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CHILDREN'S ONCOLOGY GROUP

AOST1321

Phase 2 Study of Denosumab (IND# 127430, NSC# 744010), a RANK Ligand Antibody, for Recurrent or Refractory Osteosarcoma

An Intergroup NCTN Phase 2 Study

IND sponsor for Denosumab: COG

STUDY CHAIR

[redacted]

Participating Organizations

COG / Children's Oncology Group

ALLIANCE / Alliance for Clinical Trials in Oncology

ECOG-ACRIN / ECOG-ACRIN Cancer Research Group

NRG / NRG Oncology

SWOG / SWOG

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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Email: rlrandall@ucdavis.edu

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IND sponsor for denosumab: COG

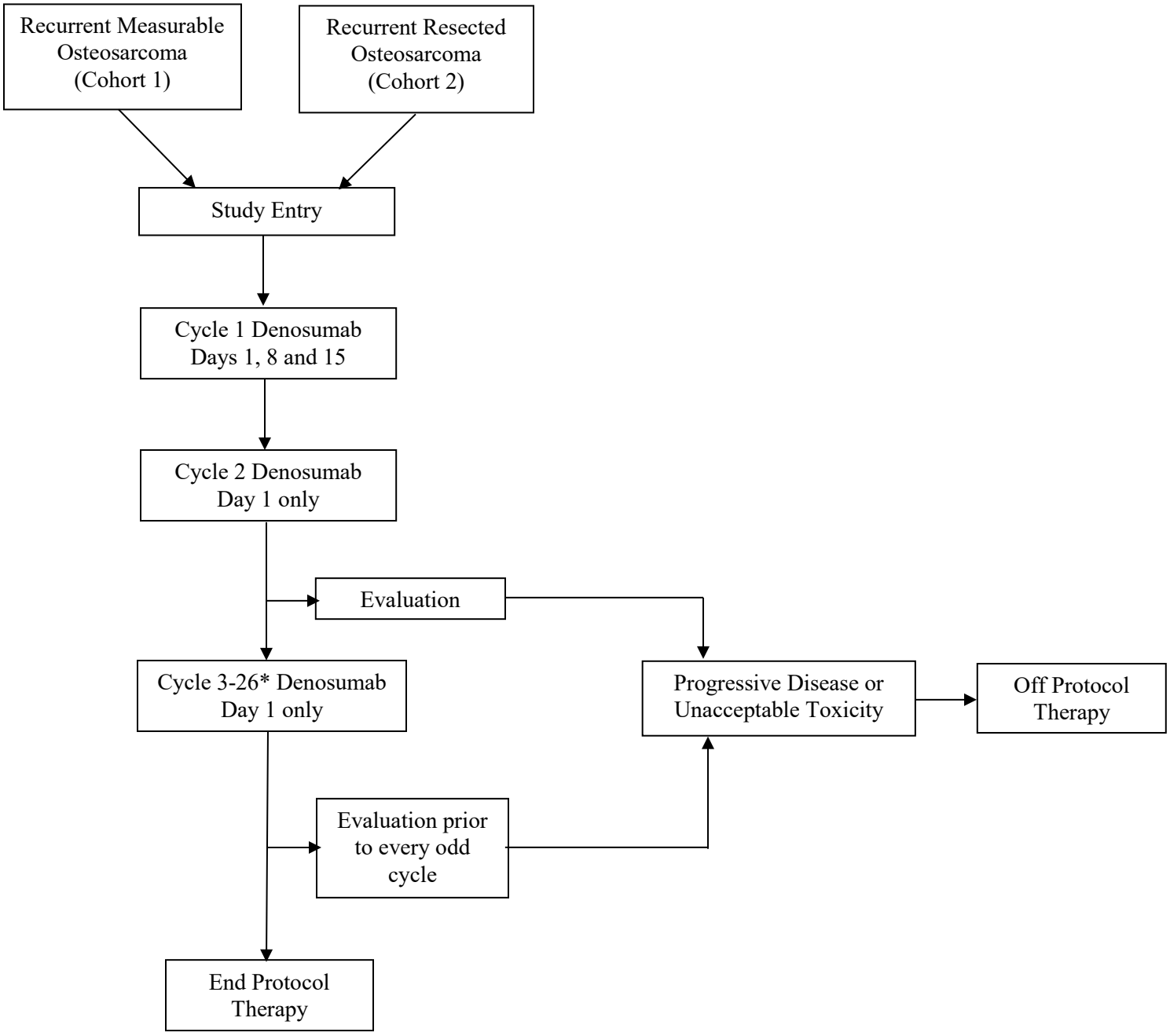
SEE SECTION 15.0 FOR SPECIMEN SHIPPING ADDRESSES.

The Children's Oncology Group has received a Certificate of Confidentiality from the federal government, which will help us protect the privacy of our research subjects. The Certificate protects against the involuntary release of information about your subjects collected during the course of our covered studies. The researchers involved in the studies cannot be forced to disclose the identity or any information collected in the study in any legal proceedings at the federal, state, or local level, regardless of whether they are criminal, administrative, or legislative proceedings. However, the subject or the researcher may choose to voluntarily disclose the protected information under certain circumstances. For example, if the subject or his/her guardian requests the release of information in writing, the Certificate does not protect against that voluntary disclosure. Furthermore, federal agencies may review our records under limited circumstances, such as a DHHS request for information for an audit or program evaluation or an FDA request under the Food, Drug and Cosmetics Act.
The Certificate of Confidentiality will not protect against mandatory disclosure by the researchers of information on suspected child abuse, reportable communicable diseases, and/or possible threat of harm to self or others.

ABSTRACT

Patients with recurrent osteosarcoma have a very poor prognosis and limited treatment options. It is a priority to identify novel agents with evidence of clinical activity in osteosarcoma. This is a prospective single arm, open-label, phase 2 trial of RANKL antibody, denosumab, in recurrent osteosarcoma with osteosarcoma-specific cohorts and osteosarcoma-specific endpoints. Patients will enroll on either the measurable disease cohort or the completely resected disease cohort. All patients will receive subcutaneous denosumab every 4 weeks with a loading dose on Days 8 and 15 of Cycle 1. Endpoints for the cohort with measurable disease (Cohort 1) are the disease control rate at 4 months as compared to historical COG experience or objective response rate. The endpoint for the fully resected cohort (Cohort 2) is the disease control rate at 12 months as compared to historical COG experience. Secondary objectives include pharmacokinetics, pharmacodynamics and tolerability of denosumab in patients with recurrent osteosarcoma. Biological markers, particularly RANK and RANKL expression will be explored.

EXPERIMENTAL DESIGN SCHEMA



*Continue Denosumab for up to 24 months or 26 cycles, whichever occurs first

1.0 GOALS AND OBJECTIVES (SCIENTIFIC AIMS)

1.1 Primary Aims

1.1.1 To determine whether denosumab therapy either increases the disease control rate at 4 months in patients with recurrent measurable osteosarcoma as compared to historical COG experience or denosumab therapy produces an objective response rate greater than 5% (Cohort 1).

1.1.2 To determine whether denosumab therapy increases the disease control rate at 12 months in patients with recurrent resected osteosarcoma as compared to historical COG experience (Cohort 2).

1.2 Secondary Aims

1.2.1 To investigate the pharmacokinetics (PK) and pharmacodynamics (PD) of denosumab in subjects with recurrent osteosarcoma.

1.2.2 To describe the tolerability of denosumab in subjects with recurrent osteosarcoma.

1.2.3 To report the disease control rate and objective response rate for patients with recurrent osteosarcoma limited to bone.

1.2.4 To investigate biological markers potentially associated with response to denosumab in patients with recurrent osteosarcoma.

2.0 BACKGROUND

2.1 Introduction/Rationale for Development

The 5-year and 10-year overall survival for patients with localized osteosarcoma is approximately 70% and 65% respectively.[1-3] Outcomes have not changed for the past 2 decades.[4] This reflects the fact that medical therapy has remained essentially unchanged during this period with no new therapies identified to complement the activity of standard therapy with high-dose methotrexate, adriamycin and cisplatin. The Children's Oncology Group (COG) Bone Tumor Committee plans to continue to investigate the impact of the addition of targeted agents to standard osteosarcoma chemotherapy in patients with localized osteosarcoma. The primary challenge is a paucity of pre-clinical and clinical data for targeted agents with demonstrated activity against osteosarcoma. The COG phase 2 studies that included osteosarcoma strata that have been completed over the past decade include ADVL0122 (imatinib), ADVL0421 (oxaliplatin), ADVL0524 (ixabepilone) and ADVL0525 (pemetrexed). Unfortunately, there was no evidence of objective drug activity in the osteosarcoma stratum for any of these trials. That being said, objective radiographic responses are rare in osteosarcoma for agents with known activity against osteosarcoma in the neoadjuvant setting. The lack of objective anti-tumor activity is likely due to the nature of the intratumoral stromal tissue that contains calcified bone matrix. Consequently, in this phase 2 trial of denosumab, an additional end point, believed to be more appropriate for assessment of activity in osteosarcoma will be utilized.

2.1.1 Recurrent Osteosarcoma

Prognosis for patients with recurrent osteosarcoma is poor. Subjects enrolled on INT-0133 who experienced recurrence had an overall survival rate of 20% at

10 years, similar outcomes are reported by other groups.[5-10] There are no standard chemotherapy agents or targeted therapies proven to improve overall survival in patients with recurrent osteosarcoma and chemotherapeutic treatment options for patients with recurrent osteosarcoma are limited. Aggressive thoracotomies are generally accepted to be standard treatment for resectable osteosarcoma recurrences limited to the lung.[11,12] Experts recommend open thoracotomy as the standard surgical approach to resection given that in 50% of cases more nodules are discovered on surgical exploration than were present on radiographic imaging. However, the rarity of this disease process, coupled with differing institutional biases and retrospective reviews on thoracic surgical management have contributed to a lack of unity in surgical approach. There are no prospectively collected data regarding management of pulmonary metastases in recurrent osteosarcoma.

