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A PHASE II TRIAL OF AVELUMAB, A FULLY HUMAN ANTIBODY THAT TARGETS CELLS EXPRESSING PD-L1 IN PATIENTS WITH RECURRENT OR PROGRESSIVE OSTEOSARCOMA

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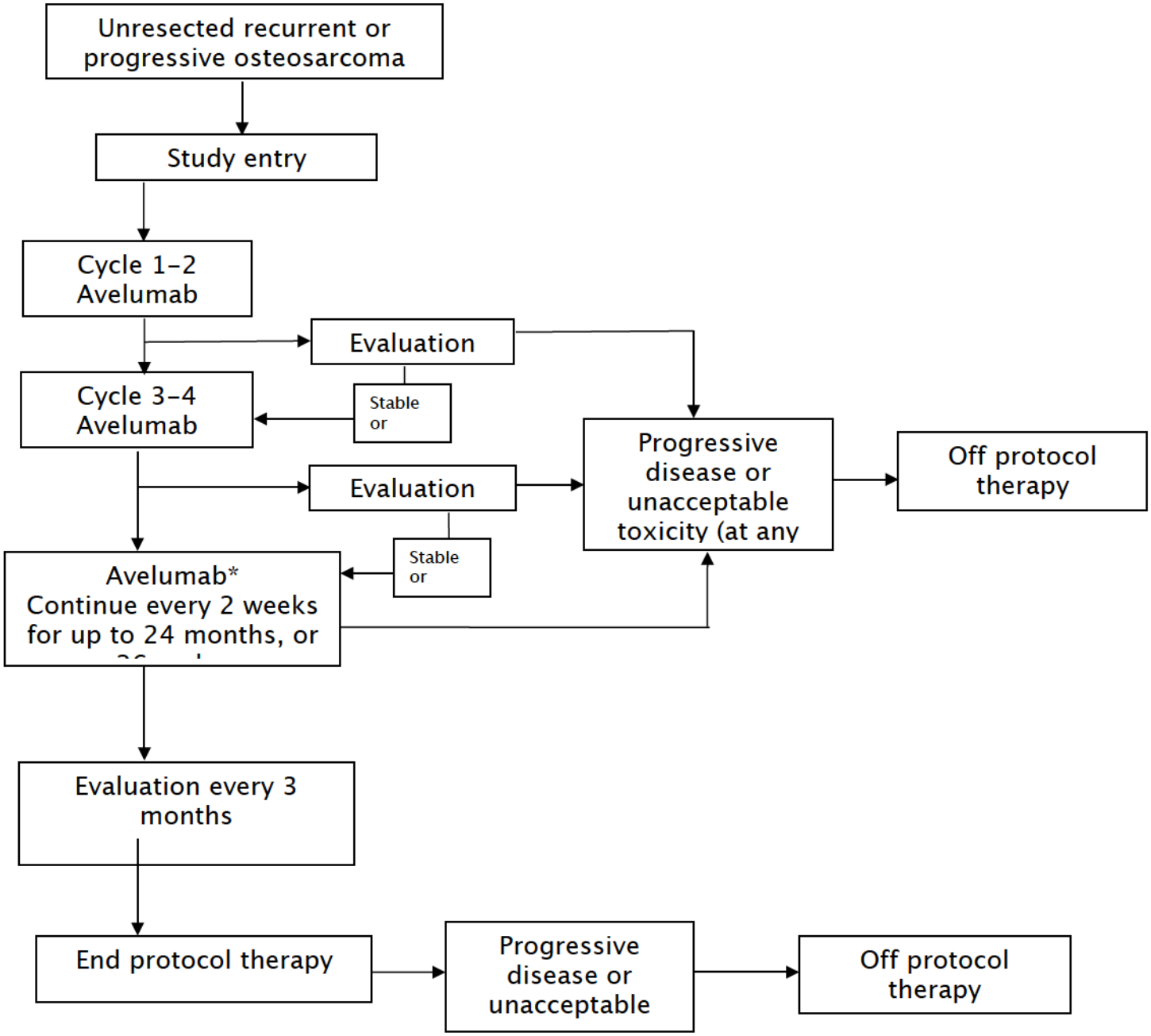
PROTOCOL SUMMARY

OSTPDL1, A Phase II Trial of Avelumab, a Fully Human Antibody that Targets Cells Expressing PDL1 in Patients with Recurrent or Progressive Osteosarcoma
Principal Investigator: Michael W. Bishop, MD, MS
IND holder: St. Jude Children's Research Hospital, IND# 132,874.
Brief background and overview of study: Patients with recurrent or progressive osteosarcoma have a poor overall survival rate and are unlikely to achieve cure without complete surgical remission. The introduction of new cytotoxic and biologic agents has failed to show efficacy in this population. Immunotherapy with checkpoint inhibitors has demonstrated dramatic changes in outcomes in several adult cancers and may provide greater benefit for tumors with high mutational load. The purpose of this phase II study is to assess the efficacy of avelumab for the treatment of patients with recurrent or progressive osteosarcoma who have measurable residual disease.
Intervention: Single agent avelumab.
Brief outline of treatment plan: Patients will receive avelumab every 2 weeks in cycles of 28 days for up to 24 months, or 26 cycles. Progression free survival and response to therapy after 4 cycles of treatment will be assessed. In addition, the toxicity profile of avelumab in this population will be closely monitored.
Study design: Multi-center phase II study.
Sample size: 32 evaluable patients; 40 total patients.
Data management: Data management and statistical analysis will be provided locally by the Comprehensive Cancer Center Developmental Biology Solid Tumors Program, and Biostatistics Department at St. Jude Children's Research Hospital.
Human subjects: The main risk to research participants will be the potential toxicities associated with the use of the investigational agent, avelumab. Research participants will be informed of toxicities which have been associated with the study drug and potential side effects of procedures recommended in this study. Adverse events will be monitored, treated, and reported following institutional and federal guidelines and regulations.

EXPERIMENTAL DESIGN SCHEMA



**Avelumab will be given on Days 1 & 15 of each 28 day cycle*

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1.3.3 To evaluate the role of T-cells in immune checkpoint blockade via measures of cell proliferation, co-inhibitory receptor expression on CD8 T cells, T cell repertoire, and epigenetic programming.

1.3.4 To assess the quality of life of patients with recurrent or progressive osteosarcoma undergoing treatment with avelumab, and to explore relationships between clinical factors and patient-reported HRQOL outcomes.

2.0 BACKGROUND AND RATIONALE

2.1 Background

Osteosarcoma is the most common bone tumor in children and adolescents. It is estimated there are approximately 400 new cases of pediatric osteosarcoma diagnosed in the United States each year.[1] Nearly 70% of children who are treated with osteosarcoma with current therapies that include multimodal chemotherapy with high dose methotrexate, doxorubicin, and cisplatin (MAP) and surgery can be cured from their disease.[2] However, patients who present with metastatic disease or relapse continue to fare poorly with estimated survival rates that do not

