduced compared to the prior dose. Full dose sargramostim should be given. If the lower dose of ch14.18 (dinutuximab) is tolerated without worsening of ocular toxicity, full dose ch14.18 (dinutuximab) should be given in subsequent cycles. If visual toxicity worsens on the 50% reduced dose, the patient should discontinue all immunotherapy.
- Dose reductions for changes in accommodation are not required

5.2.1.9 Management of Serum Sickness

- Identification of serum sickness – signs and symptoms include arthralgias/arthritis, splenomegaly, lymphadenopathy, glomerulonephritis in the presence of persistent fevers, cutaneous eruptions
- Serum sickness typically develops 1 to 3 weeks after administration of the causative agent, but can develop within 12-36 hours in patients who have previously been sensitized to the causative agent
- Patients with ≥ Grade 3 serum sickness should discontinue protocol therapy.
- For Grade 2 serum sickness, antihistamines should be prescribed

5.2.1.10 Management of Neurotoxicity

- Patients who develop Grade 4 neurotoxicity should discontinue protocol therapy.
- Ch14.18 (dinutuximab) should be discontinued for the remainder of the current cycle of therapy for patients who develop Grade 3 sensory neuropathy or Grade 3 motor neuropathy. If abnormalities

resolve by start of next cycle of therapy the patient may receive 50% total dose of ch14.18 (dinutuximab) per cycle (ie, ch14.18 (dinutuximab) dose: 35 mg/m^2). If symptoms do not completely resolve or recur with ch14.18 (dinutuximab) then the patient should discontinue protocol therapy.

5.2.2 Management of Sargramostim (GM-CSF) Related Toxicities

- Hold sargramostim if total white blood cell count is > 50,000/µL; resume at 50% dose when the count is < 20,000/µL. Administer full dose with subsequent cycles and modify again if the count exceeds 50,000/µL. Ch14.18 should be continued as per protocol in the event sargramostim is held.
- Localized skin reactions to sargramostim are common, and sargramostim can be continued when reactions are mild. Rotation of sites of injections is recommended rather than use of subcutaneous catheters, such as Insuflon for subcutaneous injection when skin reactions occur. Consider use of antihistamines. If ≥ Grade 3 injection site reactions occur, stop sargramostim for the current cycle and discontinue sargramostim for subsequent cycles of therapy.
- A syndrome characterized by respiratory distress, hypoxia, flushing, hypotension, syncope, and/or tachycardia has been reported following the administration of the first dose of sargramostim in a particular cycle. This syndrome generally resolves with symptomatic treatment and usually does not recur with subsequent doses of sargramostim in the same cycle of treatment. For safety purposes in this study, if such a "first dose reaction" occurs, the sargramostim dose will be reduced to 50% for the next dose (ie, sargramostim dose 125 mcg/m^2). If a similar reaction occurs at the 50% dose, the sargramostim will be discontinued for that patient. If the first dose at 50% does not cause any recurrent severe symptoms, subsequent doses can be escalated back to 100%. If recurrent severe symptoms are observed at 100% dose, then the dose will be reduced to 50%. If 50% is tolerated, that dose should be administered for all subsequent protocol treatment for that patient. If recurrent severe symptoms are seen at the 50% dose, the sargramostim will be discontinued for subsequent cycles of therapy.

6.0 DRUG INFORMATION

6.1 Chimeric Monoclonal Antibody 14.18 (Dinutuximab) (05/12/2017)

(Chimeric Monoclonal Antibody 14.18; human/murine anti-G_{D2} monoclonal antibody; chimeric anti-G_{D2}; chimeric mAb 14.18; ch14.18, dinutuximab, Unituxin®) NSC# 764038, IND# 4308

Source and Pharmacology:

Chimeric MOAB 14.18 (ch14.18, UTC) is an anti-G_{D2} monoclonal antibody composed of the variable region heavy and light chain genes of the murine mAb 14.G2a and the human constant region genes for heavy chain IgG_1 and light chain kappa. Ch14.18 exerts its antitumor effect by binding specifically to the disialoganglioside G_{D2}, an antigen found in human tumors of neuroectodermal origin such as melanoma and neuroblastoma. This chimeric antibody has been shown to lyse melanoma and neuroblastoma cells through the process of antibody-dependent cell-mediated cytotoxicity and complement-dependent cytotoxicity. By targeting the G_{D2} antigen on the cell surface, ch14.18 may also prevent attachment of circulating malignant cells to the extracellular matrix. Additionally, ch14.18 mediates lysis of several melanoma and neuroblastoma cell lines in a dose dependent manner in the presence of potent mediators of ch14.18-dependent cytotoxicity, such as human peripheral blood mononuclear cells and granulocytes. This is most profound with neutrophils, especially in the presence of recombinant human granulocyte-macrophage colony-stimulating factor.

The PK profile of ch14.18 has been determined in adults with melanoma and children with neuroblastoma. Although the plasma clearance for both groups of patients follow a two-compartment model, circulating antibody is cleared from the plasma at a much faster rate in children than adults (mean $t_{½}\beta = 66.6 \pm 27.4$ hours in children versus 123 ± 29 hours and 181 ± 73 hours in two adult trials, respectively). Maturation of the hepatic and renal systems with age is thought to impact on drug metabolism and elimination and could account for these differences. In general, the mAb half-life following the first course of treatment was longer than the half-lives following subsequent courses in a given patient.

Toxicity:

Comprehensive Adverse Events and Potential Risks list (CAEPR)
for
MoAb 14.18, chimeric (CH14.18, NSCs 623408 and 764038)

The Comprehensive Adverse Events and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 351 patients.* Below is the CAEPR for MoAb 14.18, chimeric (CH14.18).

NOTE: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 2.8, April 10, 2017[1]

Adverse Events with Possible Relationship to MoAb 14.18, chimeric (CH14.18) (CTCAE 4.0 Term) [n= 351]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		***Anemia (Gr 3)***
	Disseminated intravascular coagulation		***Disseminated intravascular coagulation (Gr 2)***
		Hemolytic uremic syndrome[2]	
CARDIAC DISORDERS			
		Cardiac arrest	
		Sinus bradycardia	
	Sinus tachycardia		***Sinus tachycardia (Gr 3)***
EYE DISORDERS			
		Eye disorders - Other (eye disorders)[3]	
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 3)***
	Diarrhea		***Diarrhea (Gr 3)***
	Nausea		***Nausea (Gr 2)***
	Vomiting		***Vomiting (Gr 3)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Edema limbs		***Edema limbs (Gr 2)***
Fever			***Fever (Gr 3)***
		Infusion related reaction	
Pain			***Pain (Gr 3)***
		Sudden death NOS	
IMMUNE SYSTEM DISORDERS			
	Allergic reaction		***Allergic reaction (Gr 3)***
		Anaphylaxis	
	Serum sickness		
INFECTIONS AND INFESTATIONS			
	Infection[4]		***Infection[4] (Gr 3)***
INVESTIGATIONS			
	Alanine aminotransferase increased		***Alanine aminotransferase increased (Gr 3)***
	Aspartate aminotransferase increased		***Aspartate aminotransferase increased (Gr 3)***
	Creatinine increased		***Creatinine increased (Gr 2)***
Investigations - Other (elevated c-reactive proteins)			***Investigations - Other (elevated c-reactive proteins) (Gr 2)***

Adverse Events with Possible Relationship to MoAb 14.18, chimeric (CH14.18) (CTCAE 4.0 Term) [n= 351]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Lymphocyte count decreased		***Lymphocyte count decreased (Gr 4)***
	Neutrophil count decreased		***Neutrophil count decreased (Gr 3)***
	Platelet count decreased		***Platelet count decreased (Gr 4)***
	White blood cell decreased		
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr 3)***
	Hyperkalemia		***Hyperkalemia (Gr 2)***
	Hypoalbuminemia		***Hypoalbuminemia (Gr 3)***
	Hypocalcemia		
	Hypokalemia		***Hypokalemia (Gr 4)***
	Hyponatremia		***Hyponatremia (Gr 3)***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Back pain		***Back pain (Gr 3)***
	Pain in extremity		
NERVOUS SYSTEM DISORDERS			
		Myelitis[5]	
	Neuralgia		***Neuralgia (Gr 2)***
		Peripheral motor neuropathy	
	Peripheral sensory neuropathy[6]		***Peripheral sensory neuropathy[6] (Gr 2)***
		Reversible posterior leukoencephalopathy syndrome	
RENAL AND URINARY DISORDERS			
	Proteinuria		***Proteinuria (Gr 2)***
		Renal and urinary disorders - Other (atonic urinary bladder)[6]	
	Urinary retention[6]		
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Bronchial obstruction		
Cough			***Cough (Gr 3)***
	Dyspnea		***Dyspnea (Gr 3)***
	Hypoxia		***Hypoxia (Gr 3)***
	Stridor		
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Pruritus		***Pruritus (Gr 2)***
Rash maculo-papular			***Rash maculo-papular (Gr 2)***

Adverse Events with Possible Relationship to MoAb 14.18, chimeric (CH14.18) (CTCAE 4.0 Term) [n= 351]			Specific Protocol Exceptions to Expedited Reporting (SPEER)
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Urticaria		***Urticaria (Gr 3)***
VASCULAR DISORDERS			
	Capillary leak syndrome		***Capillary leak syndrome (Gr 3)***
	Hypertension		
	Hypotension		***Hypotension (Gr 3)***

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]There have been rare instances of atypical hemolytic uremic syndrome in the absence of documented infection and resulting in renal insufficiency, electrolyte abnormalities, anemia, and hypertension.

[3]Neurological disorders of the eye including blurred vision, diplopia, cycloplegia, mydriasis, photophobia, optic nerve disorder, eyelid ptosis, and fixed pupils have been observed.

[4]Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

[5]Myelitis expressed as transverse myelitis has occurred in patients treated with chimeric MoAb 14.18. Symptoms may include weakness, paresthesia, sensory loss, or incontinence.

[6]Acute urinary retention occurs during therapy and is thought to be due to fluid shifts and narcotic administration that accompany ch14.18 administration. Atonic urinary bladder results in chronic urinary retention (CUR) that requires intermittent urethral catheterization days to weeks following chimeric MoAb 14.18 administration.