2.1.2 Historical Disease Control Rate in Recurrent Osteosarcoma

In order to determine the historical disease control rate in patients with recurrent osteosarcoma enrolled on COG phase 2 trials a retrospective analysis of data for all eligible patients with relapsed or refractory osteosarcoma enrolled on A09713, ADVL0122, ADVL0421, ADVL0524, ADVL0525, CCG-0962, P9761 and P9963 was performed. For each patient identified, event-free survival (EFS), defined as time from study enrollment until date of last contact, date of disease progression or detection of disease at a previously uninvolved site, or date of death was calculated. Patients who died or experienced disease progression were considered to have experienced an EFS event; otherwise, the patient was considered censored at the date of last follow-up. EFS as a function of time since study enrollment was estimated according to the method of Kaplan and Meier. The equality of the hazard rate for EFS-event across studies was tested using the log-rank test. The potential prognostic factors for risk of EFS-event that were examined included: (1) study; (2) age; (3) number of prior chemotherapy regimens; (4) sex; and (5) race.

Ninety-six (96) patients were identified for inclusion in this dataset, 95 of whom with some follow-up for EFS, and 93 with an EFS event. Two (2) patients were reported as alive and without EFS-event at 8 and 46 months after enrollment. Both were enrolled on ADVL0421. The EFS for patients with recurrent osteosarcoma enrolled on these seven closed phase 2 studies from the COG or its predecessor groups was 12% at 4 months with a 95% C.I. of 6-19%. There was no significant difference in the EFS across the different studies. The characteristics such as age, gender, race, and the number of prior chemotherapy regimens did not have any effect on EFS.

In addition, in an attempt to gain some insight into how our analysis of COG data might compare to data for relapsed osteosarcoma outside of COG we have evaluated a report from the Cooperative Osteosarcoma Study Group (COSS). In a 2005 analysis, Bielack et al presented data for patients with relapsed osteosarcoma.[6] Although the overall survival was very poor for patients with unresectable disease, overall survival at 2 years of 4%, it is not possible to make a valid comparison of this data to the COG data due to the differences in the data including a different start time (time of recurrence vs. time of enrollment on a phase 2 trial) and different reported outcome (overall vs. event-free survival). The COG data are more relevant to the patient population to be enrolled to Cohort 1 of this study (measurable disease).

For patients with fully resected disease (Cohort 2 of this study), the EFS data of patients enrolled on AOST0221, a phase 2 study of inhaled GMCSF in patients with first pulmonary recurrence of osteosarcoma were used to determine the historical disease control rate.[13] Osteosarcoma patients enrolled on this trial had a first recurrence restricted to resectable nodules in the lung that was completely resected. Consequently the population enrolled on AOST0221 represents the group of patients with completely resected osteosarcoma with the best possible outcome. The intervention in AOST0221 (inhaled GMCSF) was determined to lack activity on the basis of the primary outcome measure. An improvement in outcome above the baseline determined by AOST0221 would likely represent denosumab activity. Forty-two patients were enrolled on AOST0221. The 12 month EFS was 20% with a 95% confidence interval of 10-34%. In an attempt to gain some insight into how our analysis of COG data might compare to data for relapsed osteosarcoma outside of COG we have evaluated the 2005 report from the COSS. Two-year EFS in patients who achieved a second complete resection was 33%. Again it is not possible to make a valid comparison of this data to the COG data due to the difference in start time (time of recurrence vs. time of enrollment on a phase 2 trial). The COG data are more relevant to the patient population to be enrolled to Cohort 2 of this study.

2.1.3 Denosumab

Denosumab is a fully human monoclonal antibody to the receptor activator of nuclearfactor-κB ligand (RANKL). It is approved by the FDA for prevention of skeletal-related events in adults with solid tumor bone metastases (Xgeva) and for adults and skeletally mature adolescents with giant cell tumor of bone that is unresectable or where surgical resection is likely to result in severe morbidity and treatment of hypercalcemia of malignancy refractory to bisphosphonate therapy.

2.1.3.1 Denosumab PK/PD

Denosumab is administered subcutaneously and has a long half-life. The bioavailability following subcutaneous administration is 62%. The FDA approved dose for skeletal related events in patients with bone metastasis is 120 mg every 4 weeks. In phase 1 studies of denosumab in adults weight based doses up to 3 mg/kg were well tolerated. Subsequent studies utilized a fixed dose as high as 180 mg every 4 weeks.[14,15] Fixed doses have been administered to adults weighing as little as 45 kg.

Denosumab clearance and volume of distribution are proportional to body weight. At 120 mg every 4 week dosing, serum denosumab concentrations reach steady state at 6 months. In order to achieve steady state more rapidly, in the giant cell tumor study loading doses were administered on days 8 and 15. At steady state the mean elimination half-life of denosumab is 28 days.

All data on denosumab PK are from samples obtained from adult subjects. Therefore, data obtained from patients enrolled on this protocol will be used to characterize PK parameters for patients less than 18 years of age. To accomplish this, all patients enrolled onto the first stage of Cohort 1 (Stratum 1) will be required to be less than 18 years of age at enrollment. As discussed in Section 2.1.2, analyses of a group of patients

with RECIST measurable disease enrolled on previous COG single-agent phase 2 studies has demonstrated that advancing age was unrelated to increased risk of EFS-event. There were an insufficient number of RECIST responders to assess the influence of age at the start of treatment on the likelihood of response. Restricting enrollment of Stratum 1 patients to those less than 18 years of age will not result in an underestimate of the rate of disease control in this group of patients.

Denosumab rapidly inhibits bone turnover. In patients with breast cancer and bone metastases, the median reduction in urinary N-telopeptides/creatinine (uNTx/Cr) was 82% within 1 week of the first dose. Rapid decline in serum c-telopeptide is also observed.[16] In a group of patients with breast cancer receiving concurrent chemotherapy, the median duration of time to achieve > 65% reduction in uNTx/Cr was 9-13 days depending on the denosumab dose.[15]

2.1.3.2 Denosumab Toxicity

Denosumab-associated toxicity is limited. At the 120 mg every 4 week dose, the most common adverse effects are hypocalcemia and hypophosphatemia with incidences of 18% and 32%, respectively. Serum calcium concentrations of < 7 mg/dL occurs in 3.1% of adults. Hypocalcemia is observed particularly during the first 6 weeks of therapy.[17,18] In the post marketing setting, severe symptomatic hypocalcemia has been reported, including fatal cases. In clinical studies of subjects without advanced cancer with varying degrees of renal function (including patients with severe renal impairment [creatinine clearance < 30 mL/min] or receiving dialysis), there was a greater risk of developing hypocalcemia with increasing degree of renal impairment, and in the absence of calcium supplementation. Therefore, pre-existing hypocalcemia must be corrected prior to initiating therapy with denosumab 120 mg. Supplementation of calcium and vitamin D is required in all patients, unless hypercalcemia is present. Monitoring of calcium levels is recommended during treatment, especially in the first weeks of initiating therapy. In addition, a transient increase in bone remodeling associated with significant hypercalcemia following discontinuation of denosumab has been reported to occur. This off-treatment hypercalcemia has been observed up to 8 months after stopping Denosumab.[19,20]

Osteonecrosis of the jaw has been observed at a rate similar to that seen with bisphosphonates (2.2%). ln three phase 1 active-controlled clinical trials in patients with advanced malignancies involving bone, ONJ was confirmed in 1.8% of subjects in the denosumab 120 mg group (median exposure of 12.0 months; range 0.1 -40.5) and 1.3% of subjects in the zoledronic acid group. The trials in subjects with breast or prostate cancer included a denosumab 120 mg extension treatment phase (median overall exposure of 14.9 months; range 0.1-67.2). The subject-year adjusted incidence of confirmed ONJ was 1.1% during the first year of treatment and 4.1% thereafter. The median time to ONJ was 20.6 months (range: 4 - 53). In clinical trials, the incidence of ONJ was higher with longer duration of exposure. Poor oral hygiene, invasive dental

procedures, treatment with anti-angiogenic medication, local gum or oral infection were risk factors for ONJ in patients receiving denosumab 120 mg in clinical trials. Examples of invasive dental procedures that result in major disruption of the mucosal integrity and may increase the risk of ONJ include dental implants, dental or periodontal infection, dentures, tooth extraction, endodontic surgery and periodontal surgery. Osteonecrosis of the jaw may be less common in children and young adults. There are no reports of osteonecrosis of the jaw as a result of braces. There are animal models of bisphosphonates and orthodontics. These show decreased tooth movement in response to braces. In 2 papers of orthodontics in adults receiving bisphosphonates patients experienced longer treatment times and increased risk of poor parallelism but no cases of osteonecrosis of the jaw.[17,18,21,22]

Atypical femoral fracture has been reported with denosumab. Atypical femoral fractures may occur with little or no trauma in the subtrochanteric and diaphyseal regions of the femur and may be bilateral. Specific radiographic findings characterize these events. Atypical femoral fractures have also been reported in patients with certain comorbid conditions (e.g. vitamin D deficiency, rheumatoid arthritis, hypophosphatasia) and with use of certain pharmaceutical agents (e.g. bisphosphonates, glucocorticoids, proton pump inhibitors). These events have also occurred without antiresorptive therapy.

Hypersensitivity, including anaphylactic reactions, has been identified as an adverse drug reaction. Musculoskeletal pain, including severe cases, has been identified as an adverse drug reaction based on data from the postmarketing setting.