exceed 30%.[3–6] Furthermore, for patients who relapse, cure is almost impossible if they cannot be deemed surgically free of disease.[3] This observation supports the need for identification of active agents that can facilitate removal of all gross tumor in the event of a relapse. In a study by the German Cooperative Osteosarcoma Study Group, the overall 5-year survival after a first relapse of osteosarcoma was 23% and the survival for patients who did not have a second surgical complete response was 0%.[3] In a report from the same group, five year overall and event free survival rates for patients who experienced a second or subsequent relapses were only 32% and 18% at the time of second recurrence and 25% and 0% for those who experienced a third recurrence.[7] Other studies have shown similar results. For example, in a report of 235 patients treated at the Rizzoli Institute, 5 year post-relapse event-free and overall survival were 27% and 28%, respectively.[8] Strategies to improve the outcome of patients with metastatic disease and recurrent disease have been largely ineffective. In a study of 43 patients with osteosarcoma who experienced a first pulmonary recurrence and who were treated with inhaled GM-CSF, the 3-year event free and overall survival were 7.8 and 35.4% respectively.[9] In another study that utilized R1507, a monoclonal antibody against the insulin-like growth factor-1 receptor, only 2 of 38 patients with osteosarcoma experienced an objective response and the median time to progression was 5.7 weeks.[10] Recently, an analysis was conducted to assess the outcomes for children with recurrent or refractory osteosarcoma who were enrolled in a series of phase II studies within the Children's Oncology Group (COG) from 1997 to 2007. These studies included the agents topotecan, imatinib, oxaliplatin, ixabepilone, docetaxel, irinotecan and rebeccamycin. Radiographic responses were seen in only 3 trials with the highest response rate being 11% in patients who were treated with topotecan. When pooled across all 7 studies, event free survival for 96 osteosarcoma subjects with measurable disease was 12% at 4 months (95% CI 6.0%–19%).[11] This value is now being used to define a benchmark for activity of new compounds in the COG and is being incorporated into the design of phase II trials for recurrent osteosarcoma. Using this newly adopted endpoint, the COG recently conducted a study of the halichondrin B analog eribulin mesylate for patients with metastatic and recurrent osteosarcoma. This agent was selected as a result of preclinical observations of the Pediatric Preclinical Testing Program of the National Cancer Institute (NCI).[12] Despite very rapid accrual of 19 patients over a 4 month period, the efficacy endpoint described above was not met and the study is closing. However, the rapid enrollment of patients with recurrent osteosarcoma reflect the urgent need to develop novel therapies that could be incorporated not only in the relapsed setting but in patients with metastatic or newly diagnosed disease, and the willingness of these patients and families to participate in investigations of novel agents.

Other strategies aimed at improving outcome of patients with osteosarcoma based on histologic response parameters have also been largely unsuccessful. It is well known that the degree of tumor necrosis following neoadjuvant chemotherapy is a predictor of clinical outcome in childhood osteosarcoma. Thus this parameter has been used as an indirect measure of clinical outcome and has been exploited in an attempt to improve survival of these patients, via augmentation of post-operative chemotherapy for patients whose tumors demonstrate an inadequate response to pre-operative treatment. This was one of the key objectives of the recent EURAMOS trial, an international prospective clinical trial encompassing American and European collaborative oncology groups, in which patients were stratified after surgery to receive standard or modified therapy based on histologic response to upfront treatment with MAP chemotherapy. Unfortunately, the results failed to show any significant improvement in the outcome of patients who were treated with interferon after they achieved a favorable histologic response to MAP chemotherapy, or were given additional courses of chemotherapy with ifosfamide and etoposide if they experienced a poor histologic response.[13,14]

Genomic analysis of osteosarcoma has demonstrated that this tumor is genomically complex with a large number of somatic non-silent mutations averaging 25-32 mutations per case and 1.2 mutations per megabase.[15,16] In addition, osteosarcoma has a high number of structural variations that exceeds the number seen in other pediatric tumors such as embryonal rhabdomyosarcoma.[15] The majority of osteosarcomas carry an aberration in the TP53 pathway, and 50% have hypermutable regions known as kataegis.[15] Additional recurrent gene alterations found in two studies of osteosarcoma include RB1, DLG2, and ATRX. Although approximately 24% of osteosarcoma have alterations in the PI3K/mTOR pathway characterized by variable alterations of PTEN, or mutations in TSC2, NF1, PI3KCA, AKT, or PDPK1,[16] no clinical studies targeting this pathway in osteosarcoma are currently active.

The use of immunotherapy to treat malignant diseases has been advanced in recent years with the use of checkpoint inhibitors, particularly with the advent of monoclonal antibodies directed against cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1), both immunomodulatory receptors expressed on T cells,[17] as well as the ligand for PD-1 (PD-L1) that has been identified on a number of tumor types. Dramatic responses and long term cures have been documented in patients with various adult malignancies including melanoma, non-small cell lung cancer, Hodgkin lymphoma, renal cell carcinoma, and colorectal carcinoma.[18-23] Studies using checkpoint inhibitors suggest that the response to these agents might be in part related to the expression of PD-L1 in tumor tissue; however, the combined use of CTLA-4 and PD-1 inhibitors may overcome the lower

response rates seen in patients whose tumors had negative PD-L1 expression and were treated with single agent nivolumab,[24] suggesting that PD-L1 might not be an optimal surrogate biomarker of response to these agents. In addition, it is likely that inducible PD-L1 expression appears to be more stringently correlated with PD-1 blockade response as a result of adaptive immune resistance.[25] More recently, tumor specific non-synonymous mutations have been identified as being immunogenic; the majority of this "mutanome" is recognized by CD4+ T cells. To this effect, increased mutational burden in melanoma has been associated with responses to CTLA-4 blockade; however, increased mutational load alone may not explain the benefit seen in these patients. The presence of specific unique neoepitope signatures that confer higher rates of MHC I class binding appears to be essential for achievement of clinical response.[26,27] The dramatic response to checkpoint inhibitors in patients with mutations in mismatch repair deficient colorectal cancer supports the observation that a high mutational load is associated with the number of mutation-associated antigens that can trigger an immune response against the tumor.[19]

The high mutational load in osteosarcoma makes this tumor an attractive target for immunotherapy. Recent work has demonstrated that human metastatic osteosarcoma cells express PD-L1; populations of PD-L1 positive cells were observed in 12 of 16 metastatic tumors by immunofluorescence. The presence of PD-L1 may decrease the activity of cytotoxic T lymphocytes (CTLs) expressing PD-1; blockade of PD-1/PD-L1 improved CTL function *in vitro* and *in vivo*. In a K7M2 mouse model of osteosarcoma this resulted in decreased tumor burden and prolonged survival.[28] In addition, in an implantable model of metastatic osteosarcoma, T cells infiltrating PD-L1 antibody-resistant tumors up-regulate additional inhibitory receptors, notably CTLA-4, which impairs their ability to mediate tumor rejection. Combinations of α-CTLA-4 and α-PD-L1 antibody blockade in the K7M2 mouse model of metastatic osteosarcoma produces complete control of tumors in a majority of mice as well as immunity to further tumor inoculation.[29] In another study an RNA-based assay to determine PDL1 expression levels showed 32 of 38 osteosarcoma tumors expressed PDL1; high levels (3 log or more) of PDL1 were expressed in 9 tumor samples. PDL1 expression was positively correlated with tumor infiltrating lymphocytes (TILs).[30] While data is limited on the clinical activity of PD-1 or PD-L1 inhibition in patients with osteosarcoma, a recent report from a clinical trial utilizing the Merck PD-1 inhibitor pembrolizumab in patients with advanced bone and soft tissue sarcomas demonstrated a partial response in 1 of 19 enrolled osteosarcoma subjects at 8 weeks; limited data on progression free survival was available.[31]

2.2 Preclinical Studies

2.2.1 Avelumab

Avelumab (also referred to as MSB0010718C) is a fully human IgG-1 antibody which is directed against PD-L1. Avelumab binds PD-L1 and blocks the interaction between PD-L1 and its receptors PD-1 and B7-1. The interaction between PD-L1 and PD-1/B7-1 significantly inhibits activity of T cells. While PD-L1 can be detected on resting and activated T cells, B cells, macrophages, dendritic cells and mast cells, tumor cells may also express high levels of PD-L1 on their surface compared with normal tissues.[32] High levels of PD-L1 expression have been found to be associated with disease progression, increased metastasis, poor response to treatment, and decreased survival in a number of human cancers.[33] Blockade of PD-L1 removes the suppressive effects of PD-L1 on anti-tumor CD8+ T cells, resulting in the restoration of cytotoxic T cell response and elimination of tumor cells. Furthermore, as a fully human IgG1 monoclonal antibody, avelumab has the potential to facilitate tumor cell killing via tumor-directed antibody driven cellular cytotoxicity (ADCC).