Adverse events reported on MoAb 14.18, chimeric (CH14.18) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that MoAb 14.18, chimeric (CH14.18) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (thrombotic microangiopathy [e.g., thrombotic thrombocytopenic purpura [TTP] or hemolytic uremic syndrome [HUS]); Bone marrow hypocellular; Febrile neutropenia; Hemolysis
CARDIAC DISORDERS - Acute coronary syndrome; Cardiac disorders - Other (gallop on exam); Cardiac disorders - Other (N-terminal BNP); Chest pain - cardiac; Heart failure; Left ventricular systolic dysfunction; Mobitz (type) II atrioventricular block; Myocardial infarction; Palpitations; Pericardial effusion; Supraventricular tachycardia; Ventricular tachycardia
EAR AND LABYRINTH DISORDERS - Ear pain; Hearing impaired
ENDOCRINE DISORDERS - Endocrine disorders - Other (transient hypoaldosteronism); Hyperthyroidism; Hypothyroidism
EYE DISORDERS - Papilledema; Scleral disorder
GASTROINTESTINAL DISORDERS - Abdominal distension; Ascites; Cheilitis; Colitis; Constipation; Duodenal obstruction; Dysphagia; Enterocolitis; Esophageal stenosis; Esophageal ulcer; Esophagitis; Gastrointestinal disorders - Other (pneumatosis intestinalis); Gastroparesis; Hemorrhoidal hemorrhage; Ileus; Intra-abdominal hemorrhage; Lower gastrointestinal hemorrhage; Mucositis oral; Oral pain; Rectal hemorrhage; Stomach pain; Typhlitis

GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Edema face; Edema trunk; Fatigue; General disorders and administration site conditions - Other (cold and clammy); Hypothermia; Injection site reaction; Irritability; Localized edema; Non-cardiac chest pain
HEPATOBILIARY DISORDERS - Hepatobiliary disorders - Other (cholestasis)
IMMUNE SYSTEM DISORDERS - Cytokine release syndrome
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Fracture
INVESTIGATIONS - Activated partial thromboplastin time prolonged; Alkaline phosphatase increased; Blood bilirubin increased; Cardiac troponin I increased; Cholesterol high; Ejection fraction decreased; Electrocardiogram QT corrected interval prolonged; Fibrinogen decreased; GGT increased; INR increased; Investigations - Other (isolated glycosuria); Lipase increased; Lymphocyte count increased; Urine output decreased; Weight gain; Weight loss
METABOLISM AND NUTRITION DISORDERS - Acidosis; Dehydration; Hypercalcemia; Hyperglycemia; Hypermagnesemia; Hypernatremia; Hypertriglyceridemia; Hypoglycemia; Hypomagnesemia; Hypophosphatemia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthralgia; Arthritis; Bone pain; Chest wall pain; Muscle weakness lower limb; Myalgia; Neck pain
NERVOUS SYSTEM DISORDERS - Cognitive disturbance; Depressed level of consciousness; Dysesthesia; Dysgeusia; Dysphasia; Encephalopathy; Extrapyramidal disorder; Headache; Hydrocephalus; Meningismus; Movements involuntary; Nystagmus; Oculomotor nerve disorder; Paresthesia; Seizure; Somnolence; Syncope; Tremor
PSYCHIATRIC DISORDERS - Agitation; Anxiety; Confusion; Delirium; Hallucinations; Insomnia; Personality change; Restlessness
RENAL AND URINARY DISORDERS - Acute kidney injury; Chronic kidney disease; Hematuria; Renal and urinary disorders - Other (acute renal insufficiency); Renal and urinary disorders - Other (urethritis); Renal hemorrhage
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Hematosalpinx; Ovarian hemorrhage; Pelvic pain; Penile pain; Prostatic hemorrhage; Spermatic cord hemorrhage; Testicular hemorrhage; Uterine hemorrhage; Vaginal hemorrhage
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Adult respiratory distress syndrome; Apnea; Atelectasis; Bronchospasm; Laryngeal edema; Laryngopharyngeal dysesthesia; Laryngospasm; Pharyngolaryngeal pain; Pleural effusion; Pleuritic pain; Pneumonitis; Pulmonary edema; Respiratory failure; Respiratory, thoracic and mediastinal disorders - Other (tachypnea); Wheezing
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Dry skin; Erythema multiforme; Hyperhidrosis; Periorbital edema
VASCULAR DISORDERS – Flushing

Note: MoAb 14.18, chimeric (CH14.18) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

Formulation and Stability:
Please note that the following information pertains only to the ch14.18 drug product manufactured by UTC and supplied by CTEP. The UTC manufactured drug is the only supply permitted on this clinical trial.

Ch14.18 is provided as a sterile solution in single-dose vials containing 17.5 mg/5 mL (3.5 mg/mL) in 20 mM Histidine, 150 mM NaCl, 0.05% Tween 20 at pH 6.8. Intact vials should be stored in the refrigerator (2ºC to 8ºC). Stability studies of the intact vials are ongoing.

Withdraw the required dose of ch14.18 (dinutuximab) from the vial(s) and inject the exact volume for the dose (17.5 mg/m^2/dose) into a bag containing 100 mL of 0.9% sodium chloride. The use of a filter during preparation is not required. The final prepared product of ch14.18 (dinutuximab) in NS is stable at room temperature for 24 hours when diluted to a concentration between 0.044 mg/mL and 0.56 mg/mL; however, the final dosage form should be prepared immediately prior to administration as there is a maximum infusion time of 20 hours. The minimum infusion time for the antibody infusion is 10 hours. There is no need to keep empty or partially used vials of ch14.18 (dinutuximab).

Lot number information will be collected on the NCI Drug Accountability Record Form (DARF) (see the Agent Accountability section below).

Guidelines for Administration:
See Treatment, Dose Modifications and/or Supportive Care sections of the protocol.

Patient Care Implications:
Pain is one of the most common adverse effects of ch14.18. It is predominately neuropathic and manifests as abdominal cramps or back and extremity pain. Prophylactic administration of morphine by continuous infusion is required before and during the infusions of ch14.18. Other narcotics such as hydromorphone or fentanyl can be used. Gabapentin may be used in conjunction with other pain medications per institutional practice. Use of additional pain medications (lidocaine, ketamine, dexmedetomidine) in extenuating circumstances should be undertaken in consultation with pediatric pain management specialists.

Acute allergic or infusion reactions are common and may include hypotension, urticaria, hypoxia, and dyspnea. Premedication with antihistamines and acetaminophen are required for ch14.18 administration.

Human anti-chimeric antibodies (HACA) may block the effectiveness of therapy by prematurely clearing the treatment antibody and limiting further immunotherapy. HACA responses may also be associated with immune-complex related adverse events such as serum sickness and anaphylaxis. Although no increase in allergic reactions has been observed in patients treated with ch14.18 in the presence of HACA, immune complex formation may have induced serum sickness in some patients.

Supplier: Manufactured by United Therapeutics and distributed by CTEP, DCTD, NCI. **Do not use commercial supply.**

Agent Ordering:
NCI supplied agent may be requested by the Principal Investigator (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that agent be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained.) The CTEP assigned protocol number must be used for ordering all CTEP supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA form 1572 (Statement of Investigator), Curriculum Vitae, Supplemental Investigator Data Form (IDF), and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP supplied investigational

agents for the study should be ordered under the name of one lead investigator at that institution.

Active CTEP-registered investigators and investigator-designated shipping designees and ordering designees must submit agent requests through the PMB Online Agent Order Processing (OAOP) application < https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jspx >. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account < https://eapps-ctep.nci.nih.gov/iam/ > and the maintenance of an "active" account status and a "current" password. For questions about drug orders, transfers, returns, or accountability, call (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET) or email PMBAfterHours@mail.nih.gov anytime.

Agent Accountability:
Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record of the receipt, dispensing and final disposition of all agents received from PMB using the appropriate NCI Investigational Agent (Drug) Accountability Record Form (DARF) available on the CTEP forms page. Store and maintain separate NCI Investigational Agent Accountability Records for each agent, strength, formulation and ordering investigator on this protocol.

Agent Returns:
Investigators/Designees must return unused DCTD supplied investigational agent to the NCI clinical repository as soon as possible when: the agent is no longer required because the study is completed or discontinued and the agent cannot be transferred to another DCTD sponsored protocol; the agent is outdated or the agent is damaged or unfit for use. Regulations require that all agents received from the DCTD, NCI be returned to the DCTD, NCI for accountability and disposition. Return only unused vials/bottles. Do NOT return opened or partially used vials/bottles unless specifically requested otherwise in the protocol. See the CTEP web site for Policy and Guidelines for Investigational agent Returns at: http://ctep.cancer.gov/protocolDevelopment/default.htm#agents_drugs. The appropriate forms may be obtained at: http://ctep.cancer.gov/forms/docs/return_form.pdf.

Investigator Brochure Availability
The current version(s) of the IB(s) for the agent will be accessible to site investigators and research staff through the PMB Online Agent Order Processing (OAOP) application. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account and the maintenance of an "active" account status and a "current" password. Questions about IB access may be directed to the PMB IB coordinator via email.

Useful Links and Contacts

- CTEP Forms, Templates, Documents: http://ctep.cancer.gov/forms/
- NCI CTEP Investigator Registration: PMBRegPend@ctep.nci.nih.gov
- PMB policies and guidelines: http://ctep.cancer.gov/branches/pmb/agent_management.htm
- PMB Online Agent Order Processing (OAOP) application: https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jspx
- CTEP Identity and Access Management (IAM) account: https://eapps-ctep.nci.nih.gov/iam/

- CTEP Associate Registration and IAM account help: ctepreghelp@ctep.nci.nih.gov
- PMB email: PMBAfterHours@mail.nih.gov
- IB Coordinator: IBCoordinator@mail.nih.gov
- PMB phone and hours of service: (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET)

6.2 Sargramostim (11/15/2016)

(Granulocyte Macrophage Colony Stimulating Factor, rhu GM-CSF, rGM-CSF, GM-CSF, Leukine®) NSC #613795

Source and Pharmacology:

Sargramostim (recombinant human GM-CSF) is a glycoprotein produced in yeast (*S. cerevisiae*) by recombinant DNA technology. rGM-CSF is a hematopoietic growth factor which supports survival, clonal expansion, and differentiation of hematopoietic progenitor cells. rGM-CSF induces partially committed progenitor cells to divide and differentiate in the granulocyte-macrophage pathways. rGM-CSF stimulates the production of monocytes, granulocytes, erythrocytes, and sometimes, megakaryocytes in the bone marrow. It also induces mature neutrophil and monocytes to increase phagocytosis, superoxide generation, ADCC, tumoricidal killing and cytokine production (IL-1 and tumor necrosis factor). Recombinant human GM-CSF is a glycoprotein of 127 amino acids characterized by three primary molecular masses of 15500, 16800, and 19500 daltons. The amino acid sequence differs from the natural sequence by a substitution of leucine at position 23 and the CHO moiety may be different from the native protein. After subcutaneous administration of sargramostim, peak levels were obtained in 1-4 hours and were detectable at therapeutic levels for 12-16 hours post injection. The elimination $t_{½}$ ranges from 1.5-2.7 hours after SubQ or IV administration.

Toxicity:

	Common Happens to 21-100 children out of every 100	**Occasional** Happens to 5-20 children out of every 100	**Rare** Happens to < 5 children out of every 100
Immediate: Within 1-2 days of receiving drug	Headache, malaise, fatigue, rash, pruritis, bone pain, myalgia, arthralgia, fever, chills	Abdominal pain, weakness, anorexia, nausea, local injection reactions	Anaphylaxis, "first dose reaction" (hypoxia, dyspnea, hypotension, fever, tachycardia, diaphoresis, flushing, back pain), vomiting, diarrhea, phlebitis, SVT, pericardial effusion
Prompt: Within 2-3 weeks, prior to the next course		Weight gain	In high doses: capillary leak syndrome: (pleural effusion, peripheral edema, ascites, weight gain, hypotension), pneumonitis, peripheral edema, elevation of creatinine, bilirubin and hepatic enzymes in patients with pre-existing renal or hepatic dysfunction
Delayed:		Thrombocytopenia	

Any time later during therapy			
Unknown Frequency and Timing:	Fetal and teratogenic toxicities: It is not known whether sargramostim can cause fetal harm or affect reproduction capacity when administered to a pregnant woman. It is unknown whether the drug is excreted in breast milk.		

Formulation and Stability:
Sargramostim is available as a lyophilized sterile, white, preservative free powder with 250 mcg (1.4 million International Units) per vial and as a sterile, preserved injectable solution in a 500 mcg/mL (2.8 million International Units/mL) 1 mL vial. The sargramostim reconstituted lyophilized vial contains 40 mg/mL mannitol, *USP*; 10 mg/mL sucrose, NF; and 1.2 mg/mL tromethamine, *USP*, as excipients. The liquid formulation also contains 1.1% benzyl alcohol (11 mg/mL).Store refrigerated at 2-8°C (36-46°F). Do not freeze or shake.

Guidelines for Administration: See Treatment, Dose Modifications and Supportive Care sections of the protocol.