2.1.3.3 Clinical Data Regarding Long-term Denosumab

Studies of longer-term denosumab administration are just reaching maturity. Safety data are now available for a phase 3 study (vs. zolendronic acid) of prevention of skeletal-related events. In contrast to zoledronic acid, denosumab did not require monitoring or dose modification/withholding based on renal status, and was not associated with acute-phase reactions. Hypocalcaemia was more common for denosumab. Osteonecrosis of the jaw occurred at a similar rate ($P = 0.13$). The median number of denosumab doses received by participants in this study were 13, equal to 52 weeks of treatment. Rates of adverse events were not increased in the denosumab group.[23]

2.1.3.4 Clinical Data Regarding Denosumab in children and adolescents

Denosumab (120 mg) has been administered to 10 skeletally mature adolescents with giant cell tumor of bone in a prospective open-lable phase 2 study and in children.[24] In this open label phase 2 study, in Giant Cell Tumor of Bone, including 10 adolescents, adverse events were consistent with the known safety profile of denosumab. In addition there are case reports of 2 children who received denosumab: one with metastatic giant cell tumor of bone and one with McCune-Albright syndrome.[19,20]

2.2 Preclinical Studies

2.2.1 Pre-Clinical Data Supporting RANKL Inhibition in Osteosarcoma

RANKL and its receptor RANK have the physiologic function of regulating bone turnover. In response to RANKL-RANK binding, osteoclast precursors differentiate and become activated resulting in bone resorption.[25]

Osteosarcoma expresses both RANK and RANKL. In one set of studies, RANK was expressed in 57% of human osteosarcomas, most human osteosarcoma cell lines and 70% of canine osteosarcomas including 2 of 2 canine osteosarcoma skin metastases.[26,27] In contrast, results from another study presented in abstract form showed little to no RANK expression in human osteosarcoma tumor cells, though > 50% of tumor associated osteoclasts or osteoclast precursors expressed RANK.[28] This same study identified osteosarcoma tumor cell RANKL expression in 65-75% of human osteosarcoma samples. Expression was generally less intense compared with positive control samples from giant cell tumors of bone. Another study reported that RANKL is expressed in 75% of human osteosarcoma tumor specimens and the strength of expression is associated with prognosis.[29] In osteosarcoma, RANKL activates downstream signaling and modulates gene expression.[27,30]

In an orthotopic model of metastatic osteosarcoma utilizing the human cell lines HOS and SAOS2, Smad7 inhibition of TGF beta signaling reduces the development of lung metastasis and enhances tumor-associated bone formation. Smad7 inhibition of TGF beta signaling markedly reduced RANKL expression.[31] In a murine model of osteosarcoma established by p53 and Rb deletion, PTHR1 knockdown decreased invasion *in vitro*, and *in vivo*, decreased size of primary tumor and increased mineralization. When bone markers expression was evaluated, RANKL expression decreased and OPG, decoy receptor for RANKL, increased. These two studies provide inferential evidence that decreased RANK-RANKL signaling may be related to decreased invasion, reduced proliferation and increased differentiation.[32]

Further evidence supporting activity of the RANK-RANKL pathway in osteosarcoma is provided by a transgenic mouse model of osteosarcoma. In this mouse model, expression of the SV40 T/t antigen from the osteocalcin promoter results in highly penetrant osteosarcoma. In this model, heterozygous deletion of *Prkar1a* accelerates development of osteosarcoma via increased RANKL gene expression. A subset of human osteosarcomas have low Prkar1a expression and high RANKL expression.[33]

In vivo studies of denosumab are limited because the antibody does not recognize murine or canine RANKL. To circumvent these limitations, *in vivo* studies have utilized other approaches to inhibit RANKL activity including osteoprotegerin (OPG), a decoy receptor for RANKL, RANK-Fc, a chimeric protein that efficiently binds RANKL, or SiRNA directed against RANKL. In a murine orthotopic osteosarcoma model, OPG gene therapy decreased primary and metastatic tumor burden: Mice receiving OPG gene transfer did not develop osteosarcoma lung metastases while 80% of control mice developed lung metastases.[34] A study by the same group using RANK-Fc confirmed these original findings.[35]

Studies assessing the utility of RANKL directed strategies against osteosarcoma that has metastasized outside of the bone environment have contradictory results. In one experiment, gene therapy with RANK-Fc did not seem to improve survival from lung metastases in mice following retro-orbital injection of osteosarcoma cells.[35] However, experiments by another group demonstrate that RANK-Fc decreases migration and invasion *in vitro* and leads to a decreased metastatic tumor burden *in vivo*.[36] In addition, in transgenic mice that developed osteosarcoma following deletion of *Rb*, *p53* and *Prkar1a* administration of RANK-Fc resulted in improved survival (R. Khokha, unpublished data). In these two *in vivo* models, there is evidence of activity of RANKL-directed therapy in osteosarcoma that has metastasized to extra-skeletal locations. It is not clear which tumor model or which method of interfering with RANKL will be representative of denosumab activity in osteosarcoma metastatic to extra-skeletal locations in humans. Because of the limitations mentioned above, additional *in vivo* testing of denosumab by the Pediatric Preclinical Testing Program (PPTP) or other investigators is unlikely to proceed. Consequently, the Bone Tumor Committee feels the existing pre-clinical data are sufficient to support a phase 2 study of denosumab in patients with recurrent osteosarcoma with extra-skeletal metastases.

In summary, a breadth of pre-clinical data demonstrates a role for RANK-RANKL signaling in osteosarcoma and supports a potential benefit of denosumab for patients with relapsed or refractory osteosarcoma. The pre-clinical studies in murine models are summarized in the table below:

Reference (first author, year of publication, PMID)	Model	Method of inhibiting RANKL*	Osteosarcoma response (bone site)	Osteosarcoma response (lung metastasis site)
Lamora A, 2014, 25107916	Xenograft (orthotopic injection)	Smad7 overexpression	Statistically significant decrease in tumor volume	Statistically significant decrease in number of nodules
Ho PW, 2014, 25043296	Xenograft	Pthr1 knockdown	Statistically Significant decrease in tumor weight	Not applicable
Khokha, R, personal communication, 2013	Genetically engineered mouse model	RANK-FC	Improved survival	Improved survival
Akiyama T, 2010, 20383567	Xenograft (orthotopic)	RANK-FC	No change in tumor volume	Statistically significant decrease in number of nodules
Lamoureux F, 2008, 18852142	Xenograft (orthotopic injection OR retro-orbital injection)	RANK-FC	Statistically Significant decrease in tumor volume and in the % of mice with tumors	Decreased number of metastases in orthotopically injected mice No impact on metastases with retro-orbital injection.

Lamoureux F, 2007, 17671200	Xenograft (orthotopic injection)	Osteoprotegerin (OPG) transgene expression	Statistically Significant decrease in tumor volume and in the % of mice with tumors	Decrease in proportion of mice with metastases (0% vs. 80%)

*Denosumab is an antibody that does not recognize murine or canine RANKL. To circumvent this limitation in pre-clinical studies, alternate approaches to inhibit RANKL are utilized.

2.2.2 Pre-clinical Data Regarding RANKL Inhibition in the Young

Unpublished pre-clinical Amgen data suggest that administration of denosumab to skeletally immature children may result in risks to developing bone. Neonatal rats receiving OPG-Fc had decreased bone growth and tooth eruption. This effect was partially reversible upon discontinuation of OPG-Fc. Adolescent monkeys receiving 5-10 times the recommended denosumab dose had abnormal growth plates. However, in published data, inhibiting RANKL activity seems to have minimal impact on bone growth and development.[37] Denosumab therapy in skeletally immature patients may result in a risk of skeletal toxicity, but the poor prognosis of patients with recurrent osteosarcoma support the use of novel agents in this disease and the risk profile of denosumab compares favorably with other agents used in this clinical setting. Long-term follow up data regarding skeletal growth is not likely to be available from the patients enrolled on this study related to the overall prognosis of this patient population.

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 Patient Registry module in OPEN 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. For additional help or information, please contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

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, submission of regulatory documents and how to check your site's registration status.

NOTE: In order for an institution to maintain COG membership requirements, every newly diagnosed patient needs to be offered participation in ACCRN07, *Protocol for the Enrollment on the Official COG Registry, The Childhood Cancer*

Research Network (CCRN) or APEC14B1, *Project:EveryChild A Registry, Eligibility Screening, Biology and Outcome Study*.

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 (i.e., 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 **five (5)** calendar days after the date of study enrollment.

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.

3.1.6 Participation in Biology Studies

In order to minimize the potential for non-compliance once enrolled, patients/guardians must be made aware that a number of the biology research studies are mandatory and understand that a number of non-standard blood samples will be required.

3.1.7 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this study.

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), serum creatinine, calcium, magnesium and phosphorous. If the recheck is outside the limits of eligibility, the patient may not receive protocol therapy and will be considered off protocol therapy. Imaging studies, if applicable, must be obtained within 2 weeks prior to planned start of protocol therapy (repeat the tumor imaging if necessary).

See Section 4.2.1 for required studies to be obtained prior to starting protocol therapy.

3.2.1 Age

Patients must be equal to or greater than 11 years of age but less than 50 years of age at the time of enrollment.

- Female patients must have a bone age of equal to or greater than 12 years of age as determined by local read of appropriate radiographic imaging (see Section 16.0).
- Male patients must have a bone age of equal to or greater than 14 years of age as determined by local read of appropriate radiographic imaging (see

Section 16.0).