2.2.2 Preclinical Pharmacology Studies

Avelumab functionally enhances T cell activation *in vitro* and significantly inhibits the growth of PD-L1 expressing tumors *in vivo*. Avelumab binds to human PD-L1 with a high affinity of 0.7 nM and competitively blocks the interaction of PD-L1 with PD-1. *In vitro* study results showed that by binding to PD-L1, avelumab effectively enhances T cell activation as measured by interleukin-2 (IL-2) or interferon-gamma (IFN-γ) production. As a monotherapy, avelumab demonstrated anti-tumor activity against murine MC38 colon carcinoma tumors characterized by high level of PD-L1 expression with an observed dose-dependent trend when given every third day for a total of 3 doses. Dosing was determined to be 400 µg, approximating to 20 mg/kg. *In vivo* anti-tumor effects were primarily mediated by CD8+ T cells as evidenced by observation that *in vivo* depletion of CD8+ T cells abrogated anti-tumor efficacy. The contribution of ADCC as a mechanism of anti-tumor activity was demonstrated via use of a deglycosylated version of avelumab to abrogate Fc receptor binding, and via systemic depletion of natural killer (NK) cells. In both settings, loss of *in vivo* ADCC potential significantly reduced anti-tumor activity.

The combination of avelumab with commonly used cancer treatments, such as cytotoxic agents and radiation therapy, resulted in improved anti-tumor activity. The most promising combination partners for avelumab were gemcitabine or 5-fluorouracil and oxaliplatin, the core components of FOLFOX. Radiation therapy was found to be highly synergistic with avelumab, capable of causing complete regression of established tumors.

Various immunomonitoring assays were incorporated into the *in vivo* studies. Treatment with avelumab resulted in a consistent increase in the percentage of CD8+PD-1+ T cells and an increased frequency of CD8+ T cells with an effector memory (TEM) phenotype as determined by flow cytometry. These changes correlated with anti-tumor effect. Increases in tumor antigen-specific T cell responses, as measured by enzyme-linked immunosorbent spot and pentamer immunoassays, were evident following treatment with avelumab. These responses were enhanced when combined with FOLFOX or radiation. This has led to speculation that increases in CD8+PD-1+ T cells, CD8+ TEM cells, and antigen-specific T cell responses may be leveraged as pharmacodynamic biomarkers with translational relevance to the clinical setting

2.2.3 Preclinical Pharmacokinetic Studies

Avelumab demonstrated pronounced non-linear pharmacokinetic (PK) characteristics in mice and monkeys in single dose studies at doses below 20 mg/kg, suggesting a combination of first order catabolic clearance and saturable target-mediated clearance. Toxicokinetic data from repeated dose toxicity studies in mice, rats and monkeys indicated that the PK of avelumab was linear within the dose range of 20 to 140mg/kg, suggesting that the target mediated clearance could be saturated when higher doses are administered. Similar terminal half-lives of approximately 60 to 70 hours were observed in toxicity studies in mice and monkeys. A PK/pharmacodynamic (PD) study in C57BL/6 mice was used to correlate receptor occupancy data of avelumab in blood with drug combinations. A plasma concentration of 58.5 µg/mL was calculated as required for 95% target occupancy in this model.

Avelumab is immunogenic in mice, rats, and monkeys with a lower incidence of anti-drug antibodies (ADAs) at higher doses. The latter is probably due to interference of free avelumab with the immunogenicity assay (drug interference). In animals, the generated ADAs seem to have the potential to increase the clearance of the avelumab. As the fully human avelumab represents a foreign protein to the immune system of animals, the observed immunogenicity of avelumab in rodents and non-human primates is not deemed predictive for an immune response to avelumab in humans.

2.2.4 Preclinical Toxicology Studies

The toxicological profile of avelumab was evaluated in repeat-dose toxicity studies of 4-week duration with once weekly IV bolus injection/infusion of avelumab in mice, rats, and cynomolgus monkeys. A repeat-dose toxicity study with intermittent once weekly IV infusion of avelumab over 13 weeks followed by an 8-week recovery period in cynomolgus monkeys was also conducted. In addition, *in vitro* cytokine

release assays (CRA) in human and cynomolgus monkey whole blood and peripheral blood mononuclear cells (PBMCs) followed by an optimized CRA in phyto-hemagglutinin (PHA) pre-stimulated PBMCs from 16 human volunteers was completed. Tissue cross reactivity (TCR) studies in normal human and cynomolgus monkey tissues have also been performed.

On the basis of the binding affinity data, the cynomolgus monkey and the mouse were selected as relevant species for the nonclinical safety testing of avelumab. Due to severe hypersensitivity reactions after repeated administration of avelumab in mice and the low binding affinity in rats, rodent species are not considered appropriate for nonclinical safety testing of avelumab and therefore, a single species approach (cynomolgus monkey only) is applied.

In cynomolgus monkeys, clinical signs of hypersensitivity have not been seen after repeated treatment with avelumab at dose levels of 20, 60, and 140 mg/kg, respectively, in either the pilot 4-week study or in the pivotal 13-week IV repeat-dose toxicity study. For both studies, a no observed adverse effect level (NOAEL) of 140 mg/kg for systemic toxicity was established.

Initial cytokine release assays in human and cynomolgus monkey whole blood and PBMCs revealed no clear-cut evidence for release of pro-inflammatory cytokines. However, a subsequent, optimized CRA demonstrated evidence of potential cytokine release in PHA pre-stimulated PBMCs. However, clinical experience has demonstrated that the relative risk of an infusion reaction is low (approximately 2%) and with pre-medication, the incidence of infusion reaction drops to less than 1%, indicating that the optimized CRA overestimates the risk of cytokine release in the clinical setting.

2.3 Clinical Studies

2.3.1 Adult Studies

Clinical data regarding pharmacokinetics and safety for avelumab is available from four ongoing clinical trials for the treatment of adult metastatic or locally advanced solid tumors, across Phases I, II (EMR 100070-003), and III. EMR 100070-001 is a Phase I open label multiple ascending dose trial with two parts: a dose escalation cohort with a 3+3 enrollment design to determine the maximum tolerated dose (MTD) of avelumab with dose levels of 1, 3, 10 and 20 mg/kg every 2 weeks, and an expansion cohort for 16 different tumor subtypes. EMR 100070-002 is a Phase I trial to investigate the tolerability, safety, pharmacokinetics (PKs), biological and clinical activity of avelumab in Japanese patients with metastatic or locally advanced solid tumors with an expansion cohort for Asian patients with gastric cancer, and was conducted in a similar dose

escalation design as EMR 100070-001 with dose levels of 3, 10 and 20 mg/kg every 2 weeks. EMR 100070-003 is a Phase II, single arm, open-label trial investigating the activity and safety of avelumab in subjects with Merkel cell carcinoma. EMR 100070-004 is a Phase III open-label trial of avelumab versus docetaxel in subjects with NSCLC that has progressed after platinum-doublet containing therapy.

Data for dose escalation and PKs are available for Phase 1 trials EMR 100070-001 and EMR 100070-002. For EMR 100070-001, 53 subjects were included in the dose escalation cohort. Half-lives were 69, 85, 102 and 120hours for each dose level, respectively. C_{max} and AUC increased more than proportionally to dose between 1 to 10 mg/kg, but proportionally for doses above 10 mg/kg. Given no significant difference between the 10 mg/kg and 20 mg/kg groups, this PK characteristic suggests that target mediated drug disposition is involved in the clearance of avelumab and a high PD-L1 target occupancy (TO) is likely achieved at the trough concentrations for doses of 10 mg/kg. Flow cytometry of peripheral blood CD3+ T cells in healthy volunteers observed a concentration of 1 µg/mL avelumab required for >95% TO; based on trough serum levels observed in EMR 100070-001, all subjects receiving 10 mg/kg were projected to reach or exceed >95% TO throughout the entire dosing interval. The MTD was not reached. The dose escalation cohort was completed and a dose of 10 mg/kg every 2 weeks was determined to be the biologically optimal dose for further evaluation in the expansion portion. Similar results were seen for PKs in the Phase I trial EMR 100070-002. C_{max} and AUC increased proportional with dose, and half-lives were 92, 127, and 115 hours for each dose level, respectively.