Reconstitute lyophilized powder for injection with 1 mL SWFI or 1 mL Bacteriostatic Water for Injection. **Use SWFI without benzyl alcohol for neonates, infants, and children < 2 years of age or patients with hypersensitivity to benzyl alcohol**. During reconstitution, direct the diluent at the side of the vial and gently swirl the contents to avoid foaming during dissolution. Avoid excessive or vigorous agitation; do not shake. Reconstituted solutions prepared with Bacteriostatic Water for Injection (0.9% benzyl alcohol) or the liquid preserved solution may be stored for up to 20 days following the first entry into the vial at 2°-8°C (36°-46°F). Discard reconstituted solution after 20 days have elapsed. Reconstituted solutions prepared with SWFI (without preservative) should be administered as soon as possible and within 6 hours following reconstitution.

Use sargramostim for subcutaneous injection without further dilution.

Supplier: Commercially available. See package insert for more detailed information. Only sargramostim (yeast-derived recombinant human GM-CSF) will be used in this study. The *Escherichia coli*-derived product (molgramostim) will not be used.

CANADIAN SITES
Sargramostim is not commercially available in Canada. Sites may purchase and import the USA commercial supply of Liquid Leukine® (500 µg/1 mL) or Lyophylized Leukine® (250 µg/vial) manufactured by Genzyme USA via an International Distributor (Pharma Exports LLC; Contact: Pete Bigley, phone: 1-412-885-3700, fax: 1-412-885-8022, email: pharexp@aol.com or pbigley@pharma-whitehall.com) under the authority of the protocol's No Objection Letter (NOL).

Consult the product monograph for appropriate reconstitution and infusion preparation instructions. The Canadian Senior Medical Officer (SMO)'s office is responsible for coordinating the "Fax Back" approval with Health Canada's Biologics and Genetic Therapies Directorate for all lots for use in Canada on behalf of all Canadian sites. and a A list of approved lot numbers is posted on the C17 website (www.c17.ca) under the protocol titled Sargramostim. If an unapproved lot is received from the distributor, quarantine the lot and contact the COG Canada Regulatory Affairs Office at 780-492-7064.

Canadian sites must keep a drug accountability log (DAL) recording, at a minimum, manufacturer, lot number and expiry date of all formats for all doses dispensed to any registered study patient.

Note: Sargramostim may have orders placed and Drug Accountability Logs maintained on a multiple protocol basis (Multiple Protocol—Imported Biologic) as long as each protocol has an NOL and the DAL clearly indicates which protocol the subject is registered on.

7.0 EVALUATIONS/MATERIAL AND DATA TO BE ACCESSIONED

Timing of protocol therapy administration, response assessment studies, and surgical interventions are based on schedules derived from the experimental design or on established standards of care. Minor unavoidable departures (up to 72 hours) from protocol directed therapy and/or disease evaluations (and up to 1 week for surgery) for valid clinical, patient and family logistical, or facility, procedure and/or anesthesia scheduling issues are acceptable (except where explicitly prohibited within the protocol).

7.1 End of Therapy & Follow-up

See COG Late Effects Guidelines for recommended post treatment follow-up: http://www.survivorshipguidelines.org/

Note: Follow-up data are expected to be submitted per the Case Report Forms (CRFs) schedule.

Observation	End of Therapy	End of month 8 and 12 from the start of therapy	At relapse
Physical exam	X	X	
Height, weight	X	X	
CBC with diff/platelets	X	X	
AST, ALT, bilirubin, albumin	X	X	
Electrolytes, creatinine, BUN, Ca^{++}, PO_4, Mg^{++}	X	X	
Performance status	X		
MUGA or ECHO	X		
GFR or creatinine Clearance (if serum creatinine abnormal for age)	X	X	
HACA/Neutralizing antibodies	X[d]		
Imaging evaluation[a] (CT or MRI/X-ray of primary site; CT chest)	X[b]	X[c]	X
Bone scan or whole body PET/CT	X[b]	X[e]	
ctDNA[f]	X	X	X

[a] CT or MRI with gadolinium or plain X rays for patients who have undergone surgery with prosthetic implants that may cause imaging artifact.

[b] Can be performed during a 7-day window around the last day of Cycle 5 (See Section 16 for details).

[c] Can be performed during a 14-day window around the last day of Months 8 and 12 from start of protocol therapy (see Section 16 for details). Subsequently imaging is recommended every 3 months until 2 years off therapy, then per institutional policy.

[d] See Section 15.2 for details.

[e] End of month 12 from the start of therapy only.

[f] See Section 15.4 for details.

7.2 Correlative Research Studies

Study	Pre-therapy	Cycle 1	Prior to Cycle 2	Prior to Cycles 2-5	End of Cycle 2	End of Cycle 5	Within 4 weeks of the end of Cycle 5	During first 12 months from enrollment	End of therapy and end of month 8 and 12 from start of therapy	Relapse
Pharmacokinetics%		X	X							
HACA and neutralizing antibody testing	X			X		X				
Tumor GD2 assay*							X			
KIR/ FcγR genotypes and NKp30 isoforms#	X									
B7-H6 ligand#	X		X							X
Banking#	X									
ctDNA#	X				X	X			X	X

% Pharmacokinetic sampling is mandatory for the first 10 patients enrolled in this trial, and for patients enrolled after protocol amendment #2.

Patient participation is optional.

* Tumor tissue for GD2 is recommended to be submitted at enrollment but can be submitted anytime until 4 weeks after end of Cycle 5.

For additional details on research studies, see Section 15.0.

8.0 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

8.1 Criteria for Removal from Protocol Therapy

a) Progressive disease
b) Unacceptable toxicity due to protocol therapy (see Section 9.3)
c) Refusal of further protocol therapy by patient/parent/guardian
d) Completion of planned therapy
e) Physician determines it is in patient's best interest
f) Development of a second malignancy
g) Repeat eligibility studies (if required) are outside the parameters required for eligibility (see Section 3.2)

Patients who are off protocol therapy are to be followed until they meet the criteria for Off Study (see below). Follow-up data will be required unless patient is taken off study.

8.2 Off Study Criteria

a) Death
b) Lost to follow-up
c) Patient enrollment onto another COG study with tumor therapeutic intent (eg, at recurrence)
d) Withdrawal of consent for any further data submission
e) The fifth anniversary of the date the patient was enrolled on this study

9.0 STATISTICAL CONSIDERATIONS

9.1 Sample Size and Study Duration

A maximum of 39 evaluable patients are required for this study. Providing for possible ineligible and disease control inevaluable patients, the maximum enrollment is expected to be 44 patients. Based on enrollment to AOST0221, we expect 1.6 patients per month to be enrolled on study. The probability we will enroll 44 patients in 2.5 years after the study is opened is approximately 68% and that probability is 96% for an enrollment period of 3 years.

9.2 Study Design

The primary outcome measure is disease control during the first 12 months after enrollment. Patients considered evaluable for the primary outcome of disease control will be assessed during the first 12 months of enrollment, if the patient's protocol therapy is terminated for reasons other than death or disease progression prior to the completion of therapy ('evaluation period').

We are not interested in promoting the agent for further investigation if the probability of remaining analytic event free at 12 months after enrollment in any particular individual is less than or equal to 30%. We will be interested in promoting the agent for further investigation if the probability of remaining analytic event free in any particular individual

is at least 50%. The statistical analysis of AOST0221 demonstrated that the current point estimate of the 12 month EFS is 0.2 with an upper 95% confidence bound of approximately 30%, so the target 12 month EFS probability is associated with an odds ratio of 2.3 compared with the largest plausible value for 12 month EFS supported by the historical data. One group of 39 evaluable patients will be enrolled. The maximum time by which the final assessment will be made is when all patients have experienced an analytic event or the last patient enrolled is followed for 12 months, whichever occurs first. If 15 or fewer of the planned 39 patients in this cohort are confirmed disease free at 12 months after enrollment, we will have determined the therapy does not offer sufficient disease control to warrant further study. If all 39 patients are enrolled and the true 12 month disease control probability is 30%, the therapy will not be considered for further development with probability 0.91. If all 39 patients are enrolled and the true 12 month disease control probability is 50%, the therapy will be considered for further development with probability 0.90. Enrollment will be terminated in this cohort with the conclusion that ch14.18 (dinutuximab) will not be considered for further development if, at any time, more than 24 patients demonstrate disease progression or die prior to 12 months after study enrollment.

9.3 Methods of Analysis

Aim 1.1.1: The analysis for the decision rule is described in Section 9.2. Only evaluable patients will be considered in the analysis; patients who are not evaluable may be replaced for execution of the study decision rule. Only patients who can be confirmed to be free of detectable disease 12 months after enrollment, without intervening disease progression, will be considered to have experienced 12 month disease control. All other evaluable patients will not be considered to have experienced 12 month disease control.

The time to adverse event will be calculated for each evaluable patient as the time from study enrollment until disease progression, death, occurrence of a second malignant neoplasm or last patient contact, whichever comes first. Patients who experience disease progression, are diagnosed with a second malignant neoplasm or die will be considered to have experienced an event; all other patients will be considered censored at last patient contact. The probability of remaining event-free as a function of time since enrollment will be estimated by the method of Kaplan and Meier.[31] The complementary log-log transformation of the Kaplan-Meier estimate of the 12 month disease control probability will be used to construct confidence intervals of that probability.[32]

Aim 1.2.1: For the secondary objective of characterizing pharmacokinetics of dinutuximab, the parameters that will be estimated for each patient who participates in this aspect of the trial will be $t_{\frac{1}{2}\alpha}$, $t_{\frac{1}{2}\beta}$, $C_{\max}$ *and* $AUC_{0-\infty}$. The average and standard deviation of these estimates will be reported. In order to obtain the most data possible to identify any changes in PK parameters such as Cmax and serum half-life, we will require all patients who enroll subsequent to the approval of amendment #2 to consent to pharmacokinetic sampling as outlined in the amended protocol. The equality of the mean of the various PK parameters for the original schedule with that of the same parameter on the revised schedule will be tested using the Wilcoxon signed rank test. Since the sample size for these comparison is fixed by the number of post-amendment patients to be enrolled, power calculations to justify the sample size choice were not executed.

Aim 1.2.2: We will monitor each cycle for unacceptable toxicity (UT). We will base the definition of UT on Section 9.3.3 of study ANBL0931. Any of the following will be considered an "Unacceptable Toxicity":

- Grade ≥ 4 allergic reaction
- Grade ≥ 4 capillary leak syndrome
- Grade ≥ 3 peripheral motor neuropathy, duration ≥ 2 weeks
- Grade ≥ 4 pain, requiring narcotics/lidocaine AND persisting ≥ 4 days after the end of ch14.18 (dinutuximab) infusion
- Anaphylaxis
- Unexpected death where unexpected is defined as death on protocol therapy or within 30 days of the last dose of protocol therapy that is not possibly, probably, or likely related to disease.

The analytic unit for toxicity monitoring will be the patient-cycle. Any cycle in which the patient receives at least 85% of planned protocol therapy, or in which a UT is observed will be considered a reporting period in which a toxicity event has occurred. All other evaluable RPs will be considered free of a toxicity event.

We will use a Bayesian rule to monitor for excessive toxicity. We will assume a beta prior distribution with $\alpha=0.4$ and $\beta=1.6$. If this posterior probability of the chance of UT is at least 30% exceeds 80%, we will identify the regimen to the COG DSMC, Bone Tumor leadership and CTEP as associated with a toxicity profile that may require modification of the regimen. Descriptive analyses of this safety information will be performed and will include the incidence of adverse events, severe adverse events, serious adverse events, and fatal adverse events. Type, frequency, and severity of laboratory abnormalities will also be analyzed.