3.2.2 Diagnosis

3.2.2.1 Patients must have relapsed or become refractory to conventional therapy, with a regimen including some combination of high dose methotrexate, doxorubicin, cisplatin, ifosfamide and etoposide; and have had histologic verification of osteosarcoma at original diagnosis or at the time of recurrence.

3.2.2.2 Cohort 1 patients must have measurable disease according to RECIST 1.1 (see Section 10.2).

Note: Patients in Cohort 1 will be stratified as follows:

- Stratum 1: Patients ≥ 11 years of age but < 18 years
- Stratum 2: Patients ≥ 11 years of age but < 50 years

3.2.2.3 Cohort 2 patients must have had a complete resection of all sites of metastatic disease within 30 days prior to enrollment.

3.2.2.3.1 Patients will only be eligible after they have undergone complete surgical resection of suspected metastatic disease that is histopathologically confirmed to be osteosarcoma prior to enrollment.

Note: The definition of complete resections is: gross resection of all disease as per the operating surgeon. (For further details see https://www.cogmembers.org/_files/Disc/surgery/handbooks/OsteoBoneHandbook.pdf.) Post-operative imaging is not required for confirmation of complete resection.

3.2.2.3.2 Patients must undergo resection of any lung lesion meeting criteria for likely metastatic disease, defined as:

- 3 or more lesions > 5 mm in diameter OR a single lesion > 1 cm.

3.2.2.3.3 Patients with lung as the only site of resected metastatic disease must have refused participation in protocol AOST1421.

Note: This applies if AOST1421 is open to enrollment at the enrolling institution on the day the patient consents.

3.2.3 Specimen Submission

Patient must have adequate tumor specimen available for submission (see Section 15.3).

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 Organ Function Requirements

3.2.5.1 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
11 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[38] utilizing child length and stature data published by the CDC.

3.2.5.2 Adequate liver function defined as:

- Total bilirubin ≤ 1.5 x upper limit of normal (ULN) for age, and
- SGOT (AST) or SGPT (ALT) < 2.5 x ULN for age.
- Serum calcium or albumin-adjusted serum calcium ≥ 2.0 mmol/L (8.0 mg/dL) and ≤ 2.9 mmol/L (11.5 mg/dL).

3.2.6 Exclusion Criteria

3.2.6.1 Patients with known sensitivity to any of the products to be administered during the study (eg, mammalian derived products, calcium or Vitamin D).

3.2.6.2 Patients who are receiving other cancer directed therapy at the time of enrollment.

3.2.6.3 Patients who have previously received denosumab

3.2.6.4 Patients who have previously received mithramycin, strontium-89, samarium-153 or rhenium.

3.2.6.5 Patients receiving bisphosphonates.

3.2.6.6 Pre-existing conditions

3.2.6.6.1 Disorders associated with abnormal bone metabolism.

3.2.6.6.2 Hypocalcemia that is not corrected with oral calcium supplementation.

3.2.6.6.3 Vitamin D < 20 ng/mL

3.2.6.6.4 Paget's disease.

3.2.6.6.5 Prior history or current evidence of osteonecrosis of the jaw.

3.2.6.6.6 Any dental or oral condition likely to result in disruption of mucosal integrity during denosumab therapy including: active dental or jaw condition requiring oral surgery or tooth extraction; non-healed dental or oral surgery or planned invasive dental procedures during the anticipated course of study therapy.

3.2.6.6.7 Unstable systemic disease, excluding osteosarcoma, such as unstable proximal renal tubule dysfunction (Fanconi Syndrome) or congestive heart failure.

3.2.6.7 Pregnancy and Breast Feeding

3.2.6.7.1 Female patients who are pregnant, since fetal toxicities and teratogenic effects have been noted for the study drug. A pregnancy test is required for female patients of childbearing potential.

3.2.6.7.2 Lactating females who plan to breastfeed their infants while on study therapy and through 5 months after completion of study therapy.

3.2.6.7.3 Sexually active patients of reproductive potential who have not agreed to use a highly effective contraceptive method for the duration of their study participation and for 5 months after the end of study treatment.

3.2.7 Regulatory Requirements

3.2.7.1 All patients and/or their parents or legal guardians must sign a written informed consent.

3.2.7.2 All institutional, FDA, and NCI requirements for human studies must be met.

4.0 TREATMENT PLAN

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

All subjects will receive denosumab 120 mg subcutaneously every 4 weeks. Two additional loading doses will be given on Days 8 and 15 of Cycle 1 (Weeks 2 and 3) only. In the absence of progressive disease or unacceptable toxicity denosumab may be continued for up to 24 months in total or 26 cycles, whichever occurs first.

Other therapy: Although not encouraged, subjects in Cohort 1 who achieve a complete or partial response following the pre-Cycle 5 evaluation time point that is **confirmed** with imaging performed 4 weeks later will be allowed to undergo resection of sites of disease or radiation and remain on protocol therapy. Therapy will be held during surgery and resumed upon recovery from surgery, but at least 2 weeks after surgery. Denosumab therapy will be held during radiation and may be resumed upon completion of the radiation treatment course. If the patient has not recovered from surgery within 6 weeks the patient will go off protocol therapy. Surgery or radiation performed in a patient who does not achieve a **confirmed** complete or partial response will render the patient inevaluable for disease control assessment and the patient will go off protocol therapy.

Please note: Avoid invasive dental procedures during treatment with denosumab 120 mg. For patients in whom invasive dental procedures cannot be avoided, the clinical judgment of the treating physician should guide the management plan based on individual benefit/risk assessment. In addition, administration of denosumab will be withheld 30 days prior to any elective invasive oral/dental procedure and until documentation of complete mucosal healing following any invasive oral/dental procedure.

For COG Supportive Care Guidelines see:
https://childrensoncologygroup.org/index.php/cog-supportive-care-guidelines.

4.2 Therapy Delivery Map for Cycle 1

Each cycle lasts 4 weeks (28 days). Treatment may continue for up to 24 months or 26 cycles, whichever occurs first. Only Cycle 1 of this study is described in this TDM. This TDM is on one page.	_____ Patient COG ID number _____ DOB

Begin denosumab therapy only when: Cr clearance or radioisotope GFR ≥ 70 mL/min/1.73 m^2 or a serum creatinine based on age/gender as per Section 4.2.1; Total bilirubin ≤ 1.5 x upper limit of normal (ULN) for age, and SGOT (AST) or SGPT (ALT) < 2.5 x ULN for age and serum calcium or albumin-adjusted serum calcium ≥ 2.0 mmol/L (8.0 mg/dL) and ≤ 2.9 mmol/L (11.5 mg/dL). A negative pregnancy test is required for all female patients with childbearing potential.

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES
Denosumab IND #127430	Subcutaneous injection	120 mg	1, 8 & 15	Calcium and Vitamin D supplementation according to Section 4.2.2 is **required** to begin with the first dose of denosumab. Denosumab can be administered at a single site with a maximum volume of 2 mL.

Ht _____cm Wt _____kg BSA _____m^2

Date Due	Date Given	Day	Denosumab 120 mg	Studies (See details on next page)	Record Calcium and Vitamin D supplementation
			Enter actual dose administered below		
		1	_____mg	a - r	
		8	_____mg	f, m, n	
		15	_____mg	f, m, n	
		22		f, m, n	

Start Cycle 2 on Day 29 or as soon as starting criteria are met, whichever occurs later. Treatment should continue up to 24 months or 26 cycles, whichever occurs first, 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 5.0 for Dose Modifications for Toxicities and the COG Member website for Supportive Care Guidelines.

4.2.1 Required Observations Prior to and During Cycle 1

All baseline studies must be performed prior to starting protocol therapy unless otherwise indicated below.

a. History/PE/Wt /Ht
b. CBC/diff/platelets
c. Total bilirubin
d. Performance status
e. Electrolytes, Bun
f. Ca^{++}, PO_4, Mg^{++}
g. Vitamin D
h. AST, ALT, Albumin. For patients with low albumin, albumin or ionized calcium along with serum calcium should be ordered every week until normal.
i. Serum creatinine or GFR or creatinine clearance
j. Bone scan
k. CT chest
l. CT or MRI of any sites of active disease in bone or soft tissue. Bone or soft tissue sites of disease, if present, should be imaged with CT or MRI prior to the start of therapy. The same imaging modality as was used prior to the start of therapy should be used for follow-up imaging.
m. Pharmacokinetics are required for patients enrolled in Cohort 1, Stratum 1. Pharmacokinetics are optional for all other patients. Blood will be drawn prior to denosumab dosing on Day 1, 8, 15 and 22 (no denosumab is given on Day 22). See Section 15.1 for details.
n. Pharmacodynamics (required). Urine N-telopeptides (uNTx/Cr) from the second morning urine and serum c-telopeptides (sCTx). uNTx/Cr and sCTx will be obtained prior to denosumab dosing on Day 1, 8, 15 and 22 (no denosumab is given on Day 22). See Section 15.2 for details.
o. Dental exam with appropriate preventive dentistry prior to treatment with denosumab.
p. Bone age (Patients ≤ 18 years of age only).
q. Pregnancy test. Female patients of childbearing potential require a negative pregnancy test prior to starting treatment.
r. Pathology and surgical report submission is required for patients enrolled on Cohort 2 within 4 weeks of enrollment. See Sections 13.0 and 14.0.

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

Comments

Cycle 1

4.2.2 Cycle 1 Treatment Details

Cycle 1 of denosumab treatment is described below. During Cycle 1 only, denosumab is given on Days 1, 8, and 15 in order to reach steady state concentrations.

Denosumab: Subcutaneous
Days: 1, 8, and 15
Dose: 120 mg

Calcium and Vitamin D supplements
Supplementation with at least 500 mg of calcium daily and 400 IU of Vitamin D daily is **required** unless the patient has or develops hypercalcemia.