Safety data are available for 1300 subjects treated in the pooled treatment expansion cohorts of EMR 100070-001, 52 subjects in the ongoing EMR 100070-002, 88 subjects in the ongoing Phase II trial EMR 100070-003, and 75 patients treated on the ongoing Phase III trial EMR 100070-004. For EMR 100070-001, drug-related treatment-emergent adverse events (TEAEs; all grades) occurred in 62.5% of patients; the most frequent treatment-related TEAEs were fatigue (16.3%), infusion-related reaction (IRR) (16.1%), nausea (8.3%), chills (7.8%), diarrhea (6.1%), and pyrexia (5.5%). Drug-related TEAEs ≥ grade 3 were reported in 124 patients (9.5%) in the pooled expansion cohort of EMR 100070-001; the most common of these were increased gamma-glutamyl transferase (GGT) (0.7%) and IRR (0.7%). Other Grade $\geq$ 3 treatment-related TEAEs included elevated lipase, fatigue, anemia, dyspnea, increased transaminases, pneumonitis and autoimmune hepatitis. Potentially immune-mediated AEs were observed in 7.6%, including hypothyroidism (3.5%), pneumonitis (1%), hyperthyroidism (0.5%), adrenal insufficiency and dry eye (0.4%), autoimmune hepatitis and colitis (0.3%), and myositis (0.2%). 79 subjects (6.1%) had a drug-related TEAE leading to treatment

discontinuation. 25 (1.9%) of these were due to an infusion-related reaction; 5 (0.4%) were discontinued for increased GGT levels. Other events resulting in discontinuation (each in 3 patients or less) included elevated transaminases, increased creatine phosphokinase, increased lipase, arthralgia, autoimmune hepatitis, colitis, dyspnea, fatigue, and myositis. 142 patients (10.9%) experienced a TEAE leading to death; 5 (0.4%) of these were determined to be related to treatment. These adverse events included radiation pneumonitis, pneumonitis, respiratory distress, acute hepatic failure, and autoimmune hepatitis with resultant fatal liver failure.

For the 52 subjects with available safety data on EMR 100070-002, 19 serious TEAEs were experienced in 15 subjects, 14 of which were Grade 3 or higher. Eleven subjects experienced 13 IRR events, 12 of which were deemed non-serious; the lone serious IRR was related to subsequent chemotherapy after trial discontinuation and not avelumab. 3 potential immune related adverse events included hypothyroidism, hyperthyroidism and colitis. 3 subjects had TEAEs resulting in fatal outcome, one of which was treatment-related (grade 5 tumor lysis syndrome, grade 5 acute kidney injury). For 88 patients enrolled on the Phase II trial EMR 100070-003, 78 serious TEAEs were reported in 35 subjects; 57 Grade $\geq$ 3 events were reported. Eight treatment-related TEAEs were identified in 6 subjects including chondrocalcinosis, enterocolitis, synovitis, increased transaminases, tubulointerstitial nephritis, hypothyroidism, and IRR; all were grade 2 events. 2 subjects had potential immune related adverse events including hypothyroidism and grade 2 encephalopathy (not related to treatment). Preliminary safety data is available for the Phase III trial EMR 100070-004, with approximately 75 patients randomized to receive avelumab and an additional 75 receiving docetaxel. Safety data is blinded and assignment of events to either drug is not possible. A total of 70 serious TEAEs are reported in 39 patients, including 25 Grade $\geq$ 3 treatment related events. Six grade 4 treatment-related serious TEAEs were observed in 4 subjects including infusion related reaction, aortic aneurysm, decreased neutrophil count, intestinal perforation, peritonitis, and paralytic ileus. Fatal TEAEs occurred in 8 subjects, including disease progression (4), COPD/dyspnea, general physical health deterioration, and respiratory failure. Two fatal serious TEAEs (septic shock, pneumonia) in one subject were considered treatment-related.

The safety profile of avelumab is consistent with findings reported for other anti-PD-1 or anti-PD-L1 antibodies. Infusion-related reactions including hypersensitivity and immune-related AEs/autoimmune disorders have been identified as important risks for avelumab. Respective risk mitigation measures have been implemented in ongoing clinical studies with avelumab.

Clinical efficacy information of avelumab is based on data from non-small cell lung cancer (NSCLC) and ovarian cancer expansion cohorts in the ongoing Phase I trial EMR 100070-001, and for 20 subjects in the gastric cancer expansion cohort of the ongoing Phase I trial EMR 100070-002. The objective response rate (ORR) for subjects treated in the NSCLC cohort was 14.1% (26/184). Nineteen of 26 (73%) responders had their first documented response by week 12, but 7 (27%) demonstrated response by week 18 or later, suggesting a role for longer follow-up duration. The median PFS and OS for the cohort were 11.6 weeks and 8.4 months, respectively. Clinical activity of avelumab was also evaluated retrospectively by tumor PD-L1 expression. An objective response was observed in 20 of 122 subjects (16.4%) who were PD-L1 positive (defined as having at least 1% PD-L1 positive tumor cells) compared with 2 of 20 subjects (10%) who were considered PD-L1 negative (defined as having less than 1% PD-L1 positive tumor cells). A longer median PFS (12 vs. 5.9 weeks) and OS (8.9 vs. 4.6 months) were both observed in PD-L1 positive compared with PD-L1 negative subjects. For the ovarian cancer expansion cohort, the objective response rate based on confirmed and unconfirmed responses for treated subjects was 10.7% (8 of 75 subjects). The median PFS for the ovarian cancer cohort was 11.4 weeks. Preliminary efficacy data for gastric cancer patients on EMR 100070-002 as of a data cutoff of March 11th 2015 demonstrated 3 of 20 subjects with confirmed partial responses (PRs), with a median PFS of 11.9 weeks. The presence of human anti-human antibodies (HAHAs) were also assessed, as this may impact drug efficacy; incidence was low with 11 out of 377 subjects with post-treatment samples testing positive for the presence of HAHAs.

Currently there are additional clinical trials open or in development for avelumab in adults at various levels. These include single agent use or combinations with chemotherapies and/or immunotherapies for a variety of adult malignancies. Data are not available for these studies.

2.3.2 Pediatric Studies

There have been no pediatric phase I or II studies using avelumab that have been conducted to date. Therefore this trial will evaluate avelumab using a dosage and schedule of administration that has been shown to be safe in adults with refractory solid tumors, and we will closely monitor for toxicities in all patients. Patients with osteosarcoma are typically diagnosed in the second and third decade of life. Several other studies have allowed for the use of adult dosing to be extended to patients age 12 years or greater due to body surface area requirements that are more closely aligned with adult patients, including recent COG phase II studies specifically targeting patients with recurrent or progressive osteosarcoma with agents such as eribulin mesylate and glembatumumab vedotin.

2.4 Rationale for this Study

This study will evaluate avelumab in patients with osteosarcoma using dosing that has previously been determined in adult studies. Review of COG phase II trials enrolling osteosarcoma patients reveals that over 80% of enrolled patients have been age 12 years or older. Objective response using traditional criteria such as the Response Evaluation Criteria in Solid Tumors (RECIST) has proven challenging for osteosarcoma, as its dense calcified structure limits its ability to demonstrate a radiographic representation of drug activity. Using disease control (defined as extended stabilization of disease for 4 cycles of therapy or greater) as an additional endpoint may allow for the ability to detect a signal of activity. Therefore objective response will be evaluated as a primary endpoint, but stable disease control will also be monitored.