Examples of situations in which this rule will indicate unacceptable toxicity have been noted and are presented below:

Number of Toxicity-Evaluable Cycles	Number of Cycles with Unacceptable Toxicity Observed	P ($p_{\text{Unacceptable Toxicity}} > 0.30 \mid \text{Data}$)
5	3	0.95
10	4	0.90
15	5	0.86
20	6	0.83
25	7	0.80
30	9	0.88
35	10	0.87
40	11	0.85

Feasibility of delivery of treatment according to protocol will be evaluated in every eligible patient who receives at least one dose of protocol therapy. Such patients will be designated as feasibility-evaluable patients. Any feasibility-evaluable patient: (1) who has protocol therapy terminated because of unacceptable toxicity (see Section 8.1 (b)); (2) who dies on protocol therapy or within 30 days of the termination of protocol therapy for a cause considered possible, probably or likely related to protocol therapy will be considered have experienced a feasibility-event.

At any time, if more than 6 individuals experience a feasibility-event, we will identify the regimen to the COG DSMC, Bone Tumor leadership and CTEP as associated with a feasibility profile that may require modification of the regimen. If 39 patients are enrolled and the true feasibility-event rate is 0.10, the rule will determine the therapy to have an excessive feasibility-event rate with probability 8.9%. If 39 patients are enrolled and the true feasibility-event rate is 0.25, the rule will determine the therapy to have an excessive feasibility-event rate with probability 89%.

9.4 Evaluability for Disease Control

Any eligible patient who receives at least one dose of ch14.18 (dinutuximab) will be considered evaluable for disease control.

9.5 Evaluability for Toxicity

Only eligible patients will be considered in the assessment of toxicity. The analytic unit for unacceptable toxicity monitoring will be the patient-cycle. Any cycle in which the patient receives at least 85% of planned protocol therapy, or in which a UT is observed will be considered a reporting period in which a toxicity event has occurred. All other evaluable RPs will be considered free of a toxicity event.

9.6 Statistical Considerations for Exploratory Aims

9.6.1 To Assess the Relationship between Probability of Disease Control and Tumor GD2 Expression

Aim 1.3.1: Submission of archival tissue will be required for this study. The IHC result will be coded as an integer between 0 and 3 with 0 being no GD2 expression and 3 indicating strong GD2 expression. The outcome measure will be DC success (Yes *v.* No). Logistic regression using the categorical IHC result will be fitted to the data. The fitted coefficients from the logistic regression, and the p-value for the test of the hypothesis of no relationship between IHC result and probability of DC success will be used to characterize this exploratory analysis. Trend will also be assessed using the actual IHC numerical value.

9.6.2 To Assess KIR and FcγR Genotypes, NKp30 Isoforms and its Circulating Ligand, B7-H6, and their Relationships to the Probability of Disease Control

Aim 1.3.2: We expect at least 70% of patients will submit a peripheral blood specimen prior to the start of chemotherapy for the assessment of KIR, NKp30 and FcγR genotype. Only eligible patients with evaluable KIR and/ or FcγR results will be considered in this analysis. The FcγR genotype result characterized as H/R, R/R or H/H will be considered as the predictor variable of interest. KIR/KIR ligand mismatch will be categorized as present or absent. Presence of the immunosuppressive isoform NKp30c will be categorized as present or absent. The outcome measure will be DC success (Yes *v.* No). Logistic regression using the categorical FcγR result will be fitted to the data. The fitted coefficients from the logistic regression, and the p-value for the test of the hypothesis of no relationship between FcγR result and probability of DC success will be used to characterize this exploratory analysis. Logistic regression using the categorical KIR/KIR ligand mismatch (present *v.* absent) will be fitted to the data. The fitted coefficients from the logistic regression, and the p-value for the test of the hypothesis of no

relationship between FcγR result and probability of DC success will be used to characterize this exploratory analysis.

The analytic plan for circulating ligand B7-H6 entails quantifying the change in the ligand between enrollment and the end of the first cycle of therapy. Additionally, serum for the evaluation of ligand levels at relapse will be requested. The primary analytic endpoint for this aim will be the change in B7-H6 levels between enrollment and the end of the first cycle. The change will be estimated by the average intra-patient change and the variance will be estimated by the variance of the intra-patient differences. Since some enrolled patients will not be assessable at the end of the first cycle of treatment because the patient will have been removed from protocol treatment prior to that time, and that this loss likely will be at most 10%, we expect at least 63% of patients will provide samples for this goal.

9.6.3 Banking of Tumor Samples.

Aim 1.3.3: No specific statistical plan currently exists for the banking aim of the study. Individual statistical plans will be developed for future studies answering a specific question using these banked tumor specimens.

9.6.4 To Determine a Descriptive Profile of Human Anti-Chimeric Antibody (HACA) During Immunotherapy

HACA will be determined using a sample of blood taken before the start of each cycle and within 30 days after the end of Cycle 5. All patients will contribute data from each cycle for the qualitative and quantitative evaluation of human anti-chimeric antibody (HACA). At each time point, the optical density of the ELIZA bridging assay will be calculated. Patients will be identified as HACA positive if there is any optical density above background levels and the quantitative level of HACA activity will be taken as the optical density (OD) at a wavelength of 405 or 492 nm. The number of patients who are HACA positive divided by the number of patients with a successful HACA assay at each time point will be used to quantify the fraction of patients displaying HACA activity. We will also graph the average OD of the ELIZA assay over all patient at each time point, where time point is the number of days from the start of treatment with the study agent. For patients found to be positive for HACA, neutralizing antibodies will also be assessed in the same blood sample.

9.6.5 Circulating Tumor DNA (ctDNA)

Serial plasma samples from consenting patients will be stored at the BPC for future studies to evaluate the utility of ctDNA detection in osteosarcoma. Separate biology proposals for access of these samples for the conduct of such studies will be required and reviewed by the bone tumor committee and statistical considerations for specific studies will be provided as part of these proposals.

9.7 Gender and Minority Accrual Estimates

The gender and minority distribution of the study population is expected to be:

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	0	0	0	0	**0**
Asian	1	0	0	0	**1**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	1	6	0	0	**7**
White	14	20	1	1	**36**
More Than One Race	0	0	0	0	**0**
Total	**16**	**26**	**1**	**1**	**44**

This distribution was derived from AOST0221.

10.0 EVALUATION CRITERIA

10.1 Common Terminology Criteria for Adverse Events (CTCAE)

This study will utilize version 4.0 of the CTCAE of the National Cancer Institute (NCI) for toxicity and performance reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm). Additionally, toxicities are to be reported on the appropriate case report forms.

Please note: 'CTCAE v4.0' is understood to represent the most current version of CTCAE v4.0 as referenced on the CTEP website (ie, v4.02 and all subsequent iterations prior to version 5.0).

10.2 Disease Evaluation

For the purposes of this study, patients will be evaluated for disease progression following Cycle 2, Cycle 5 and then at months 8 and 12 from the start of therapy.

Disease progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1).[33]

10.2.1 Definition of Progressive Disease (PD)

The appearance of one or more new lesions is considered progression. Please see Section 16.1.1 for further details.

11.0 ADVERSE EVENT REPORTING REQUIREMENTS

11.1 Purpose

Adverse event (AE) data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. Certain adverse events must be reported in an expedited manner to allow for timelier monitoring of patient safety and care. The following sections provide information about expedited reporting.

11.2 Determination of Reporting Requirements

Reporting requirements may include the following considerations: 1) whether the patient has received an investigational or commercial agent; 2) the characteristics of the adverse event including the *grade* (severity), the *relationship to the study therapy* (attribution), and the *prior experience* (expectedness) of the adverse event; 3) the Phase (1, 2, or 3) of the trial; and 4) whether or not hospitalization or prolongation of hospitalization was associated with the event.

An investigational agent is a protocol drug administered under an Investigational New Drug Application (IND). In some instances, the investigational agent may be available commercially, but is actually being tested for indications not included in the approved package label.

Commercial agents are those agents not provided under an IND but obtained instead from a commercial source. The NCI, rather than a commercial distributor, may on some occasions distribute commercial agents for a trial.

When a study includes both investigational and commercial agents, the following rules apply.

- *Concurrent administration*: When an investigational agent is used in combination with a commercial agent, the combination is considered to be investigational and expedited reporting of adverse events would follow the guidelines for investigational agents.
- *Sequential administration*: When a study includes an investigational agent and a commercial agent on the same study arm, but the commercial agent is given for a period of time prior to starting the investigational agent, expedited reporting of adverse events that occur prior to starting the investigational agent would follow the guidelines for commercial agents. Once therapy with the investigational agent is initiated, all expedited reporting of adverse events follow the investigational agent reporting guidelines.

11.3 Expedited Reporting Requirements – Serious Adverse Events (SAEs)

To ensure compliance with these regulations/this guidance, as IND/IDE sponsor, NCI requires that AEs be submitted according to the timeframes in the AE reporting tables assigned to the protocol, using the CTEP Adverse Event Reporting System (CTEP-AERS).

Any AE that is serious qualifies for expedited reporting. An AE is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. A Serious Adverse Event (SAE) is any adverse drug event (experience) occurring at any dose that results in ANY of the following outcomes:

1) Death.
2) A life-threatening adverse drug experience.
3) An adverse event resulting in inpatient hospitalization or prolongation of existing hospitalization (for ≥ 24 hours). This does not include hospitalizations that are part of routine medical practice.
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

11.4 Special Situations for Expedited Reporting

11.4.1 SAEs Occurring More than 30 Days After Last Dose of Study Drug

Any Serious Adverse Event that occurs more than 30 days after the last administration of the investigational agent/intervention **and** has an attribution of a possible, probable, or definite relationship to the study therapy must be reported according to the CTEP-AERS reporting tables in this protocol.

11.4.2 Persistent or Significant Disabilities/Incapacities

Any AE that results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions (formerly referred to as disabilities), congenital anomalies or birth defects, must be reported via CTEP-AERS if it occurs at any time following treatment with an agent under a NCI IND/IDE since these are considered to be serious AEs.

11.4.3 Death

Reportable Categories of Death

- Death attributable to a CTCAE term.
- Death Neonatal: A disorder characterized by cessation of life during the first 28 days of life.
- Sudden Death NOS: A sudden (defined as instant or within one hour of the onset of symptoms) or an unobserved cessation of life that cannot be attributed to a CTCAE term associated with Grade 5.

- Death NOS: A cessation of life that cannot be attributed to a CTCAE term associated with grade 5.
- Death due to progressive disease should be reported as Grade 5 "Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (Progressive Disease)" under the system organ class (SOC) of the same name. Evidence that the death was a manifestation of underlying disease (e.g., radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

Any death occurring ***within 30 days*** of the last dose, regardless of attribution to the investigational agent/intervention requires expedited reporting within 24 hours.

Any death occurring ***greater than 30 days*** after the last dose of the investigational agent/intervention requires expedited reporting within 24 hours **only if** it is possibly, probably, or definitely related to the investigational agent/intervention.

11.4.4 Secondary Malignancy

A ***secondary malignancy*** is a cancer caused by treatment for a previous malignancy (e.g., treatment with investigational agent/intervention, radiation or chemotherapy). A metastasis of the initial neoplasm is not considered a secondary malignancy.

The NCI requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy
- Myelodysplastic syndrome
- Treatment related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) must also be reported via the routine reporting mechanisms outlined in this protocol.

11.4.5 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine reporting via CDUS unless otherwise specified."

11.4.6 Pregnancy, Fetal Death, and Death Neonatal

NOTE: When submitting CTEP-AERS reports for "Pregnancy", "Pregnancy loss", or "Neonatal loss", the Pregnancy Information Form, available at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/PregnancyReportForm.pdf, needs to be completed and faxed along with any additional medical information to 301-230-0159. The potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agent(s) should be documented in the "Description of Event" section of the CTEP-AERS report.