Criteria to start Cycle 1:

- 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
11 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[38] utilizing child length and stature data published by the CDC.

- Adequate liver function defined as:
  - Total bilirubin ≤ 1.5 x upper limit of normal (ULN) for age, and
  - SGOT (AST) or SGPT (ALT) < 2.5 x ULN for age.

- Serum calcium or albumin-adjusted serum calcium ≥ 2.0 mmol/L (8.0 mg/dL) and ≤ 2.9 mmol/L (11.5 mg/dL)

See Section 5.0 for Dose Modifications based on Toxicities.

Start the next cycle on Day 29 (Day 1 of Cycle 2) or as soon as starting criteria are met, whichever occurs later. Treatment should continue up to 24 months or 26 cycles, whichever occurs first. See Section 4.3 for details of Cycles 2-26.

Cycle 1

4.3 Therapy Delivery Map for Cycles 2-26

Each cycle lasts 4 weeks (28 days). Treatment may continue for up to 24 months or 26 cycles, whichever occurs first. One cycle of treatment is described in this TDM. This TDM is on one page. Use a copy of this page once for each cycle (please note cycle number below).	_____ Patient COG ID number _____ DOB

Begin denosumab therapy only when: Cr clearance or radioisotope GFR ≥ 70 mL/min/1.73 m^2 or a serum creatinine based on age/gender as per Section 4.3.1; Total bilirubin ≤ 1.5 x upper limit of normal (ULN) for age, and SGOT (AST) or SGPT (ALT) < 2.5 x ULN for age and Serum calcium or albumin-adjusted serum calcium ≥ 2.0 mmol/L (8.0 mg/dL) and ≤ 2.9 mmol/L (11.5 mg/dL).

DRUG	ROUTE	DOSAGE	DAYS	IMPORTANT NOTES
Denosumab IND #127430	Subcutaneous injection	120 mg	1	Calcium and Vitamin D supplementation according to Section 4.3.2 **required** to begin with the first dose of denosumab. Denosumab can be administered at a single site with a maximum volume of 2 mL.

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

Date Due	Date Given	Day	Denosumab 120 mg	Studies (See details on next page)	Record Calcium and Vitamin D supplementation
			Enter actual dose administered below		
		1	_____mg	a-n	
		8		d	
		15		d, j	
		22		d	

Start the next cycle on Day 29 or as soon as starting criteria are met, whichever occurs later. Treatment should continue up to 24 months or 26 cycles, whichever occurs first, 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 5.0 for Dose Modifications for Toxicities and the COG Member website for Supportive Care Guidelines.

4.3.1 Required Observations during Cycles 2-26

All baseline studies must be performed prior to starting protocol therapy unless otherwise indicated below.

a. History/PE/Wt/Ht.
b. Total bilirubin
c. Electrolytes, Bun
d. Ca^{++}, PO_4, Mg^{++} weekly for the first 8 weeks only and then prior to the start of every cycle. It is strongly recommended that Ca^{++} continue to be monitored weekly for any patient with hypocalcemia on any prior blood draw or in patients with evidence of rapid bone turnover as assessed by Urine N-telopeptides or serum c-telopeptides.
e. AST, ALT, Albumin. For patients with low albumin, albumin or ionized calcium along with serum calcium should be ordered every week until normal.
f. Serum creatinine or GFR or creatinine clearance
g. Bone Scan every 6 months.
h. CT Chest prior to the start of every odd numbered cycle only.
i. CT or MRI of any sites of active disease in bone or soft tissue prior to the start of every odd numbered cycle only. The same imaging modality as was used prior to the start of therapy should be used for follow-up imaging.
   - For patients who undergo a surgical resection following Cycle 4, either a CT scan or MRI post-surgery to establish a new baseline is strongly recommended before re-initiating denosumab therapy.
   - Patients who have a RECIST response (CR or PR) on any evaluation following Cycle 4 will have confirmatory imaging 4 weeks after the evaluation upon which the RECIST response was detected (additional imaging time point).
j. Pharmacokinetics are required for patients enrolled in Cohort 1, Stratum 1. Pharmacokinetics are optional for other patients. Blood will be drawn prior to denosumab dosing on Day 1 of Cycles 2, 3, 4 and 7 only and on Day 1 and 15 of Cycle 6 only (no denosumab is given on Day 15). Even when PK is required, the Cycle 6 Day 15 time point is only for patients who have provided consent. See Section 15.1 for details.
k. Pharmacodynamics (required). Blood and urine collection for Urine N-telopeptides (uNTx/Cr) from the second morning urine and serum c-telopeptides (sCTx). uNTx/Cr and sCTx will be obtained prior to denosumab dosing on Day 1 of Cycle 2, 3, 4 and 7. See Section 15.2 for details.
l. Dental exam every 6 months.
m. Bone age every 6 months only in patients without epiphyseal closure at the time of enrollment.
n. Pregnancy test. Female patients of childbearing potential require a negative pregnancy test prior to each cycle of treatment.

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

Comments

Cycle 2-26

4.3.2 Treatment Details for Cycle 2 and All Subsequent Cycles

One cycle of denosumab treatment is described below. A cycle may be repeated every 28 days if the patient has at least stable disease and has again met laboratory parameters as defined below.

Denosumab: Subcutaneous
Day: 1 of each 28 day cycle
Dose: 120 mg

Calcium and Vitamin D supplements
Supplementation with at least 500 mg of calcium daily and 400 IU of Vitamin D daily is **required** unless the patient has or develops hypercalcemia.

Criteria to start each cycle:

- 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
11 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[38] utilizing child length and stature data published by the CDC.

- Adequate liver function defined as:
  - Total bilirubin ≤ 1.5 x upper limit of normal (ULN) for age, and
  - SGOT (AST) or SGPT (ALT) < 2.5 x ULN for age.

- Serum calcium or albumin-adjusted serum calcium ≥ 2.0 mmol/L (8.0 mg/dL) and ≤ 2.9 mmol/L (11.5 mg/dL)

See Section 5.0 for Dose Modifications based on Toxicities.

Cycle 2-26

5.0 DOSE MODIFICATIONS FOR TOXICITIES

Patients who have Grade 3 or 4 allergic reaction or anaphylaxis or recurrence of Grade 2 allergic reaction with appropriate pre-medication (anti-histamine such as diphenhydramine and steroid such as dexamethasone) must be removed from protocol therapy.

5.1 Denosumab Dose Delay

Hold denosumab until starting criteria to begin the cycle are met. Specifically, Denosumab should not be given on Day 1 of any cycle or on Days 8 and 15 of Cycle 1 if serum calcium is less than 2.0 mmol/L (8.0 mg/dL). Denosumab will be held for Grade 3 or 4 adverse events per CTCAE reported by investigator as related to denosumab. Denosumab may be resumed when the event resolves to Grade 1 or returns to baseline. The start of a cycle may be held for a maximum of 2 weeks. If toxicity prevents the start of a cycle following a 2 week delay, the patient will be removed from protocol therapy.

6.0 DRUG INFORMATION

6.1 DENOSUMAB

(AMG 162, Xgeva®, NSC# 744010, IND# 127430) (09/18/18)

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]	[redacted]
[redacted]	[redacted]

[redacted]

[redacted]

[redacted]

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

Subjects who discontinue investigational product will have an End of Treatment visit approximately 4 weeks after the last dose of investigational product (see below). Thereafter, they will complete safety follow-up visits approximately every month for the first year off protocol therapy. Serum calcium should be checked at each safety follow-up visit due to possible occurrence post-treatment hypercalcemia. Reporting of adverse events observed at safety follow-up visits is as per Section 11.0.

STUDIES TO BE OBTAINED	End of Therapy
History	X
Physical exam with VS	X
Ht, Wt, BSA	X
Performance status	X
Pregnancy test	X
CBC, differential, platelets	X
Electrolytes including Ca^{++}, PO_4, Mg^{++}	X
Creatinine, SGPT, total bilirubin	X
Tumor disease evaluation	X

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

Note: Follow-up data must be submitted in accordance with the Case Report Forms (CRFs) schedule.

8.0 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

8.1 Criteria for Removal from Protocol Therapy

a) Progressive disease.
b) Osteonecrosis of the jaw.
c) Fracture excluding pathological fracture at the site of osteosarcoma bone metastasis or fracture at the site of prior orthopedic surgery.
d) Refusal of further protocol therapy by patient/parent/guardian.
e) Completion of planned therapy.
f) Physician determines it is in patient's best interest.
g) Development of a second malignancy.
h) Pregnancy.
i) Surgery or radiation performed in a patient who has not achieved a confirmed complete or partial response.
j) Repeat eligibility studies (if required) prior to the initiation of protocol therapy are outside the parameters required for eligibility (see Section 3.2).
k) Unacceptable toxicity due to protocol therapy (see Section 5.0).

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) Fifth anniversary of the date the patient was enrolled on this study.

9.0 STATISTICAL CONSIDERATIONS

9.1 Statistical Design

Cohort 1 (Evaluation of Aim 1.1.1): A maximum of 41 evaluable patients will be enrolled in two stages. In the first stage, 15 disease control and RECIST response evaluable patients will be enrolled. Only those patients enrolled in Stratum 1 will be considered eligible for Stage 1 of Cohort 1.

Each patient will be evaluated for both outcome measures: (1) disease control success; and (2) RECIST response (CR or PR *v.* not CR or PR). If 2 or fewer disease control successes and no RECIST responses are observed, enrollment to this cohort will be terminated with the conclusion that denosumab is not associated with sufficient activity for further single-agent investigation. Otherwise, an additional 26 response evaluable patients will be enrolled at which point cohort enrollment will be terminated. If, cumulatively, 12 or fewer disease control successes and 4 or fewer RECIST responses are observed, we will conclude denosumab is not associated with sufficient activity for further single-agent investigation. Otherwise, we will conclude denosumab is associated with sufficient activity for further single-agent investigation.