2.5 Overview of Proposed Study

This is a Phase 2 study using a traditional Simon two-stage design. Patients 12 years or greater with recurrent/refractory osteosarcoma will be administered avelumab at a dose of 10 mg/kg intravenously (IV) over 60 minutes on days 1 and 15 of each cycle, with a cycle lasting 28 days. Evaluations to assess disease control and response will be completed after cycles 2 and 4, and then will be completed every 3 cycles. Correlative biologic studies will be obtained and will include measures of immune activation, including phenotypic and functional analysis of peripheral blood mononuclear cells (PBMCs) and serum analyses of cytokines, chemokines, soluble CD-27, soluble CD40L, antibodies, and tumor-associated antigens.

In addition to assessment of disease control, response assessments will use standard RECIST criteria. Determination of progression will use measurements according to RECIST criteria at 16 weeks.

2.6 Rationale for Correlative Biology Studies

As an IgG-1 antibody targeting PD-L1, avelumab may potentially cause anti-tumor activity through inhibition of the PD-1/PD-L1 interaction and resultant removal of inhibition of the immune system to target tumor cells, or may be related to ADCC properties; therefore the mechanism of action of avelumab is not fully understood. Therefore, correlative biology studies will investigate the activation status of the immune system through evaluation of serum markers at baseline and after treatment with avelumab. This will include cytokines (IFN-γ, IL-10, IL-12, IL-2, IL-4, TGF-β), chemokines, soluble CD27, soluble CD40 ligand, and tumor-associated antigens. Proliferation of 123 unique subsets of PBMCs will be evaluated by flow cytometry at baseline and after exposure to avelumab.

Increased numbers of mutations have been associated with of tumors responsive to immune blockade treatment (including CTLA-4 and PD-1). The proposed mechanism of action in immune blockade therapy is the activation of host CD4 and CD8 T cell responses against tumor antigens, with tumor neoantigens considered to be the primary targets of efficacious responses.[27,34] In order for a tumor mutation to generate a neoantigen, it must occur within a peptide context that can bind to a host major histocompatibility complex (MHC). Further, this mutation-bearing peptide still needs to be processed and presented efficiently and elicit a cognate T cell response. Therefore, it is expected that tumors with high numbers of mutations are more likely to generate individual mutations that meet all the requirements to be immunogenic neoepitopes. In the small number of studies that have been conducted using computationally predicted neoepitopes, ~2-10% of the predicted epitopes were confirmed to have elicited detectable T cell responses by tetramer staining, with some variation likely due to differences in the method of prediction.[35]

Host T cells play a critical role in controlling many forms of cancer, but can progressively lose their anti-tumor functions during prolonged exposure to high levels of antigen and inflammation.[36] Repression of CD8 T cell effector functions, commonly referred to as T cell exhaustion, is a major obstacle for current therapeutic strategies aimed at utilizing the host immune system.[37] Acquisition of the functionally exhausted state in antigen-specific T cells is controlled by the expression of inhibitory receptors.[38,39] These recent advances in our mechanistic understanding of T cell exhaustion have led to immune checkpoint blockade therapies that reverse the exhausted state by blocking the ligation of inhibitory receptors expressed on the surface of tumor-specific CD8 T cells.[40] It is now apparent that the development of the fully exhausted phenotype is correlated to the successive up-regulation of multiple inhibitory receptors, including co-expression of PD-1 with Lag3, 2B4, and Tim3.[41,42] Simultaneous expression of all four receptors identifies a fully exhausted cell, while cells that express a smaller subset of these receptors might still retain some function. After checkpoint blockade, the loss of inhibitory receptor expression will serve as correlate of rescued functionality.

Studies have also demonstrated that increased PD-1 expression can be used as a surrogate marker for cells exposed to antigen, which has recently been employed as a strategy to identify tumor-specific CD8 T cells[43]; in this work, all tumor-specific cells expressed PD-1. Few data are available regarding the percentage of CD8+ T cells that are PD-1 positive in pediatric patients with cancer; between 30 and 70% of CD8+ T cells were found to be positive for PD-1 in 27 healthy adult samples[44]. Based on the role PD-1 expression plays in T cell exhaustion, as well as its utility in identifying activated tumor-specific CD8 T cells, we will perform correlative studies to determine changes in the immunophenotypic

profile of PD-1^{high} CD8+ T cells from serial peripheral blood samples obtained before and during treatment. We will be measuring the change in the percentage of PD-1^{high} CD8+ T cells in peripheral blood samples obtained before and 6 weeks after start of therapy.

Emerging evidence indicates that immune checkpoint blockade results in expansion of a clonal population of tumor-specific cells.[45] While this expansion is groundbreaking, the oligoclonality of T cells will provide a selective pressure against specific antigens and may eventually enhance tumor escape. Therefore we must better understand the mechanisms for the restricted clonality of rejuvenated CD8 T cells in individuals that receive immune checkpoint blockade therapy in order to develop strategies the allow for a greater breadth of tumor-specific T cells to respond to immune checkpoint blockade.

Repertoire diversity has been established as a key correlate of efficacious immune responses against infections in mice and humans.[46,47] This includes diversity of epitope targets, as well as repertoire diversity within an epitope-specific response. We hypothesize that the generation of successful anti-tumor immune responses after checkpoint blockade requires the activation of diverse T cell repertoires. Conversely, the inability to recruit and reactivate a sufficiently diverse repertoire may be a key correlate of treatment failure. The characterization of the anti-tumor T cell repertoire has to date been performed by growing T cell lines, or using predictive algorithms to detect potential T cell responses based on neoepitopes. In the latter case, these are almost exclusively Class I-restricted responses, though a few reports have demonstrated a robust role for anti-tumor Class II-restricted T cells.[48,49] We propose to use an unbiased approach to define the T cell repertoire in tumor-associated T cells (where tumor tissue is available) or in antigen-exposed (PD-1^{+}) T cells from the peripheral blood of tumor patients before and after treatment with avelumab. Using single cell techniques, we will comprehensively characterize the repertoire of PD1+ cells at baseline and following treatment, with a focus on identifying clonotypes that expand after treatment. Selected clonotypes that expand following treatment will be cloned and expressed in a cell line that will report out T cell receptor activation using a Nur77-GFP construct. In patients where tumor tissue or tumor sequence is available, we will initially screen the reporter lines against the neoantigens present in the tumor, using transgenic antigen presenting cells transfected with the appropriate HLA and a synthetic construct containing all the identified neoantigens. If positive hits are identified, the specific neoantigen(s) will be characterized.