11.4.6.1 **Pregnancy**

Patients who become pregnant on study risk intrauterine exposure of the fetus to agents that may be teratogenic. For this reason, pregnancy needs to be reported in an expedited manner via CTEP-AERS as **Grade 3 "*Pregnancy, puerperium and perinatal conditions - Other (pregnancy)*"** under the ***Pregnancy, puerperium and perinatal conditions*** SOC.

Pregnancy needs to be followed **until the outcome is known**. If the baby is born with a birth defect or anomaly, then a second CTEP-AERS report is required.

11.4.6.2 **Fetal Death**

Fetal death, defined in CTCAE as "*A disorder characterized by death in utero; failure of the product of conception to show evidence of respiration, heartbeat, or definite movement of a voluntary muscle after expulsion from the uterus, without possibility of resuscitation*", needs to be reported expeditiously, as **Grade 4 "*Pregnancy, puerperium and perinatal conditions - Other (pregnancy loss)*"**. Do NOT report a fetal death as a Grade 5 event since CTEP-AERS recognizes any Grade 5 event as a patient death.

11.4.6.3 **Death Neonatal**

Neonatal death, defined in CTCAE as *"A disorder characterized by cessation of life occurring during the first 28 days of life*" needs to be reported expeditiously, as **Grade 4 "*General disorders and administration - Other (neonatal loss)*" when the death is the result of a patient pregnancy or pregnancy in partners of men on study**. Do NOT report a neonatal death resulting from a patient pregnancy or pregnancy in partners of men on study as a Grade 5 event since CTEP-AERS recognizes any Grade 5 event as a patient death.

11.5 Reporting Requirements for Specialized AEs

11.5.1 Baseline AEs

Although a pertinent positive finding identified on baseline assessment is not an AE, when possible it is to be documented as "Course Zero" using CTCAE terminology and grade. An expedited AE report is not required if a patient is entered on a protocol with a pre-existing condition (eg, elevated laboratory value, diarrhea). The baseline AE must be re-assessed throughout the study and reported if it fulfills expedited AE reporting guidelines.

a. If the pre-existing condition worsens in severity, the investigator must reassess the event to determine if an expedited report is required.
b. If the AE resolves and then recurs, the investigator must re-assess the event to determine if an expedited report is required.
c. No modification in grading is to be made to account for abnormalities existing at baseline.

11.5.2 Persistent AEs

A persistent AE is one that extends continuously, without resolution between treatment cycles/courses.

ROUTINE reporting: The AE must be reported only once unless the grade becomes more severe in a subsequent course. If the grade becomes more severe the AE must be reported again with the new grade.
EXPEDITED reporting: The AE must be reported only once unless the grade becomes more severe in the same or a subsequent course.

11.5.3 Recurrent AEs

A recurrent AE is one that occurs and resolves during a cycle/course of therapy and then reoccurs in a later cycle/course.
ROUTINE reporting: An AE that resolves and then recurs during a subsequent cycle/course must be reported by the routine procedures.
EXPEDITED reporting: An AE that resolves and then recurs during a subsequent cycle/course does not require CTEP-AERS reporting unless:

1) The grade increases OR
2) Hospitalization is associated with the recurring AE.

11.6 Exceptions to Expedited Reporting

11.6.1 Specific Protocol Exceptions to Expedited Reporting (SPEER)

SPEER: Is a subset of AEs within the Comprehensive Adverse Events and Potential Risks (CAEPR) that contains a list of events that are considered expected for CTEP-AERS reporting purposes. (Formerly referred to as the Agent Specific Adverse Event List (ASAEL).)

AEs listed on the SPEER should be reported expeditiously by investigators to the NCI via CTEP-AERS ONLY if they exceed the grade of the event listed in parentheses after the event. If the CAEPR is part of a combination IND using multiple investigational agents and has an SAE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

11.6.2 Special Situations as Exceptions to Expedited Reporting

An expedited report may not be required for a specific protocol where an AE is listed as expected. The exception or acceptable reporting procedures will be specified in the protocol. The protocol specific guidelines supersede the NCI Adverse Event Reporting Guidelines. These special situations are listed under the CTEP-AERS reporting Table A for this protocol.

11.7 Reporting Requirements - Investigator Responsibility

Clinical investigators in the treating institutions and ultimately the Study Chair have the primary responsibility for AE identification, documentation, grading, and assignment of attribution to the investigational agent/intervention. It is the responsibility of the treating physician to supply the medical documentation needed to support the expedited AE reports in a timely manner.

Note: All expedited AEs (reported via CTEP-AERS) must also be reported via routine reporting. Routine reporting is accomplished via the Adverse Event (AE) Case Report Form (CRF) within the study database.

11.8 **General Instructions for Expedited Reporting via CTEP-AERS**

The descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting and are located on the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm. All appropriate treatment areas should have access to a copy of the CTCAE.

An expedited AE report for all studies utilizing agents under an NCI IND/IDE must be submitted electronically to NCI via CTEP-AERS at: https://eapps-ctep.nci.nih.gov/ctepaers.

In the rare situation where Internet connectivity is disrupted, the 24-hour notification is to be made to the NCI for agents supplied under a CTEP IND by telephone call to 301-897-7497.
In addition, once Internet connectivity is restored, a 24-hour notification that was phoned in must be entered into the electronic CTEP-AERS system by the original submitter of the report at the site.

- Expedited AE reporting timelines are defined as:
  - **24-Hour; 5 Calendar Days -** The AE must initially be reported via CTEP-AERS within 24 hours of learning of the event, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
  - **7 Calendar Days -** A complete expedited report on the AE must be submitted within 7 calendar days of the investigator learning of the event.

- Any event that results in a persistent or significant incapacity/substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect, or is an IME, which based upon the medical judgment of the investigator may jeopardize the patient and require intervention to prevent a serious AE, must be reported via CTEP-AERS **if the event occurs following investigational agent administration.**

- Any death occurring within 30 days of the last dose, regardless of attribution to an agent/intervention under an NCI IND/IDE requires expedited reporting **within 24 hours**.

- Any death occurring greater than 30 days of the last dose with an attribution of possible, probable, or definite to an agent/intervention under an NCI IND/IDE requires expedited reporting **within 24 hours**.

CTEP-AERS Medical Reporting includes the following requirements as part of the report: 1) whether the patient has received at least one dose of an investigational agent on this study; 2) the characteristics of the adverse event including the *grade* (severity), the *relationship to the study therapy* (attribution), and the *prior experience* (expectedness) of the adverse event; 3) the Phase (1, 2, or 3) of the trial; and 4) whether or not hospitalization or prolongation of hospitalization was associated with the event.

Any medical documentation supporting an expedited report (eg, H & P, admission and/or notes, consultations, ECG results, etc.) MUST be faxed within 48-72 hours to the NCI. NOTE: English is required for supporting documentation submitted to the numbers listed below in order for the NCI to meet the regulatory reporting timelines.

Fax supporting documentation **for AEs related to investigational agents supplied under a CTEP IND** to: **301-230-0159** (back-up: 301-897-7404).

Also: Fax or email supporting documentation to COG for **all** IND studies (Fax # 310-640-9193; email: COGAERS@childrensoncologygroup.org; Attention: COG AERS Coordinator).

- **ALWAYS include the ticket number on all faxed documents.**
- **Use the NCI protocol number and the protocol-specific patient ID provided during trial registration on all reports.**

11.9 Reporting Table for Late Phase 2 and Phase 3 Studies – Table A

Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention [1]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)

NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)

An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death.
2) A life-threatening adverse event.
3) Any AE that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours. This does not include hospitalizations that are part of routine medical practice.
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6.)

ALL SERIOUS adverse events that meet the above criteria **MUST** be immediately reported to the NCI via CTEP-AERS within the timeframes detailed in the table below.

Hospitalization	Grade 1 Timeframes	Grade 2 Timeframes	Grade 3 Timeframes	Grade 4 & 5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	**7 Calendar Days**			**24-Hour Notification 5 Calendar Days**
Not resulting in Hospitalization ≥ 24 hrs	**Not Required**		**7 Calendar Days**	

NOTE: Protocol specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR. Additional Special Situations as Exceptions to Expedited Reporting are listed below.

Expedited AE reporting timelines are defined as:

"24-Hour; 5 Calendar Days" - The AE must initially be reported via CTEP-AERS within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour notification.

"7 Calendar Days" - A complete expedited report on the AE must be submitted within 7 calendar

days of learning of the AE.
[1]SAEs that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows: **Expedited 24-hour notification followed by complete report within 5 calendar days for:** • All Grade 4, and Grade 5 AEs **Expedited 7 calendar day reports for:** • Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization • Grade 3 adverse events

11.10 **Protocol Specific Additional Instructions and Reporting Exceptions**

- **Grades 1-4 myelosuppression (anemia, neutropenia, thrombocytopenia) do not require expedited reporting.**
- **Grade 1-4 fever does not require expedited reporting**
- **Grades 1-2 AST/ALT elevations do not require expedited reporting.**
- **Grade 1-2 visual changes or Grade 3 visual changes that resolve within 14 days of onset do not require expedited reporting**
- **Grade 1-3 hypotension does not require expedited reporting**

11.11 **Reporting of Adverse Events for Commercial Agents – CTEP-AERS Abbreviated Pathway**

The following are expedited reporting requirements for adverse events experienced by patients on study who have not received any doses of an investigational agent on this study. Commercial reporting requirements are provided in Table B.

COG requires the CTEP-AERS report to be submitted **within 7 calendar days** of learning of the event.

Table B
Reporting requirements for adverse events experienced by patients on study who have NOT received any doses of an investigational agent on this study.

CTEP-AERS Reporting Requirements for Adverse Events That Occur During Therapy With a Commercial Agent or Within 30 Days[1]

Attribution	**Grade 4**		**Grade 5**
	Unexpected	Expected	
Unrelated or Unlikely			CTEP-AERS
Possible, Probable, Definite	CTEP-AERS		CTEP-AERS
[1]This includes all deaths within 30 days of the last dose of treatment with a commercial agent, regardless of attribution. Any death that occurs more than 30 days after the last dose of treatment with a commercial agent that can be attributed (possibly, probably, or definitely) to the agent and is not due to cancer recurrence must be reported via CTEP-AERS.			

11.12 **Routine Adverse Event Reporting**

Note: The guidelines below are for routine reporting of study specific adverse events on the COG case report forms and do not affect the requirements for CTEP-AERS reporting.

Routine reporting is accomplished via the Adverse Event (AE) Case Report Form (CRF) within the study database. For this study, routine reporting will include all toxicities reported via CTEP-AERS and all Grade 3 and higher Adverse Events. In addition, the following AEs will require routine reporting: Grade 2 allergic reactions, hypotension, capillary leak syndrome, dyspnea, cytokine release syndrome and serum sickness.

12.0 STUDY REPORTING AND MONITORING

The Case Report Forms and the submission schedule are posted on the COG web site with each protocol under "*Data Collection/Specimens*". A submission schedule is included.

12.1 CDUS

This study will be monitored by the Clinical Data Update System (CDUS). Cumulative CDUS data will be submitted quarterly to CTEP by electronic means. Reports are due January 31, April 30, July 31 and October 31. This is not a responsibility of institutions participating in this trial.

12.2 Data and Safety Monitoring Committee

To protect the interests of patients and the scientific integrity for all clinical trial research by the Children's Oncology Group, the COG Data and Safety Monitoring Committee (DSMC) reviews reports of interim analyses of study toxicity and outcomes prepared by the study statistician, in conjunction with the study chair's report. The DSMC may recommend the study be modified or terminated based on these analyses.