Each patient enrolled will be evaluated for: (1) complete or partial response as defined by the RECIST criteria where the first evaluation of CR or PR is made at or before the end of the fifth cycle of study therapy (denoted as R below); or (2) stable disease four months after study enrollment (denoted as S below). We will not be interested in promoting the agent for further investigation if the probability of response in any particular individual is less than or equal to 0.05 (P(R)≤0.05) and the probability of remaining analytic event free in any particular individual is less than or equal to 0.20 (P(S)≤0.20). We will be interested in promoting the agent for further investigation if the probability of response in any particular individual is at least 0.20 (P(R)≥0.20) **or** the probability of remaining analytic event free in any particular individual is at least 0.40 (P(S)≥0.40).

For the calculations below, it is assumed $\Pr(S \mid R) = 0.90$.

The statistical characteristics of this design are:

Probability of four month disease control	Probability of RECIST response	Probability of Stopping After Stage 1 (and concluding the drug is ineffective)	Probability of Concluding the Drug is Ineffective at the Conclusion of the Trial	Probability of Concluding the Drug is Effective at the Conclusion of the Trial
0.20	0.05	0.25	0.91	0.09
0.40	0.20	0.0062	0.029	0.971
0.40	0.05	0.020	0.11	0.89
0.20	0.20	0.035	0.088	0.912

Cohort 2 (Evaluation of Aim 1.1.2): 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 protocol therapy or 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 12 months of therapy ('evaluation period').

Each patient enrolled will be evaluated for stable disease after 12 cycles of study therapy. We are not interested in promoting the agent for further investigation if the probability of remaining analytic event free 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 prior phase 2 COG studies demonstrates 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 patients will be enrolled in Cohort 2. The maximum time by which the final assessment for this stratum will be made is until 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 demonstrate disease control at 12 months, denosumab will not be considered for further development in this cohort, otherwise, denosumab will be considered of sufficient activity 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 denosumab will not be considered for further development if, at any time, more than 23 patients demonstrate disease progression or die prior to 12 months after study enrollment.

Potential factors that could be related to risk for EFS-event included are age group at enrollment, patient sex, race, ethnicity, time from diagnosis to first recurrence, initial diagnosis of metastatic disease and site of initial recurrence (lung only, bone only, lung and bone, other). For categorical characteristics, we will estimate the relative hazard rate and 95% confidence interval associated with the various categories using the proportional hazards regression model with the characteristic of interest as the only variable in the model. The logrank statistic will be used to quantify statistical significance. Age at enrollment will be treated as a continuous variable and we will also survey the peer-reviewed literature and test age cutoffs reported in reliable publications.

9.2 Patient Accrual and Expected Duration of Trial

Cohort 1: A review of enrollment on COG single-agent phase 2 studies demonstrates that approximately 2 patients per month without regard for patient sex or age can be expected for enrollment. An assessment of enrollment to ADVL0421, ADVL0524 and ADVL0525 indicates more than 65% of enrollments conformed to the age and sex requirements for AOST1321. More recently, enrollment on AOST1322 was 4.75 patients per month. 58% or 2.75 patients per month were under age 18. We assume that as in the predecessor studies, only 65% of the enrollees on AOST1321 would meet the developmental age requirements of AOST1321. Thus, we expect at least 1.5 patients per month to be enrolled. A maximum of 41 outcome evaluable patients will be required in this cohort. Providing for possible ineligible and inevaluable patients, the maximum enrollment will be 46 patients.

With these entry rates, the probability we will accrue 46 patients in 36 months is in excess of 93%. The study will likely require at most 3 years to enroll sufficient patients. A maximum of 46 patients is anticipated for this cohort.

Cohort 2: 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. We expect 1.6 patients per month to be enrolled on study, thus enrollment to this cohort is expected to be completed within 2.5 years of opening the study.

9.3 Statistical Analysis Methods

Evaluability

Response (Cohort 1 Patients Only): Any eligible patient who receives at least one dose of denosumab will be considered evaluable for response with the following exception: if a patient receives non-protocol anti-cancer therapy during the response evaluation period after the patient is considered as having a partial or complete response but prior to confirmation of this status by tumor imaging and before progressive disease is noted, the individual will be considered inevaluable for the response endpoint. The exception to this exception is patients who stop denosumab after the 1st evaluation due to toxicities or death, and these patients will be considered evaluable for the response evaluation.

Disease Control (Cohort 1 and Cohort 2 Patients): Any eligible patient who receives at least one dose of denosumab will be considered evaluable for disease control, with the exception noted above for "Response Evaluation". Only patients who are inevaluable for the response endpoint may be replaced for the purpose of the statistical rule.

Aim 1.2.1: To investigate the pharmacokinetics (PK) and pharmacodynamics (PD) of denosumab in subjects with recurrent osteosarcoma.

All eligible patients who receive at least one dose of denosumab and for whom at least one PK or PD sample is submitted will be considered evaluable for PK or PD, respectively. The presentation of PK values will be segregated according to time point at which the denosumab was administered. Sample means, medians and variances will be calculated. Clearance and volume of distribution will be determined. PD will be characterized by uNTx/Cr ratio and c-telopeptide levels. Both of these characteristics will be modeled according to a repeated measures linear regression model. Depending on the fit of the quadratic model, additional terms in powers of time since enrollment may be added to explore how the PD parameters vary with time.

Aim 1.2.2: Evaluating the Tolerability of Denosumab

Tolerability of Denosumab - An eligible patient will be considered for toxicity monitoring if one of the following occurs: (1) complete one cycle of denosumab prior to receiving non-protocol anticancer therapy; (2) die on protocol therapy for a reason considered possibly, probably or likely related to denosumab; or (3) are removed from protocol therapy because of an adverse experience possibly, probably or likely related to denosumab. A toxicity-evaluable patient will be considered in the analysis during the interval from study enrollment until the termination of protocol therapy. A toxicity-evaluable patient will be considered to have experienced an excessive toxicity event if: (1) the patient dies on protocol therapy for a reason considered possibly, probably or likely related to denosumab; or (2) experiences a dose-limiting toxicity. DLTs will be: any ≥ Grade 3 event not considered attributable to osteosarcoma except hypocalcemia ≥ Grade 3 corrected with medical therapy, hypophosphatemia ≥ Grade 3 corrected with medical therapy, ≥ Grade 3 cardiac toxicity in a subject who previously received Adriamycin and ≥ Grade 3 ototoxicity in a subject who previously received cisplatin. Osteonecrosis of the jaw (ONJ) and fractures (excluding pathologic fracture at the site of an osteosarcoma bony metastasis) will be considered DLTs and will require cessation of therapy. Serum calcium, magnesium and phosphate will be monitored at the start of every cycle and weekly during the first 8 weeks. Bone age and assessment for epiphyseal closure will be performed every 6 months in those patients without epiphyseal closure at the time of enrollment. Patients will be subject to routine AE reporting while on protocol therapy and for up to 30 days off protocol therapy. Expedited reporting of serious adverse events according to Section 11 of the

protocol will continue as long as a patient is considered on study according to Section 8 of the protocol.

The analytic unit for monitoring for excessive toxicity will be the patient-cycle: Each cycle where the patient receives denosumab and does not receive non-protocol anticancer therapy will be considered in the analysis. We will use a Bayesian rule to monitor for excessive toxicity. We will assume a beta prior distribution with $\alpha=0.6$ and $\beta=1.4$. If this posterior probability of the chance of DLT 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. Safety analyses will be performed in aggregate, by cohort, and by age group (≤ 18 years and in patients > 18 years of age). The safety of denosumab in adults and adolescents will be compared. In addition a subset analysis of the safety of denosumab in patients ≤ 15 years and in patients > 15 years of age will be performed.

Aim 1.2.3: Estimation of the Response and Disease Control Rates
Cohort 1: P(R) and P(S) will be estimated using the maximum likelihood estimates, *viz.,*

$$P(R) \doteq \hat{p}_R = \frac{Number\ of\ Patients\ Considered\ as\ PR\ or\ CR}{Number\ of\ Evaluable\ Patients\ in\ Cohort\ 1};$$

$$P(S) \doteq \hat{p}_S = \frac{Number\ of\ Patients\ With\ Disease\ Control\ at\ 4\ Months}{Number\ of\ Evaluable\ Patients\ in\ Cohort\ 1}$$

Confidence intervals will be constructed using the approximate normal distribution of each of the estimates and their asymptotic variances:

$$V(\hat{p}_R) \doteq \frac{\hat{p}_R(1-\hat{p}_R)}{Number\ of\ Evaluable\ Patients\ in\ Cohort\ 1};$$

$$V(\hat{p}_S) \doteq \frac{\hat{p}_S(1-\hat{p}_S)}{Number\ of\ Evaluable\ Patients\ in\ Cohort\ 1}$$

Cohort 2: The proportion of patients who experience 12 month disease control will be estimated by the method of Kaplan and Meier.[39] 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.[40]

9.4 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	1	0	0	**2**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	4	13	0	0	**17**
White	21	40	3	7	**71**
More Than One Race	0	0	0	0	**0**
Total	**26**	**54**	**3**	**7**	**90**

This distribution was derived from ADVL0821, ADVL0921, ADVL1221, 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 Response Criteria for Patients with Solid Tumors

For the purposes of this study, patients should be evaluated for response prior to Cycle 3, Cycle 5 and every odd cycle thereafter.