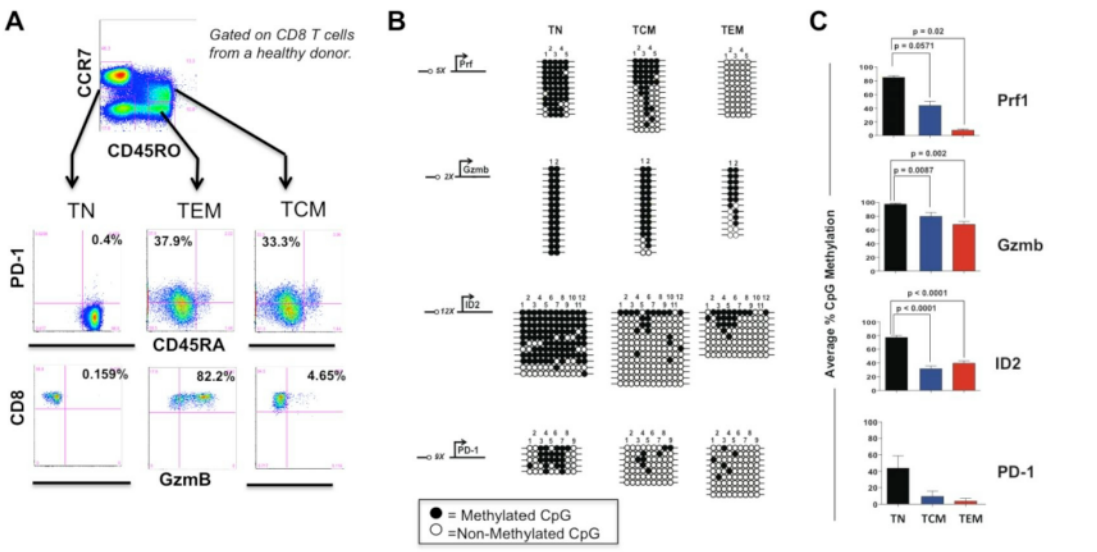

Figure 1: Differential DNA methylation programming among human naïve and memory CD8 T cell subsets is coupled to their functional state. **(A)** FACS based isolation strategy to purify naïve and memory CD8 T cells subsets from the peripheral blood of healthy individuals. TN = naïve, TEM = effector-memory, TCM = central-memory CD8 T cells. Representative FACS-based gating strategy to identify the following CD8 T cells: Naïve = $CCR7^{+}$ $CD45RO^{-}$, TEM= $CCR7^{-}$ $CD45RO^{+}$, and TCM= $CCR7^{+}$ $CD45RO^{+}$. **(B)** Bisulfite sequencing analysis of Id2, Prf1 (Perforin), PD-1, and GzmB (Granzyme B) regulatory loci from genomic DNA of FACS sorted naïve, TEM, and TCM CD8 T cells. Cartoon representation of the promoter region is depicted to the left of the representative methylation profiling for each region. Open circles represent unmethylated CpG site while closed circles represent methylated CpG. Each horizontal line represents a sequenced clone and each number represents a CpG site displayed in the cartoon representation of transcriptional start. **(C)** Graphical summary for the average % CpG

Several studies have demonstrated that CD8 T cell exhaustion gene expression programs can become reinforced and heritably maintained.[50-52] Transcriptional memory is often mediated by epigenetic modification to the genome, therefore we hypothesize that acquired exhaustion-specific epigenetic programs must be erased in order for PD-1 blockade to be effective on all exhausted T cells. In support of this hypothesis, we have recently determined that conditional deletion of the DNA methyltransferase 3a (Dnmt3a cKO) inhibits acquisition of de novo DNA methylation programs in antigen-specific CD8 T cells and prevents T cell exhaustion during chronic viral infection (Youngblood B, unpublished data). Importantly, Dnmt3a deficient antigen-specific CD8 T cells retain their ability to mount a polyclonal effector response, and ultimately control the chronic pathogen. Moreover, Dnmt3a cKO cells remain resistant to T cell exhaustion even when the cKO cells are forced to persist in an environment that has artificially high levels of antigen for several months. Our data serve as proof of principle that acquired epigenetic programs promote T cell exhaustion, and have significant implications for generating a greater breadth of long-lived tumor-specific T cell responses after immune therapy. We have recently developed protocols for performing whole-genome methylation analysis using low input genomic DNA. We have applied this methodology to

determine the epigenetic signature of functional human memory CD8 T cells (Figure 1). From our whole-genome analysis we have identified differentially methylated regions among the T cell subsets. We have developed a PCR based assay to measure the epigenetic signature of human memory CD8 T cell subsets (Figure 1B & 1C). These data provide insight into the poised state for expression of critical effector molecules and inhibitory receptors among the memory CD8 T cell subsets. We propose to perform loci-specific and whole genome bisulfite sequencing methylation analysis of PD-1+ CD8 T cells from patients before and after therapy with avelumab to identify epigenetic programs that are coupled to the ability of host T cells to be rejuvenated following PD-1 blockade therapy.

2.7 Rationale for Quality of Life Studies

Cancer and its treatment can cause medical and psychosocial complications that adversely affect a patient's quality of life (physical, emotional, social and cognitive functioning) during and after treatment. Once complications and their outcomes are specified and measured, interventions designed to prevent or diminish the complications can be developed, implemented in clinical care, and their effects evaluated. Until recently, the majority of studies evaluating quality of life in patients with childhood cancer had been conducted in survivorship populations.[53-56] Many of these studies included single time points of assessments, cross-sectional designs, inclusion of only one or a limited number of quality of life domains, or included a heterogeneous sample not specific to any diagnosis.[57,58] More recently, several studies have emerged implementing quality of life measures during active treatment.[59-63] The majority of reports of quality of life in osteosarcoma patients tend to involve long-term survivors rather than patients who are actively undergoing treatment.[64-66] Two studies included more than a single data point and the selected time points varied, ranging from end of treatment to 8 years after the end of treatment or at the time of definitive surgery and then annually for the next three years.[67,68]

A number of additional studies exist which include patients with osteosarcoma along with a variety of other diagnoses, or include patients with both pediatric and adult osteosarcoma; data are reported in group fashion and it is impossible to extract scores specific to patients with osteosarcoma.[69-78] A prospective study of patients with high grade, localized osteosarcoma evaluated health-related quality of life (HRQOL) and symptom distress at four time points including diagnosis, prior to definitive surgery, approximately 2 months post-surgery, and 3 to 6 months after completion of treatment. Significant improvements in most domains and decreases in symptom distress were demonstrated across time points during therapy.[63] The international collaborative EURAMOS trial for newly diagnosed osteosarcoma patients included self- and

parent-reported quality of life measures using the EORTC QLQ-C30 questionnaire; results have not yet been reported.[79] Repeated quality of life measurements that are linked to specific clinical indicators may result in more clinically useful and interpretable findings. Furthermore, little to no data exist examining health-related quality of life measures in patients with recurrent osteosarcoma. Better understanding of HRQOL outcomes may identify opportunities to improve treatment-related morbidities, facilitate decision making, establish effective communication strategies and improve satisfaction with care for patients and their families.[80,81] In the setting of a disease which has previously demonstrated poor prognosis after progression or recurrence, HRQOL measures may aid in the identification of reasonable therapeutic approaches that allow for optimization of patients' quality of life in balance with treatment goals.

2.7.1 Patient Reported Outcome Measurement Information System (PROMIS) – Pediatric and Adult Profiles

The PROMIS is a set of questionnaires utilized to assess patient reported outcomes (PROs) consistent with goals established by the National Institute of Health (NIH). The PROMIS Pediatric Profile (self-report) is validated for use with individuals ages 8 to 17 years, and the PROMIS Adult Profile (self-report) is validated for individuals ages 18 years and older. Both the PROMIS Pediatric and Adult Profiles include PROs across a number of domains including: 1) physical function mobility, 2) anxiety, 3) depressive symptoms, 4) fatigue, 5) peer relationships, and 6) pain interference. On domains other than the physical function mobility domain, respondents rate how often statements describe their functioning. For the physical function mobility domain, participants use a 5-point Likert rating scale to rate how much difficulty is experienced with engagement in various activities. Scoring tables are then used to convert raw scores for each domain into age-normed T-scores. The PROMIS measures have demonstrated strong psychometric properties.[82] Literature is available for its use in pediatric oncology research,[83,84] as well as in adults with lower extremity bone metastases.[85] The PROMIS measures have shown known-group validity with ability to distinguish subgroups of children with a chronic health condition from other conditions as well as general population samples.[86,87] Additionally, the ability to determine a clinical minimally important difference has been established with the PROMIS questionnaires, which has been a methodological limitation of other HRQOL instruments.[88]

Patients will complete the PROMIS Pediatric or Adult Profile (based on their age at time of administration) at four time points during the study: at diagnosis prior to the start of treatment, at the end of cycle 2 (8 weeks $\pm$ 1 week), at the end of cycle 4 (16 weeks $\pm$ 1 week), and at end of avelumab therapy. Mixed effect models will be performed to assess changes in quality of life domains over time and to assess for impact of clinical factors on scores in quality of life domains.

3.0 ELIGIBILITY CRITERIA AND STUDY ENROLLMENT

According to institutional and NIH policy, the study will accession research participants regardless of gender and ethnic background. Institutional experience confirms broad representation in this regard.

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 7 days at the start of therapy. 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) and serum creatinine. Imaging studies, where applicable, must be obtained within 2 weeks prior to enrollment.