Toxicity monitoring is also the responsibility of the study committee and any unexpected frequency of serious events on the trial are to be brought to the attention of the DSMC. The study statistician is responsible for the monitoring of the interim results and is expected to request DSMC review of any protocol issues s/he feels require special review. Any COG member may bring specific study concerns to the attention of the DSMC.

The DSMC approves major study modifications proposed by the study committee prior to implementation (eg, termination, dropping an arm based on toxicity results or other trials reported, increasing target sample size, etc.). The DSMC determines whether and to whom outcome results may be released prior to the release of study results at the time specified in the protocol document.

12.3 CRADA/CTA

NCI/DCTD Standard Language to Be Incorporated into All Protocols Involving Agent(s) Covered by a Clinical Trials Agreement (CTA), a Cooperative Research and Development Agreement (CRADA) or a Clinical Supply Agreement, hereinafter referred to as Collaborative Agreement:

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between the Pharmaceutical Company(ies) (hereinafter referred to as "Collaborator(s)") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator"

(http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.

2. For a clinical protocol where there is an investigational Agent used in combination with (an)other Agent(s), each the subject of different Collaborative Agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data"):

   a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NCI, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.

   b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own Agent.

   c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own Agent.

3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order as described in the IP Option to Collaborator (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm). Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/media presentation should be sent to:

   Email: ncicteppubs@mail.nih.gov

   The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.

13.0 SURGICAL GUIDELINES

Timing of protocol therapy administration, response assessment studies, and surgical interventions are based on schedules derived from the experimental design or on established standards of care. Minor unavoidable departures (up to 72 hours) from protocol directed therapy and/or disease evaluations (and up to 1 week for surgery) for valid clinical, patient and family logistical, or facility, procedure and/or anesthesia scheduling issues are acceptable (except where explicitly prohibited within the protocol).

See COG Surgical Guidelines for osteosarcoma at:
https://members.childrensoncologygroup.org/_files/Disc/surgery/handbooks/OsteoBoneHandbook.pdf

Please note: banking of tumor samples is encouraged from patients who undergo biopsy or resection of suspected metastatic disease recurrence while on protocol therapy or during the first 12 months from enrollment.

14.0 PATHOLOGY GUIDELINES AND SPECIMEN REQUIREMENTS

All patients enrolling on this protocol require institutional histological confirmation of osteosarcoma at the time of original diagnosis. In addition, pathologic confirmation of metastatic recurrence of osteosarcoma in at least one of the resected lung nodules is required.

Please note: At enrollment, all patients must have adequate tumor specimen available for submission as detailed in Section 15.1. Submission of tissue for banking of tumor samples is strongly encouraged (see Section 15.4 for details).

Autopsy
In the event of patient death on AOST1421, a complete unrestricted postmortem examination is strongly encouraged.

COG sites: For patients enrolled on AOST06B1, tissue submission at autopsy is requested. (See AOST06B1 protocol.)

15.0 SPECIAL STUDIES SPECIMEN REQUIREMENTS

15.1 Tumor GD2 Expression (Mandatory Participation)

15.1.1 Required Specimen

Submission of a formalin-fixed, paraffin-embedded (FFPE) sample of tumor (tissue block or 4 unstained slides) from the most recent surgery is mandatory. The tumor material is strongly recommended to be submitted at enrollment but can be submitted up until 4 weeks from the last cycle of protocol therapy. The paraffin block/slides will not be returned to the institution.

Note: Although FFPE tissue is preferred, tumor tissue can be provided frozen instead.

15.1.2 Sample Collection and Processing

FFPE samples and slides can be stored indefinitely at room temperature prior to shipment.
Frozen tissue should be frozen (at -70ºC or colder) until shipped on dry ice.

15.1.3 Sample Labeling and Shipping

All tumor material must be labeled with the following information:

- GD2 assay
- COG patient ID number
- specimen type (P for primary or M for metastatic)
- surgical pathology ID (SPID) number
- block number
- collection date

Please include a COG Specimen transmittal form and the corresponding pathology report with each shipment.

FFPE samples can be batched and shipped at room temperature. Instructions to hold and ship tissue that is frozen are described below.

The BPC will provide a specimen procurement kit upon request. This kit is for the shipment of frozen specimen submitted to the BPC. If you are shipping ambient specimens (blocks or slides), you must use your own shipping container and supplies. Blocks and slides are shipped using the institution's courier account or regular mail.

Kits are ordered via the BPC Kit Management application. To access the application click on the 'Biopathology Center Application' link on either the Protocol or the CRA Home Page of the COG web site. On the Biopathology Center Applications page, select the BPC Kit Management link to enter the Kit Management application.

The Biopathology Center provides Specimen Procurement Kits to institutions in the US and Canada. Institutions outside of the US and Canada are expected to provide their own supplies for submission of samples.

Please note that tissue sample submissions to the BPC for AOST1421 must include the following documentation:

1. Institutional pathology report
2. Institutional operative report (Can be provisional as long as final report is sent when available)
3. COG Generic Specimen Transmittal Form

Snap frozen tissue must be sent on dry ice. Using at least 4 lbs. total, layer ½ the dry ice in the bottom of the compartment until it is approximately one third full. Place the frozen specimens on top of the dry ice. Cover the specimens with dry ice until the compartment is almost completely full. Secure the foam lid. Remember to include the COG Specimen Transmittal Form, pathology report and operative report with each shipment.

Close the outer lid of the kit and tape with filament or other durable packing tape. COG sites will access the Kit Management application to print a FedEx shipping label. Non-COG sites should contact the BPC by phone (800-347-2486) or email (BPCBank@nationwidechildrens.org) to request a shipping label. A blank label is provided in the kit to use when printing the shipping label. Attach the label to the top of the kit. Complete the dry ice label (UN 1845) and secure both this label and the Exempt Human Specimen label to the side of the box. A shipping label will only be provided when frozen tissue is submitted. Blocks and slides must be shipped using the institution's courier account or regular mail.

Send samples to:

Biopathology Center
Nationwide Children's Hospital
Protocol AOST1421
700 Children's Drive, WA1340*
Columbus, OH 43205
Phone: (614) 722-2865
Fax: (614) 722-2897
Email: BPCBank@nationwidechildrens.org

**Be sure to include the room number. Packages received without the room number may be returned to the sender.*

Specimens should be shipped Monday through Thursday for delivery on Tuesday through Friday. Do not ship specimens the day before a holiday.

15.1.4 BPC Sample Processing

The BPC will forward tumor tissue specimen for the GD2 assay to

Cambridge Biomedical
1320 Soldiers Field Road
Boston, MA 02135

15.1.5 Methodology

Immunohistochemistry will be performed on whatever osteosarcoma tissue is received for GD2 expression.

15.1.6 Banking of Leftover Specimens

If the patient consents, any specimens left over after the required GD2 expression tests are performed will be banked at the BPC for future research studies.
Left-over tumor material must be labeled with:

- the patient's COG patient ID number
- specimen type (primary or metastatic)
- collection date

Include the surgical pathology ID (SPID) number and block number when submitting a block or slides.

15.2 Ch14.18 (Dinutuximab) Pharmacokinetics and HACA sampling

The first 10 evaluable patients and patients enrolled after protocol amendment #2 are **required** to provide serial blood samples for the assessment of ch14.18 (dinutuximab) serum concentrations. **Providing blood samples for HACA testing is mandatory for all patients**.

15.2.1 Timing of Pharmacokinetic Sampling

Blood will be drawn during Cycles 1 and 2 for the PK study. Timing of pharmacokinetic sampling will be as follows:

Cycle	Day	Time
1	**4***	• Prior to infusion of ch14.18 (dinutuximab) • 4-6 hours after the start of the first 10 hour infusion • Within one hour of the end or at the end of the day 4 infusion
	7*	• 4-6 hours after the start of the 4th 10 hour infusion • Within one hour of the end or at the end of the day 7 infusion • 4-8 hours following the termination of infusion[#] • 4-10 days following the termination of infusion
2	**0 or 1**	• Prior to the start of Cycle 2.

***Note: if there is any change in infusion rate during an ongoing infusion, please record the date, time and new infusion rate. If there is an interruption during a planned infusion, please record the stop/start date and time.**

15.2.2 Timing of HACA Sampling

Sampling for the HACA study will be prior to ch14.18 (dinutuximab) infusion for Cycles 1-5 and can occur anytime between Day 1 and Day 4, and during the end-of-therapy visit after Cycle 5.

15.2.3 Sample Collection and Processing

15.2.3.1 Pharmacokinetics Study

1. Collect approximately 2 mL of blood in preservative-free heparin (green top tube) and mix thoroughly. Record the calendar date and exact time of the blood draw on the AOST1421 PK/HACA Specimen Transmittal Form.
2. Within 12 hours of collection, centrifuge the specimen for 10-15 minutes at 3000 rpm.
3. Label 2 cryovials as detailed below in Section 15.2.3. One tube will serve as a backup specimen for storage at the clinical site.
4. Pipette approximately 0.5 mL plasma aliquots into each cryovial.

5. Freeze samples immediately at < -10°C until shipment to the bioanalytical laboratory on dry-ice.

NOTE: Blood samples can be stored at 2-8ºC for up to 72 hours after drawing, and then be processed as described above.

15.2.3.2 HACA study

1. Collect approximately 2 mL of blood in preservative-free heparin (green top tube) and mix thoroughly. Record the calendar date and exact time of the blood draw on the AOST1421 PK/HACA Specimen Transmittal Form.
2. Within 12 hours of collection, centrifuge the specimen for 10-15 minutes at 3000 rpm.
3. Label two 2 mL cryovials as detailed below in Section 15.2.3. One tube will serve as backup specimen for storage at the clinical site.
4. Pipette approximately 0.5 mL plasma aliquots into each cryovial.
5. Freeze samples immediately at < -10°C until shipment to the bioanalytical laboratory on dry-ice.

15.2.4 Sample Labeling and Shipping

Each tube should have a freezer-safe label with the following information:

- AOST1421
- COG patient ID number
- Sampling date and time
- Cycle and day of therapy blood was drawn
- Test type (PK or HACA)

NOTE: Samples should be batched and shipped once all specimens for a patient have been collected. The backup specimens will be retained at the site and transported later if necessary.

All shipments must be accompanied by a completed AOST1421 PK/HACA Specimen Transmittal Form (provided in the CRF packet on the protocol webpage). Please note on the form any specimens that are hemolyzed.

Package the shipping tubes to prevent breakage and contamination in Styrofoam boxes containing a generous supply of dry ice that will allow for 3 days in transit.

Contact the bioanalytical laboratory prior to the shipment of specimens, by phone, fax or email (details below). An advanced electronic copy of the AOST1421 PK/HACA Specimen Transmittal Form should also be provided to the laboratory. In addition, a copy of the completed AOST1421 PK/HACA Specimen Transmittal Form must be uploaded into RAVE.

When possible, samples should be shipped overnight, on dry ice, on a Monday, Tuesday or Wednesday via Federal Express Priority Overnight to:

BioAgilytix Labs
2300 Englert Drive
Durham, NC 27713

Phone: (919) 287-1538
Fax: (919) 381-6099
Email: Krystal.alligood@bioagilytix.com

15.3 Determination of FcR and Kir Genotype, NKp30 Isoforms and its Circulating Ligand, B7-H6

15.3.1 Study Prioritization

The 10 mL blood sample in an EDTA tube will be used for FcR and Kir genotyping, NK cell receptors, NKp30 SNP determination and detection of its ligand, B7-H6, in plasma. Blood samples in PAXgene™ tubes will be used for NKp30 isoform determination.