Response and 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).[41] Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

10.2.1 Definitions

10.2.1.1 Evaluable for objective response: See Section 9.3

10.2.2 Disease Parameters

10.2.2.1 Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm by chest x-ray, as ≥ 10 mm with CT scan, or ≥ 10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

10.2.2.2 Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be ≥ 15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

10.2.2.3 Non-measurable disease: All other lesions (or sites of disease), including small lesions (longest diameter < 10 mm or pathological lymph nodes with ≥ 10 to < 15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

10.2.2.4 Target lesions: All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

10.2.2.5 Non-target lesions: All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline.

Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

10.2.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

10.2.3.1 Clinical lesions: Clinical lesions will only be considered measurable when they are superficial (eg, skin nodules and palpable lymph nodes) and ≥ 10 mm diameter as assessed using calipers (eg, skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

10.2.3.2 Chest x-ray: Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

10.2.3.3 Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (eg, for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

10.2.3.4 Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound

examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

10.2.3.5 Endoscopy, Laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

10.2.3.6 Cytology, Histology: These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (eg, residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

10.2.4 Response Criteria

10.2.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

10.2.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (< 10 mm short axis).

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

10.2.4.3 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Patients with Measurable Disease (ie, Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥ 4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥ 4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	documented at least once ≥ 4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

*See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
**Only for non-randomized trials with response as primary endpoint.
***In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration.*" Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients with Non-Measurable Disease (ie, Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

10.2.5 Duration of Response

10.2.5.1 Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

10.2.5.2 Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

11.0 ADVERSE EVENT REPORTING REQUIREMENTS

11.1 Purpose

Adverse event 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 Expedited Reporting Requirements – Serious Adverse Events (SAEs)

To ensure compliance with these regulations/this guidance, 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.

11.3 Specific Examples for Expedited Reporting

11.3.1 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.

11.3.2 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, COG, or industry sponsor IND/IDE since these are considered to be serious AEs.

11.3.3 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 "*Disease progression*" in the system organ class (SOC) "*General disorders and administration site conditions*." 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.

11.3.4 Secondary Malignancy

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

All secondary malignancies that occur following treatment need to 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.

11.3.5 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.

11.3.6 Pregnancy, Pregnancy Loss, 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 (310) 640-9193. 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.

11.3.6.1 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.

11.3.6.2 Pregnancy Loss (Fetal Death)

Pregnancy loss is defined in CTCAE as "*Death in utero.*" Any pregnancy loss should be reported expeditiously, as **Grade 4 "*Pregnancy loss*" under the "*Pregnancy, puerperium and perinatal conditions*" SOC**. Do NOT report a pregnancy loss as a Grade 5 event since CTEP-AERS recognizes any Grade 5 event as a patient death.

11.3.6.3 Death Neonatal

Neonatal death, defined in CTCAE as "*Newborn death occurring during the first 28 days after birth*" should be reported expeditiously as **Grade 4 "*Death neonatal*" under the "*General disorders and administration*" SOC 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.

11.4 Reporting Requirements for Specialized AEs

11.4.1 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.

11.4.2 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.

11.4.3 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.

11.5 Exceptions to Expedited Reporting

11.5.1 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.

11.6 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.

11.7 General Instructions for Expedited Reporting via CTEP-AERS

The reporting methods described below are specific for clinical trials evaluating agents for which the IND is held by COG, an investigator, or a pharmaceutical company. It is important to note that these procedures differ slightly from those used for reporting AEs for clinical trials for which CTEP holds the IND.

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

An expedited AE report must be submitted electronically via CTEP-AERS at: https://eapps-ctep.nci.nih.gov/ctepaers

- 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 requires expedited reporting **within 24 hours** via e-mail to the COG CTEP-AERS Coordinator and Study Chair.
- Any death occurring greater than 30 days of the last dose with an attribution of possible, probable, or definite to an agent/intervention requires expedited reporting **within 24 hours** via e-mail to the COG CTEP-AERS Coordinator and Study Chair.

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.

Fax or email supporting documentation **for AEs related to investigational agents** to COG:
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.**

11.8 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 (COG) **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.

<table>
<tr><th>Hospitalization</th><th>Grade 1 Timeframes</th><th>Grade 2 Timeframes</th><th>Grade 3 Timeframes</th><th>Grade 4 & 5 Timeframes</th></tr>
<tr><td>Resulting in Hospitalization ≥ 24 hrs</td><td colspan="3">7 Calendar Days</td><td rowspan="2">24-Hour Notification
5 Calendar Days</td></tr>
<tr><td>Not resulting in Hospitalization ≥ 24 hrs</td><td colspan="2">Not Required</td><td>7 Calendar Days</td></tr>
</table>

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

11.9 Routine Reporting of Adverse Events

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 CTEP-AERS reportable events and Grade 3 and higher Adverse Events.

12.0 RECORDS AND REPORTING

See the Case Report Forms 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.

13.0 SURGICAL GUIDELINES

Patients who have had a complete resection of all sites of metastatic disease within 30 days prior to enrollment (Cohort 2) must upload the surgical report into RAVE within 4 weeks of enrollment.

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

14.0 PATHOLOGY GUIDELINES AND SPECIMEN REQUIREMENTS

Patients who have had a complete resection of all sites of metastatic disease within 30 days prior to enrollment (Cohort 2) must upload the pathology report into RAVE within 4 weeks of enrollment.

All patients enrolling on this protocol require institutional histological confirmation of osteosarcoma at the time of original diagnosis or at the time of recurrence. For enrollment on Cohort 1, histologic confirmation of recurrence/relapse is strongly encouraged but NOT required. For enrollment on Cohort 2 histologic confirmation of recurrence is required as detailed in Section 3.2.2.3.

Please note: all patients must have adequate tumor specimen available for submission as detailed in Section 15.3.

COG sites: For patients enrolled on AOST06B1 or APEC14B1 who have a biopsy or who undergo surgical resection, tissue submission is encouraged. (See AOST06B1 protocol or APEC14B1 Manual of Procedures.)

Autopsy

In the event of patient death on AOST1321, a complete unrestricted postmortem examination is strongly encouraged. For patients enrolled on AOST06B1 or APEC14B1 at COG sites, tissue submission is requested. (See AOST06B1 protocol or APEC14B1 Manual of Procedures.)

15.0 SPECIAL STUDIES SPECIMEN REQUIREMENTS

15.1 Denosumab Pharmacokinetics

The first 15 evaluable patients < 18 years of age enrolled on Cohort 1 (ie, Stratum 1) are **required** to have serial blood samples for the assessment of denosumab serum concentrations. Pharmacokinetic sampling will be optional for all other patients.

15.1.1 Timing of pharmacokinetic sampling

Serum will be drawn **prior** to denosumab dosing at the following time points.	
Cycle 1	• Day 1 • Day 8 • Day 15 • Day 22
Cycle 2	• Day 1
Cycle 3	
Cycle 4	
Cycle 6	• Day 1 • Day 15*
Cycle 7	• Day 1

*Cycle 6 Day 15 sampling time point requires patient consent from all patients, including those enrolled on Stratum 1. Collection at this time point will permit measurement of denosumab clearance and volume of distribution.

15.1.2 Sample Collection and Processing

At each protocol specified time point, fill one appropriately labeled (waterproof ink) 5.0 mL Serum Separator drawing tube (SST), containing no anti-coagulant until the vacuum is exhausted and blood flow ceases. Gently invert the filled tube 5 times and allow to clot for 30 to 60 minutes (maximum clotting time 1 hour) at room temperature.

Centrifuge at 1500 x g for 15 minutes at room temperature (if you have a temperature controlled centrifuge set to 20^0C).

Prepare two 4-mL polypropylene storage tubes per subject per time point. Each tube should have a freezer-safe label with:

- AOST1321
- COG patient ID number
- Study Day
- Sampling Date
- One tube will be labeled “A” and the other “B”

Using a pipette, remove serum from the top of the tube without disturbing the blood cells and transfer an ***equal volume*** (ideally 2 mL each) into each of the two labeled 4 mL cryovials**.**

Note: If there is an inadequate amount of serum for 2 mL per cryovial then split the available serum volume equally into all cryovials.

Immediately place the 4 mL cryovials containing the serum sample in a -70 ± 10^0C freezer. All tubes should be stored in an upright position.

The serum sample must be frozen within 2 hours of blood collection.

Following the plasma harvest, the remaining buffy coat and red blood cell layers should be discarded as biological specimen.

15.1.3 Sample Labeling and Shipping

Each tube should have a freezer-safe label with the details listed in Section 15.1.1.

When possible, primary "A" tubes and backup "B" tubes should be shipped on different days. All "A" tubes will be shipped frozen in appropriately insulated containers with enough dry ice to last 3 days. The tubes should be shipped on the first Monday, Tuesday, or Wednesday following the last scheduled sample collection by **overnight delivery** such that they reach PPD before or by Thursday of that week. A study-specific paper PK transmittal form should be completed in RAVE, printed and sent with the specimen to the following address:

PPD Sample Management, Attention James Rhyne
2246 Dabney Road
Richmond, VA 23230
Tel: (804) 977-8430
E-mail: RichmondSMOpeners@ppdi.com

If feasible, specimens should be collected at the clinical site and shipped in a batch to PPD. Specimens must be shipped in accordance with rules and laws governing the shipment of human diagnostic specimens

PPD should be notified by e-mail that a shipment is on its way. The courier and tracking number should be provided.

No shipments should be made within 3 days of a holiday.

15.2 Denosumab Pharmacodynamics

Pharmacodynamic sampling will be **required** of all patients and will be analyzed by the institution's laboratory as would be performed for clinical testing. A serum sample and a urine sample will be required at each time point.