3.1 Inclusion Criteria

3.1.1 Patients must be ≥ 12 years of age but < 50 years of age at the time of enrollment.

3.1.2 Patients must have histologic verification of osteosarcoma at initial diagnosis or relapse.

3.1.3 Patients must have had evidence of having relapsed, progressed or become refractory to conventional therapy.

3.1.4 Patients must have measurable disease, documented by clinical, radiographic or histologic criteria. Disease must be bi-dimensionally measurable by computed tomography (CT) or magnetic resonance imaging (MRI).

3.1.5 Patients must have a performance status of ≥ 50 using the Karnofsky scale for patients > 16 years of age and the Lansky scale for patients ≤ 16 years of age.

3.1.6 Patients must have a life expectancy of ≥ 6 weeks.

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

a. Myelosuppressive chemotherapy: must not have received within 3 weeks of entry onto this study
b. Biologic (anti-neoplastic agent): at least 7 days since the completion of therapy with a biologic agent.
c. Immunotherapies: at least 42 days must have elapsed since a prior therapy that included a monoclonal antibody or any other type of immunotherapy (e.g. chimeric antigen receptor (CAR) T cell therapy).
d. Radiation therapy (RT): ≥ 2 weeks for local palliative RT (small port); ≥ 6 months must have elapsed if prior craniospinal RT or if ≥ 50% radiation of the pelvis; ≥ 6 weeks must have elapsed if other substantial bone marrow (BM) radiation.

3.1.8 Organ Function Requirements:

a. Adequate bone marrow function defined as:

- Peripheral absolute neutrophil count (ANC) ≥ 1500/mm^3
- Platelet count ≥ 100,000/mm^3 (transfusion independent)
- Hemoglobin ≥ 9.0 g/dL (may receive RBC transfusions)

b. Adequate renal function defined as:

- Creatinine clearance or radioisotope GFR ≥70 mL/min/1.73m^2 OR
- Serum creatinine based on age/gender as follows: (threshold creatinine values were derived from the Schwartz formula for estimating GFR)[89]

Age	Maximum creatinine (mg/DL)	
	Male	Female
12 to < 13 years	1.2	1.2
13 to <16 years	1.5	1.4
≥ 16 years	1.7	1.4

c. Adequate liver function defined as:

- Total Bilirubin ≤ 1.5x the institutional upper limit of normal (IULN) for age
- ALT (SGPT) and AST (SGOT) ≤ 2.5 x IULN for age (or < 5 x IULN for patients with documented metastatic disease to the liver)
- Serum albumin > 2 g/dL

d. Serum lipase ≤ upper limit of normal (IULN).

e. Patients must have documented pulse oximetry ≥ 92% on room air.

3.1.9 Female patients of childbearing potential must have a negative serum or urine pregnancy test within 7 days of enrollment.

3.1.10 Male or female patients who are sexually active and of reproductive potential must agree to use an effective contraceptive method throughout the study and for at least 60 days after last avelumab treatment administration. Abstinence is an acceptable form of contraception.

3.1.11 Patients must not currently be using other investigational agents.

3.1.12 Patients must not currently be using other anti-cancer agent.

3.1.13 Patients must be able to comply with the safety monitoring of the study in the opinion of the investigator.

3.1.14 Written, informed consent and assent following Institutional Review Board, NCI, FDA and OHRP guidelines.

3.2 Exclusion Criteria

3.2.1 Central nervous system (CNS) metastases.

3.2.2 Current use of immunosuppressive medication, EXCEPT for the following: a. intranasal, inhaled, topical steroids, or local steroid injection (e.g., intra-articular injection); b. Systemic corticosteroids at physiologic doses ≤ 10 mg/day of prednisone or equivalent; c. Steroids as premedication for hypersensitivity reactions (e.g., CT scan premedication).

3.2.3 Active autoimmune disease that might deteriorate when receiving an immuno-stimulatory agent. Patients with diabetes type I, vitiligo, psoriasis, or hypo- or hyperthyroid diseases not requiring immunosuppressive treatment are eligible.

3.2.4 Active infection requiring systemic therapy.

3.2.5 Known history of testing positive for HIV or known acquired immunodeficiency syndrome.

3.2.6 Hepatitis B virus (HBV) or hepatitis C virus (HCV) infection at screening (positive HBV surface antigen or HCV RNA if anti-HCV antibody screening test positive).

3.2.7 Patient who has received vaccination within 4 weeks of the first dose of avelumab and while on trials is prohibited except for administration of inactivated vaccines.

3.2.8 Known prior severe hypersensitivity to investigational product or any component in its formulations, including known severe hypersensitivity reactions to monoclonal antibodies (NCI CTCAE v4.03 Grade ≥ 3).

3.2.9 Clinically significant (i.e., active) cardiovascular disease: cerebral vascular accident/stroke (< 6 months prior to enrollment), myocardial infarction (< 6 months prior to enrollment), unstable angina, congestive heart failure (≥ New York Heart Association Classification Class II, see Appendix II), or serious cardiac arrhythmia requiring medication.

3.2.10 Persisting toxicity related to prior therapy (NCI CTCAE v. 4.03 Grade > 1); however, alopecia, sensory neuropathy Grade ≤ 2, or

other Grade ≤ 2 not constituting a safety risk based on investigator's judgment are acceptable

3.2.11 Other severe acute or chronic medical conditions including colitis, inflammatory bowel disease, pneumonitis, pulmonary fibrosis or psychiatric conditions including recent (within the past year) or active suicidal ideation or behavior; or laboratory abnormalities that may increase the risk associated with study participation or study treatment administration or may interfere with the interpretation of study results and, in the judgment of the investigator, would make the patient inappropriate for entry into this study.

3.2.12 Patients with active diarrhea > CTCAE v4.03 Grade 2.

3.2.13 Patients who have previously received a prior organ transplantation, including allogeneic stem cell transplantation.

3.2.14 Female patients who are pregnant or actively breastfeeding.

3.2.15 Patients who have previously received anti-PD1 or anti-PD-L1 therapy. Patients who have previously received anti-CTLA-4 therapy (e.g. ipilimumab) are eligible for study.

3.3 Recruitment and Screening

Participants will be recruited by study investigators through their clinical practice.

3.4 Enrollment at St. Jude

A member of the study team will confirm potential participant eligibility as defined in Section 3.1-3.2, complete and sign the 'Participant Eligibility Checklist'. The study team will enter the eligibility checklist information into the Patient Protocol Manager (PPM) system. Eligibility will be reviewed, and a research participant-specific consent form and assent document (where applicable) will be generated. The complete signed consent / assent form(s) must be faxed or emailed to the CPDMO at 595-6265 to complete the enrollment process.

The CPDMO is staffed 7:30 am-5:00 pm CST, Monday through Friday. A staff member from the St. Jude Cerner Millennium (MILLI) helpline is on call Saturday, Sunday, and holidays from 8:00 am to 6:00 pm. If you have a prospective research enrollment and need assistance releasing your consent, please call the MILLI helpline (901-338-0596) on call number.

3.5 Enrollment at Collaborating Sites

Collaborating Site research participants should be registered at St. Jude within 24 hours of enrollment at the site. The completed Eligibility Checklist and entire signed Informed Consent should be faxed to 901-595-6265. Please call 901-595-2568 if confirmation of the enrollment information is needed. The Protocol Eligibility Coordinator will then register the research participant in the Patient Protocol Manager (PPM) system.

4.0 TREATMENT PLAN

4.1 Overview of Treatment Plan

This is a single arm phase 2 study with a two stage design and an endpoint specific to osteosarcoma. Avelumab will be given on days 1 and 15 of each 4 week cycle. Treatment will be discontinued if there is clear evidence of progression with increase in tumor size greater than 20%, or with drug related dose limiting toxicity that requires removal from therapy, as defined in Section 10.1. Therapy may otherwise continue for a maximum duration of 24 months or 26 cycles, whichever occurs first. Initial radiographic assessments will take place after cycle 2 and cycle 4 and will be compared to imaging obtained prior to initiation of therapy. For patients who remain on protocol therapy, imaging thereafter will take place every 3rd cycle.