If there is an inadequate amount of blood for both the KIR/FcR study and the NKp30 isoforms/B7-H6 study, the blood in PAXgene™ tubes for NKp30 isoform study will be omitted.

15.3.2 Sample Collection and Processing

Within 1 week prior to the first sargramostim injection, collect the following:

- 10 mL blood (7 mL if < 12 kg) in an EDTA-containing (purple top) tube
- 5 mL blood divided into two PAXgene™ Blood RNA Tubes. Note: If PAXgene™ Blood RNA Tubes are not available, one EDTA-containing (purple top) tube can be used instead.

In addition, prior to the start of Cycle 2 therapy and at relapse, collect 5 mL blood in an EDTA tube for B7-H6 measurement.

15.3.3 Sample Labeling and Shipping

Each tube should be labeled with:

- AOST1421
- COG patient ID number
- specimen type (blood)
- date blood was drawn

NOTE: EACH specimen should be sent with a completed COG Specimen Transmittal form.

Freshly-obtained samples should be sent at room temperature. Care should be taken to ship the blood samples with ice packs in the summer to prevent overheating and with extra insulation in the winter to prevent freezing.

Samples should be shipped Monday through Thursday only. It is recommended that the patient is seen between Monday and Thursday of the week before starting GM-CSF on Friday of that week to facilitate adequate sample collection.

Samples should be sent via Priority Overnight shipping using the COG Courier account referenced in the usage guidelines (refer to https://members.childrensoncologygroup.org/_files/reference/FEDEXmemo.pdf) to:

Alice Yu, MD, PhD (Attention Jenna Mielke or Dr. Diccianni)
UCSD Medical Center
Clinical Teaching Facility, B-114*
212 W. Dickinson Street
San Diego, CA 92103-8447
Phone (619)-543-2438 (Lab)
Fax: (619)-543-5413 (Lab)
E-mail: yulab@ucsd.edu
Lab contact: Jenna Mielke

*Be sure to include the room number. Packages received without the room number may be returned to sender.

15.4 Circulating Tumor DNA (ctDNA)

15.4.1 Streck Cell-Free DNA BCT Tube Ordering

IMPORTANT: If the patient has consented to ctDNA study, tubes MUST be ordered immediately following patient enrollment to allow time for processing and shipment from Streck. See the Streck tube order form in the CRF packet. The form can be completed and sent by email, please follow the instructions provided on the form.

15.4.2 Specimen Collection

Peripheral blood samples should be obtained as follows:

- Blood should be collected in Streck Cell-Free DNA BCT tubes
- For patients > 5 kg collect 20 mL (10 mL per tube x 2 tubes)
- For patients > 2 kg but < 5 kg collect 10 mL (one tube)
- For patients < 2 kg research samples will not be collected

In all cases, blood draw volumes should strictly adhere to institutional limitations, taking other blood draws into consideration. However, if a reduction in volume is required, samples should be collected in 10 mL increments (ie. 0, 10, or 20 mL should be collected such that each Streck Cell-Free DNA BCT is completely filled).

15.4.3 Time points for ctDNA sample collection

If patient consents for this biology study, peripheral blood will be required at the following time points:

- Study entry (within 7 days prior to treatment start)
- Cycle 2 any time prior to the infusion of Day 4 Ch14.18
- At time of tumor evaluations at the end of Cycle 2 and 5 of therapy
- At Month 8 and 12 from start of protocol therapy
- At time of relapse

Established institutional guideline should be followed for blood collection via vascular access devices. Heparin should be avoided in pre-collection flush procedures. If therapeutic heparin dosing contamination is a possibility, venipuncture is recommended as a first choice collection method. If a Streck Cell-Free DNA BCT tube immediately follows a heparin tube in the draw order, we

recommend collecting an EDTA tube as a waste tube prior to collection in the Streck Cell-Free DNA BCT.

For patients who do not have indwelling catheters, blood should be collected via venipuncture. To guard against backflow, observe the following precautions:

- Keep patient's arm in the downward position during the collection procedure.
- Hold the tube with the stopper in the uppermost position so that the tube contents do not touch the stopper or the end of the needle during sample collection.
- Release tourniquet once blood starts to flow in the tube, or within 2 minutes of application.
- Fill tube completely.
- Remove tube from adapter and immediately mix by gentle inversion 8 to 10 times. Inadequate or delayed mixing may result in inaccurate test results.

15.4.4 Specimen Labeling and Shipment

Tubes must be labeled with the patient's COG patient ID number, BPC number, specimen type (blood), and date.

Specimen should be shipped by FedEx Priority Overnight at room temperature to the COG Biopathology Center for immediate separation, extraction, and storage of plasma and cellular DNA. Samples should be shipped from Monday through Friday for Tuesday through Saturday delivery. If blood is collected in the Streck tube over the weekend or on the day before a holiday, the sample can be stored at room temperature until shipped on the next business day.

Ship to the COG Biopathology Center (BPC) using a Federal Express shipping label obtained through the BPC Kit Management application. See Section 15.1.3 for instructions on accessing the BPC Kit Management application.

A COG Generic Specimen Transmittal form must be included with each shipment. The time point listed on the transmittal form must be written on the transmittal form exactly as listed in Section 15.4.3.

Ship specimens to the following address:

Biopathology Center
Nationwide Children's Hospital
Protocol AOST1421
700 Children's Drive, WA1340*
Columbus, OH 43205
Phone: (614) 722-2865

**Be sure to include the room number. Packages received without the room number may be returned to the sender.*

For questions about this correlative study, please contact the study chair, Dr. Hingorani. For questions about sample processing and shipping, please contact the BPC directly.

15.4.5 Methodology

Serial plasma samples from consenting patients will be stored at the BPC for future studies to evaluate the utility of ctDNA detection in osteosarcoma.

15.5 Banking of Tumor Tissue

Submission of additional tumor tissue to be banked for future research studies is strongly encouraged. Tumor tissue obtained as a result of biopsy or resection of a suspected disease recurrence site prior to therapy and during the first 12 months from the time of enrollment is requested for banking. Snap frozen, blocks or slides (at least 10 cut sequentially from the same block) will be accepted.

Note: this is a separate submission from the required submission of tumor samples for the GD2 assay.

Sample Labeling and Shipping

Label biology specimens with:

- the patient's COG ID number
- specimen type (primary or metastatic)
- collection date.

Include the surgical pathology ID (SPID) number and block number in the labeling when submitting a block or slides.

A specimen procurement kit may be requested from the BPC for shipping frozen tissue. See Section 15.1.3 for shipping details.

Include a transmittal form, pathology report and operative report with each shipment.

16.0 IMAGING STUDIES REQUIRED AND GUIDELINES FOR OBTAINING

Timing of protocol therapy administration, response assessment studies, and surgical interventions are based on schedules derived from the experimental design or on established standards of care. Minor unavoidable departures (up to 72 hours) from protocol directed therapy and/or disease evaluations (and up to 1 week for surgery) for valid clinical, patient and family logistical, or facility, procedure and/or anesthesia scheduling issues are acceptable (except where explicitly prohibited within the protocol).

16.1 Required Osteosarcoma Imaging

16.1.1 Overview of Required Imaging Studies

Site	Anatomic Imaging	Functional Imaging	Timing
Primary tumor site	CT or MRI with gadolinium or plain X rays for patients who have undergone surgery with prosthetic implants that may cause imaging artifact.		1. Within 6 weeks prior to enrollment* 2. During a 7-day window around the last day of Cycle 5 3. During a 14-day window around the last day of months 8 and 12 from start of protocol therapy
Chest	CT		1. Baseline post-operative CT within 7 days prior to enrollment** 2. Between Day 24-28 of Cycle 2 3. During a 7-day window around the last day of Cycle 5 4. During a 14-day window around the last day of Months 8 and 12 from start of protocol therapy
Whole body		MDP bone scintigraphy OR FDG-PET/CT	1. Within 6 weeks prior to enrollment 2. During a 7-day window around the last day of Cycle 5 3. During a 14-day window around the last day of Month 12 from start of protocol therapy

* Repeat tumor imaging if necessary.

** For patients with bilateral lung metastases, who undergo consecutive lung surgeries for metastasectomies, baseline post-operative CT chest should be performed after the last surgical procedure performed to achieve complete remission.

NOTE: Patients with new lesions or progression of any pre-existing small lesions, on any follow up imaging: if these lesions do not unequivocally represent tumor progression as per RECIST 1.1 (e.g., very small size; positive

bone scan or PET scan without associated CT/MRI changes) then patients should undergo confirmatory imaging 4 weeks after the initial evaluation demonstrating a confirmed progression (additional imaging time point) or histological confirmation of progression by biopsy or surgical resection.

16.1.2 Technical Guidelines for Imaging Studies

CT and MRI guidelines are available on the COG Member site at: http://members.childrensoncologygroup.org/_files/reference/RefMaterial/DiagnosticImagingGuidelines_MRI&CT.pdf

If possible, MRI should include diffusion weighted scans (parameters e.g. (TR/TE/b-value=6400ms/55ms/0, 600-800). Apparent diffusion coefficient (ADC) maps should be generated and submitted for central review. Tumor ADC values will be calculated for different time points before and after therapy.

16.1.3 X-ray

Two-view plain radiographs of the primary tumor site can be performed for patients who have a metallic prosthetic implant instead of CT or MRI if a significant metal artifact would occur by those imaging modalities. If there is suspicion for tumor recurrence on plain radiographs, CT or MRI should be performed to confirm tumor recurrence.

16.1.4 Bone Scan

MDP bone scintigraphy

- Whole body bone scintigraphy should be performed and include planar images of the skeleton, including anterior and posterior views of the axial skeleton. Anterior and/or posterior views should be obtained of the appendicular skeleton.
- Delayed (skeletal phase) images should be performed in all cases with flow and blood pool images as per local custom and clinical need.
- Dose Administration: Dose administered should be according to standard weight-based protocols. Injection site should be away from lesion extremity or contralateral extremity if flow imaging is to be performed. Three-phase imaging is not required unless warranted by symptoms for a focal lesion to assess hyperemia.
- Imaging Parameters: Whole body delayed imaging is acquired 2-3 hours after injection of the radiopharmaceutical. Spot views should be acquired of specific sites of symptoms or of any sites of abnormality as warranted by the whole body views.
- Single-photon emission computed tomography (SPECT) is recommended, but not required, particularly in cases with suspicion of lung metastases.
- SPECT Imaging: SPECT should be performed of the lesion site. SPECT imaging should be performed as recommended by the camera manufacturer. Typical acquisition and processing parameters are 360° circular orbit, 60–120 stops, 64 ´ 64 ´ 16 or greater matrix, and 10–40 s/stop. An equivalent total number of counts should be acquired if continuous acquisition is used.
- Special Consideration: Imaging of pelvis can be difficult due to overlying bladder activity. To lessen this problem, repeat imaging can be performed immediately after patient voiding. Bladder catheterization may be used, but should be reserved for patients in whom visualization of the pelvis is

essential. For SPECT acquisition of the pelvis: Single or multiple rapid (5-10 min/acquisition) SPECT acquisition(s) are preferred to avoid artifacts caused by changing activity in the bladder. Bladder artifacts are exaggerated in the plane in which the SPECT acquisition begins and ends. Beginning SPECT acquisition with the camera heads in the left and right lateral positions (for dual-head camera) or posterior position (for single-head camera) will help reduce bladder filling artifact.

16.1.5 [^{18}F]–Fluorodeoxyglucose Positron Emission Tomography (FDG PET) Imaging (Recommended)

^{18}FDG PET or bone scan is required at baseline and following Cycle 5 and end of months 8 and 12. It is recommended to obtain MRI or CT scan of any lesion positive on bone or ^{18}FDG PET scan.