15.2.1 Timing of Pharmacodynamic Observation:

<table>
<tr><td colspan="2">uNTx/Cr and sCTx will be obtained prior to denosumab dosing at the following time points.</td></tr>
<tr><td>Cycle 1</td><td>• Day 1
• Day 8
• Day 15
• Day 22</td></tr>
<tr><td>Cycle 2</td><td rowspan="4">• Day 1</td></tr>
<tr><td>Cycle 3</td></tr>
<tr><td>Cycle 4</td></tr>
<tr><td>Cycle 7</td></tr>
</table>

15.2.2 Specimen Processing

A urine N-telopeptide (uNTx/Cr) from the second morning urine and serum c-telopeptide (sCTx) will be analyzed by the institution's laboratory as would be performed for clinical testing.

15.2.3 Methodology

Changes in urine N –telopeptide (uNTx/Cr) and serum c-telopeptide (sCTx) will be used to evaluate the impact of denosumab on bone turnover (pharmacodynamics).

15.3 Evaluation of RANK / RANKL Expression in Pre-Treatment Tumor Specimens

Immunohistochemical analyses (IHC) of RANK and RANKL expression will be performed by Clarient Diagnostic Services Inc., Aliso Viejo, CA using prototype assays, which have been developed and optimized by Dako on their automated staining platform. A total of 7 slides will be required for the RANK IHC [H&E stain (one slide), RANK IHC plus negative control (2 slides), RANKL IHC plus negative control (2 slides), slides to potentially repeat a failed RANK/RANKL IHC (2 slides)].

15.3.1 Required Specimens

Submission of a formalin fixed paraffin embedded (FFPE) sample of tumor is **required**. A block is preferred. If a block is not available, 7 unstained slides must be provided from the most recent recurrence (preferred), a more distant recurrence (second choice) or original diagnosis (third choice). The handling and age of tumor tissue slides will adversely affect the results of the RANK and RANKL IHC due to epitope instability. The tumor material must be sent within 4 weeks of patient enrollment.

15.3.2 Specimen Processing

If slides are being sent in place of a block, section from FFPE blocks at 5 µm the day before shipment to the Biopathology Center (BPC) and mount on superfrost

plus coated slides. Slides are to be air dried overnight and placed in a rigid slide box and shipped ambient temperature on the next business day.

15.3.3 Specimen Labeling and Shipment

All tumor material must be labeled with the patient's COG patient ID number, specimen type, surgical pathology ID, block number and collection date.

All tumor material and associated pathology report(s) for that sample should be sent to the BPC at room temperature by regular mail or by the submitting institution's courier account. A study specific specimen transmittal form should be completed in RAVE, printed and sent with the specimen to the following address:

Biopathology Center (BPC)
Nationwide Children's Hospital
Protocol AOST1321
700 Children's Drive, WA1340*
Columbus, OH 43205
Phone: (614) 722-2865
Fax: (614) 722-2897

For questions about this correlative study, please contact the study chair, Dr. Janeway. For questions about shipping the slides, please contact the BPC directly.

15.3.4 Methodology

RANK / RANKL expression in pre-treatment specimens with an emphasis on metastatic lesions (when available) will be performed by immunohistochemistry. Utilizing pre-treatment specimens (when available), gene profiling will be performed and genomic data will be analyzed to determine patterns associated with a favorable response to denosumab. If there is variable expression of RANK / RANKL between tumors, expression array data will be analyzed for genes associated with RANK / RANKL expression.

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 and Recommended Osteosarcoma Imaging

16.1.1 Overview of required and recommended imaging studies

Site	Anatomic Imaging	Functional Imaging	Timing
Bone or soft tissue sites of disease	CT or MRI with gadolinium		Prior to starting protocol therapy and prior to every odd cycle*
Bone age (patients ≤ 18 years of age)	AP radiograph of the left hand		Prior to starting protocol therapy and every 6 months only in patients without epiphyseal closure at the time of enrollment.
Chest	CT		Prior to starting protocol therapy and prior to every odd cycle
Whole body		MDP bone scintigraphy	Prior to starting protocol therapy and every 6 months

*For patients who undergo a surgical resection following Cycle 4, either a CT scan or MRI post-surgery to establish a new baseline is strongly recommended before re-initiating denosumab therapy.

NOTE: patients who have a RECIST response (CR or PR) on any evaluation following Cycle 4 will have confirmatory imaging 4 weeks after the initial evaluation demonstrating a RECIST response (additional imaging time point).

16.1.2 Technical Guidelines for Imaging Studies

CT and MRI guidelines are available on the COG Member site at: https://www.cogmembers.org/_files/reference/RefMaterial/DiagnosticImagingGuidelines_MRICT.pdf.

16.1.3 X-ray

Bone age will be determined based on an AP radiograph of the left hand and with reference to the atlas of Greulich & Pyle (GP). Bone age is calculated by comparing the left wrist radiographs of the subject with the nearest matching reference radiographs provided in the atlas, which are standard for different ages provided in the atlas.

The hand radiographs are quite safe to obtain as the effective dose of radiation received during each exposure is between 0.0001-0.1 mSV. This dose is less than 20 minutes of natural background radiation or the amount of radiation received by an individual on a 2 minute transatlantic flight.

The pattern of ossification in the hand and wrist bones is in a fairly predictable manner and age specific until end of adolescence when the elongation of bone is complete. Thus, the standards of bone age have been derived by comparing the level of maturation of hand and wrist bones with normal age levels. The method

based on "The *Radiographic Atlas of Skeletal Development of the Hand and Wrist"*, by Dr. William Walter Greulich and Dr. Sarah Idell Pyle, its last edition published in 1959, is still one of the most commonly used atlas for bone age measurement. It contains reference images of male and female standards of the left wrist and hand from birth till 18 years for females and 19 years for males. Also, explanation regarding the gradual age related changes observed in the bone structure is provided with each standard image.

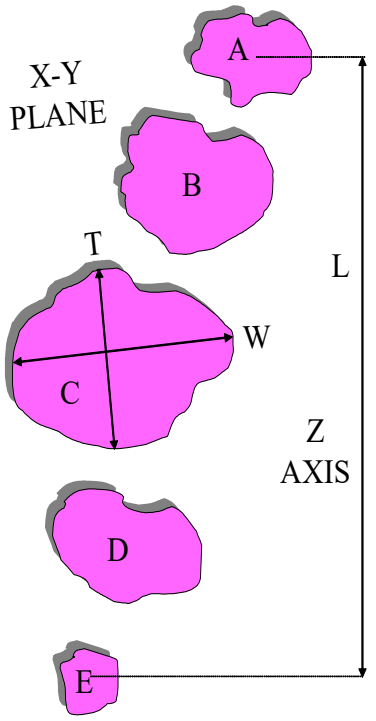
16.1.4 Bone Scan

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.

RELATIONSHIP BETWEEN CHANGE IN SINGLE DIAMETER (RECIST), PRODUCT OF TWO DIAMETERS (WHO), AND THREE PERPENDICULAR DIAMETERS ("VOLUME")

	Diameter, 2R	Product, $(2R)^2$	Volume, $4/3\pi R^3$
Response	**Decrease**	**Decrease**	**Decrease**
	30%	50%	65%
	50%	75%	87%
Disease Progression	**Increase**	**Increase**	**Increase**
	12%	25%	40%
	20%	44%	73%
	25%	56%	95%
	30%	69%	120%



COG GUIDELINE: TUMOR SIZE MEASUREMENT BASED ON CROSS-SECTIONAL IMAGING

A, B, C, D, & E are contiguous parallel slices in the X-Y plane (usually axial) showing the tumor

W and T are the maximal perpendicular diameters on the slice (C in this example) showing the largest surface area

Tumor length in the Z-axis (L) (perpendicular to X-Y plane) can be obtained either by the [a] (difference in table position of the first and last slices showing the tumor *plus* one *slice* thickness), or [b] the product of ([slice thickness + gap] and the number of slices showing the tumor) *minus* one *gap* distance

- WHO criteria: TxW is used
- RECIST: the larger of the two (T & W) is used (W in this example)
- Elliptical model volume=0.5 LxWxT
- The same modality and measurement method used in the initial imaging should be used in follow ups

Target lesions at baseline must measure greater than 1 cm; if these target lesions decrease in size to below 1 cm, care should be taken in measuring and inadvertently progressing a patient due to minimal changes in measurement from a nadir value below 1 cm, which may be within measurement error. When multiple primary or metastatic masses are present, all masses will be described. However, up to 5 target masses should be measured, using the same method in subsequent follow ups.

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 AOST1321 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 AOST1321
- Click on the Site Registration Documents link

Requirements for AOST1321 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)

Submitting Regulatory Documents:

Submit required forms and documents 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 AOST1321
(for subjects from 11 to 17 years of age)

A trial using denosumab to treat osteosarcoma that has not responded to treatment or 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. 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. 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 a new drug to treat recurrent osteosarcoma. We do not know how well the new drug will work in children, teens and young adults. That is why we are doing this study.

3. Children and teens and young adults who are part of this study will be given a new drug. You will also have imaging tests to see if the cancer is getting worse, staying the same or getting better. There is a possibility that you will also have surgery or radiation therapy (high energy X-rays) while you are part of this study. Surgery and radiation therapy are often used to treat osteosarcoma and they are not experimental.

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 you are able to get rid of the cancer. 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. 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. Together you can decide if you want to take part in the study or not. 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 trying to learn how child and teenage bodies handle the new drug denosumab. We also want to see if there are ways to tell how the cancer will respond to treatment. To do this, we will collect extra blood, urine and tumor samples from you for research tests. Most of these samples will be taken when other standard blood draws, urine collection or tumor surgery are being performed but there may be extra blood draws. These additional blood tests may help children, teens and adults who receive this drug in the future.

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