Patient Guidelines

The patient should fast for at least 4 hours prior to injection of FDG. FDG PET imaging may follow a MUGA study on the same day, or FDG PET imaging may be performed on the day preceding this study. Plasma glucose should be checked and, if the patient is hyperglycemic (plasma glucose > 250 mg/dL), appropriate treatment with small doses of insulin may be given to bring the plasma glucose into the normal range prior to FDG PET imaging. However, insulin administration may result in excessive muscle uptake of FDG and consequent tumor non-visualization. If possible, the study should be postponed until the plasma glucose is under better control.

Good hydration is required, as the primary route of FDG excretion is renal. The patient should drink water or receive intravenous fluids (not containing dextrose) after FDG injection to promote urinary excretion of the radioactive substrate. After injection, the patient must be kept in a resting state for 45-60 minutes prior to imaging. The patient should empty the bladder immediately prior to imaging.

Imaging Technique

The technique will vary by local institutional guidelines. In general, FDG is administered intravenously at a dose of 0.125-0.200 mCi/kg or by algorithms that adjust the dose by body surface area, with a minimum total dose of 2.0 mCi and maximum total dose of 20.0 mCi.

The body should be imaged from the top of the ears to the bottom of the feet. If there is suspicion of involvement of the skull or skull contents, the volume that is imaged should be expanded.

Imaging with a dedicated positron emission tomograph/computed tomography (PET/CT) camera is standard.

The length of time needed to perform head to toe CT will depend on the patient's height but will be approximately 45 seconds. Contiguous axial images should be obtained at 5 mm thickness using 90 mA and 120 Kv and adjusted for local institutional protocol. No oral or IV contrast is required but either or both are permissible and may be of benefit in cases where intraabdominal or pelvic pathology is a specific concern. With regard to patient positioning, the arms can

be placed in a comfortable position at the patient's sides as long as they fit into the field of view. If the patient is large it may be necessary to lay the arms across the abdomen and hold in position with a stabilizing device.

Study Processing

The FDG PET study is processed for display by an iterative reconstruction algorithm. FDG activity should be corrected for attenuation, scatter, and radioactive decay. Attenuation correction is necessary, as apparent uptake will otherwise vary with depth of the lesion in the body and the nature of surrounding tissues. The procedure used for attenuation correction should be recorded. The level of tumor uptake is assessed subjectively by visual inspection and semi-quantitatively by determination of standardized uptake values (SUV). Uptake time, glucose levels, and partial volume effects influence both methods. The SUV method is also dependent on body weight, and correction of SUV by normalizing for body surface area (BSA) reduces this dependency on body weight. SUVs should be calculated for lesions known to be 1.2 cm or larger in diameter. Smaller lesions may have underestimated SUVs due to partial volume averaging effects at typical scanner resolutions (0.6-1.2 cm).

To calculate the SUV, a region of interest (ROI) should be carefully drawn around as much of the area of elevated FDG uptake as can be done. The SUV should be calculated as SUV_{BSA} = ROI activity concentration (nCi/cc) x BSA / injected activity (nCi). SUV_{MAX} is obtained by determining the activity of the pixel with the highest FDG uptake.

The BSA is calculated from body mass (kg) and height (cm) using an appropriate algorithm. The SUV_{BSA} for each measured lesion should be recorded and the technique for assessing SUV_{BSA} should be consistent on follow-up studies.

APPENDIX I: CTEP AND CTSU REGISTRATION PROCEDURES

CTEP INVESTIGATOR REGISTRATION PROCEDURES

Food and Drug Administration (FDA) regulations and National Cancer Institute (NCI) policy require all investigators participating in any NCI-sponsored clinical trial to register and to renew their registration annually.

Registration requires the submission of:

- a completed ***Statement of Investigator Form*** (FDA Form 1572) with an original signature
- a current Curriculum Vitae (CV)
- a completed and signed ***Supplemental Investigator Data Form*** (IDF)
- a completed ***Financial Disclosure Form*** (FDF) with an original signature

Fillable PDF forms and additional information can be found on the CTEP website at <http://ctep.cancer.gov/investigatorResources/investigator_registration.htm>. For questions, please contact the ***CTEP Investigator Registration Help Desk*** by email at <pmbregpend@ctep.nci.nih.gov>.

CTEP Associate Registration Procedures / CTEP-IAM Account

The Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) application is a web-based application intended for use by both Investigators (i.e., all physicians involved in the conduct of NCI-sponsored clinical trials) and Associates (i.e., all staff involved in the conduct of NCI-sponsored clinical trials).

Associates will use the CTEP-IAM application to register (both initial registration and annual re-registration) with CTEP and to obtain a user account.

Investigators will use the CTEP-IAM application to obtain a user account only. (See CTEP Investigator Registration Procedures above for information on registering with CTEP as an Investigator, which must be completed before a CTEP-IAM account can be requested.)

An active CTEP-IAM user account will be needed to access all CTEP and CTSU (Cancer Trials Support Unit) websites and applications, including the CTSU members' website.

Additional information can be found on the CTEP website at <http://ctep.cancer.gov/branches/pmb/associate_registration.htm>. For questions, please contact the ***CTEP Associate Registration Help Desk*** by email at <ctepreghelp@ctep.nci.nih.gov>.

CTSU REGISTRATION PROCEDURES

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

Downloading Site Registration Documents:

Site registration forms may be downloaded from the AOST1421 protocol page located on the CTSU members' website. Permission to view and download this protocol and its supporting documents is restricted and is based on person and site roster assignment housed in the CTSU RSS.

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password
- Click on the Protocols tab in the upper left of your screen
- Click on the COG link to expand, then select trial protocol AOST1421.
- Click on the Site Registration Documents link

Requirements for AOST1421 Site Registration:

- CTSU IRB Certification (for sites not participating via the CIRB)
- CTSU IRB/Regulatory Approval Transmittal Sheet (for sites not participating via the NCI CIRB)
- Verification that training has been completed by appropriate institutional personnel.

Submitting Regulatory Documents:

Submit completed forms along with a copy of your IRB Approval to the CTSU Regulatory Office, where they will be entered and tracked in the CTSU RSS.

Regulatory Submission Portal: www.ctsu.org (members' area) → Regulatory Tab →Regulatory Submission

When applicable, original documents should be mailed to:
CTSU Regulatory Office
1818 Market Street, Suite 1100
Philadelphia, PA 19103

Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 in order to receive further instruction and support.

Checking Your Site's Registration Status:

Check the status of your site's registration packets by querying the RSS site registration status page of the members' section of the CTSU website. (Note: Sites will not receive formal notification of regulatory approval from the CTSU Regulatory Office.)

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password
- Click on the Regulatory tab at the top of your screen
- Click on the Site Registration tab
- Enter your 5-character CTEP Institution Code and click on Go

APPENDIX II: YOUTH INFORMATION SHEETS

INFORMATION SHEET REGARDING RESEARCH STUDY AOST1421
(for subjects from 7 through 12 years of age)

A study of a new drug to treat children with osteosarcoma that has come back.

1. We have been talking with you about your illness, osteosarcoma. Osteosarcoma is a type of cancer that grows in the cells that produce bones. Your cancer came back after treatment. After it came back you had surgery to remove tumors.

2. We are asking you to take part in a research study because your cancer came back. A research study is when doctors work together to try out new ways to help people who are sick. We are trying to learn more about how to treat cancer that came back after treatment. We will do this by giving you two drugs. We do not know if this treatment will keep the cancer from coming back. That is why we are doing this study.

3. Children and teens on this study will be given two drugs. You will also have tests to see if the cancer comes back during your treatment. We also want to learn more about how children's bodies handle one of the drugs you will receive. To do this, we will collect extra blood samples. These samples will be drawn through your central line if you have one. Collecting these blood samples may involve extra needle sticks.

4. Sometimes good things can happen to people when they are in a research study. These good things are called "benefits." We hope that a benefit to you of being part of this study is your cancer will not come back but we don't know for sure if there is any benefit of being part of this study.

5. Sometimes bad things can happen to people when they are in a research study. These bad things are called "risks." One risk to you is that the study treatment may not work or stop your tumor from growing or coming back. Another risk is that you may have more side effects than you would on another therapy. We do know that the study treatment can cause a lot of pain so you will also be given drugs to help lower the pain. The drugs that fight pain will be given before, during and after treatment. Other things may happen to you that we don't yet know about.

6. Your family can choose to be part of this study or not. Your family can also decide to stop being in this study at any time once you start. There may be other treatments for your illness that your doctor can tell you about. Make sure to ask your doctors any questions that you have.

7. We want to see if there are ways to tell how the cancer will respond to treatment. We are asking your permission to collect extra blood samples. These samples would be taken when other blood samples are being taken, so there would be no extra needle sticks. We would also like to collect tumor tissue to keep for other studies later on. This tissue would be taken when you have surgery, so there would be no extra surgeries. You can still take part in this study even if you don't allow us to collect the extra blood samples or tissue for research.

INFORMATION SHEET REGARDING RESEARCH STUDY AOST1421
(for teens from 13 through 17 years of age)

A study of ch14.18 (dinutuximab) and sargramostim (GM-CSF) to treat patients with recurrent osteosarcoma.

1. We have been talking with you about your osteosarcoma. Osteosarcoma is a type of cancer that grows in the bones. Recurrent means that the cancer has come back after treatment. After doing tests, we have found that you have this type of cancer.

2. We are asking you to take part in a research study because you have recurrent osteosarcoma and the tumor(s) that came back have now been removed by surgery. A research study is when doctors work together to try out new ways to help people who are sick. In this study, we are trying to learn more about how to treat osteosarcoma that has come back. We will do this by giving an experimental drug called ch14.18 (dinutuximab) along with a drug called sargramostim. Both drugs are FDA-approved, but not for osteosarcoma treatment. We don't know for sure if these two drugs together will keep the tumor from coming back. That is why we are doing this study.

3. Children, teens and young adults who are part of this study will be given ch14.18 (dinutuximab) and sargramostim. You will have imaging tests to see if the cancer comes back during your treatment. We will collect blood samples for research tests. We are trying to learn how child, teenage and young adult bodies handle dinutuximab. This study may help subjects who receive this drug in the future. These blood tests will be drawn through your central line if you have one. Otherwise, collecting these blood samples will involve extra needle sticks.

4. Sometimes good things can happen to people when they are in a research study. These good things are called "benefits." We hope that a benefit to you of being part of this study is that your cancer will not come back, but we don't know for sure if there is any benefit of being part of this study.

5. Sometimes bad things can happen to people when they are in a research study. These bad things are called "risks." Being in this study may involve special risks, which your doctor will discuss with you. The risks to you from this study are that the study treatment may not stop your tumor from growing or coming back. It is also possible that the study treatment may cause more side effects than other therapies. Your doctors will monitor you closely for signs of any side effects. We do know that the study treatment can cause a lot of pain so you will be given pain medication to help reduce the pain. Pain medication will be given before, during and after treatment as needed. Other things may happen to you that we don't yet know about.

6. You or your family can choose to be part of this study or not. Your family can also decide to stop being in this study at any time once you start. Please talk this over with your parents. There may be other treatments for your illness that your doctor can tell you about. Make sure to ask your doctors any questions that you have.

7. We are asking your permission to collect additional blood. We want to see if there are ways to tell how the cancer will respond to treatment. These blood samples would be taken when other standard blood tests are being performed, so there would be no extra needle sticks. We would also like to collect tumor tissue to keep for other studies later on. This tissue would be taken when you have surgery, so there would be no extra surgeries. You can still take part in this study even if you don't allow us to collect the extra blood samples or tissue for research.

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