4.2 Treatment During Cycle 1 and Subsequent Cycles

One cycle of avelumab treatment is described below. A cycle may be repeated every 28 days if the patient has at least stable disease and has met laboratory parameters as defined in the eligibility section, and detailed below.

4.2.1 Premedication

In order to mitigate infusion-related reactions, **a premedication with an antihistamine and with paracetamol (acetaminophen) 30 to 60 minutes prior to each dose of avelumab is mandatory** (for example, 25-50 mg diphenhydramine and 500-650 mg paracetamol IV or oral). This may be modified based on local treatment standards and guidelines, as appropriate.

4.2.2 Setting

Avelumab should be administered in a setting that allows for immediate access to an intensive care unit or equivalent environment and administration of therapy for anaphylaxis, such as the ability to implement immediate resuscitation measures. Steroids (dexamethasone 10mg), epinephrine (1:1,000 dilution), allergy

medications (IV antihistamines), bronchodilators, or equivalents, and oxygen should be available for immediate access.

4.2.3 Observation Period

Following avelumab infusions, patients must be **observed for 2 hours post-infusion** for potential infusion-related reactions.

4.2.4 Avelumab Administration

Prior to avelumab infusion:

Diphenhydramine: IV over 5-15 minutes (per institutional guidelines)
Days: 1 and 15
Dose: 1 mg/kg (MAX 50mg)
Note: Equivalent antihistamine therapy may be considered.

Acetaminophen: By mouth (PO)
Days: 1 and 15
Dose: 10-15 mg/kg (MAX 1000mg)
Note: In the setting of limited PO intake and per institutional guidelines, IV acetaminophen may be considered.

Avelumab infusion:

Avelumab: Intravenously over 60 minutes
Days: 1 and 15
Dose: 10 mg/kg/dose

4.3 Criteria for Starting Subsequent Cycles

A cycle may be repeated every 28 days if the patient has again met the parameters for organ function requirements as defined in the eligibility criteria (Section 3.0), has not met any of the criteria for removal from therapy, and has not experienced a dose limiting toxicity. A delay of up to 7 days from the scheduled date of the next scheduled treatment dose may be allowed.

5.0 DOSE MODIFICATIONS FOR TOXICITIES

5.1 Adverse Drug Reactions Requiring Avelumab Discontinuation or Modification

Any Grade 4 ADRs require treatment discontinuation with avelumab except for single laboratory values out of normal range that are unlikely related to study treatment as assessed by the Investigator, do not have any clinical correlate, and resolve within 7 days with adequate medical management.

Any Grade 3 ADRs require treatment discontinuation with avelumab except for any of the following:

- Transient (≤ 6 hours) Grade 3 flu-like symptoms or fever, which is controlled with medical management
- Transient (≤ 24 hours) Grade 3 fatigue, local reactions, headache, nausea, emesis that resolves to Grade ≤ 1
- Single laboratory values out of normal range (excluding Grade ≥ 3 liver function test increase) that are unlikely related to study treatment according to the Investigator, do not have any clinical correlate, and resolve to Grade ≤ 1 within 7 days with adequate medical management
- Tumor flare phenomenon defined as local pain, irritation, or rash localized at sites of known or suspected tumor
- Grade 3 rash that resolves to Grade ≤ 1 with steroid treatment
- Change in ECOG PS to ≥ 3 that does not resolve to £ 2 within 14 days (infusions should not be given on the following cycle, if the ECOG PS is ≥ 3 on the day of study drug administration)

Any Grade 2 ADR should be managed as follows:

- If a Grade 2 ADR resolves to Grade ≤ 1 by the last day of the current cycle, treatment may continue.
- Upon the second occurrence of the same Grade 2 ADR (except for hormone insufficiencies that can be managed by replacement therapy) in the same participant, treatment with avelumab has to be permanently discontinued.

5.2 Treatment Modification for Symptoms of Infusion-Related Reactions

In order to mitigate infusion-related reactions, all participants will receive pretreatment with H1 blockers and acetaminophen 30 to 60 minutes prior to each infusion. A premedication regimen of 1 mg/kg diphenhydramine (MAX 50 mg) and 10-15mg/kg acetaminophen (MAX 1000 mg) is recommended prior to each dose of drug. Participants must be observed for 2 hours post-infusion. This regimen may be modified based on local treatment standards and guidelines as appropriate.

NCI-CTCAE Grade	**Treatment Modification for Study Drug**
Grade 1 - mild Mild transient reaction; infusion interruption not indicated; intervention not indicated.	Decrease the study drug infusion rate by 50% and monitor closely for any worsening. The total infusion time for study drug should not exceed 120 minutes.
Grade 2 - moderate Therapy or infusion interruption indicated but responds promptly to symptomatic treatment (for example, antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for £ 24 h.	Stop study drug infusion. Resume infusion at 50% of previous rate once infusion-related reaction has resolved or decreased to at least Grade 1 in severity, and monitor closely for any worsening.
Grade 3 or Grade 4 - severe or life-threatening Grade 3: Prolonged (for example, not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for clinical sequelae. Grade 4: Life-threatening consequences; urgent intervention indicated.	Stop the study drug infusion immediately and disconnect infusion tubing from the participant. Participants have to be withdrawn immediately from study drug treatment and must not receive any further study drug treatment.
· Once the avelumab infusion rate has been decreased by 50% or interrupted due to an infusion-related reaction, it must remain decreased for all subsequent infusions. · If the patient has a second infusion-related reaction Grade ≥ 2 on the slower infusion rate, the infusion should be stopped and the patient should be removed from study treatment. · If hypersensitivity reaction occurs, the patient must be treated according to the best available medical practice.	

5.2.1 Severe Hypersensitivity Reaction

Hypersensitivity reactions must be treated according to best medical practices. Patients should also be instructed to report any delayed reactions to the investigator immediately:

a. Symptoms: Impaired airway, decreased oxygen saturation (<92%), confusion, lethargy, hypotension, pale/clammy skin, cyanosis
b. Management: epinephrine injection, dexamethasone (or equivalent) infusion, continuous monitoring, and potential ICU management

Patients who experience an acute severe hypersensitivity reaction should no longer receive avelumab therapy.

5.2.2 Influenza-Like Symptoms

For prophylaxis of influenza-like symptoms, patients may receive a non-steroidal anti-inflammatory drug (NSAID) such as ibuprofen 10mg/kg (MAX 800 mg) or naproxen sodium 10mg/kg (MAX 500 mg) may be administered 2 hours before and 8 hours after the start of each avelumab infusion.

5.2.3 Tumor Lysis Syndrome

Because avelumab can induce ADCC, there is a potential risk of tumor lysis syndrome. Should this occur, participants should be treated as per local guidelines for tumor lysis syndrome including IV fluids, frequent monitoring of electrolytes (uric acid, K^+, Ca^{++}, PO_4), and use of allopurinol or rasburicase as needed.

5.3 Management of Immune-Mediated Adverse Reactions

Since inhibition of PD-L1 stimulates the immune system, immune-related AEs (irAEs) may occur. Treatment of irAEs is mainly dependent upon severity (NCI-CTCAE grade):

- Grade 1 to 2: treat symptomatically or with moderate dose steroids, more frequent monitoring.
- Grade 1 to 2 (persistent): manage similar to high grade AE (Grade 3 to 4)
- Grade 3 to 4: treat with high dose corticosteroids

Treatment of gastrointestinal, dermatological, pulmonary, hepatic and endocrine irAEs should follow guidelines set forth below.

For Grade 2 toxicities requiring delay of avelumab therapy as listed in the tables below, study drug should be held until ≤ Grade 1. Patients who

experience Grade 2 toxicities that do not resolve to ≤ Grade 1 despite symptomatic/medical intervention within 14 days should have study drug discontinued; in the setting of clinical benefit, investigators may discuss continuation of study drug with the PI on a case-by-case basis.

Gastrointestinal irAEs		
It is recommended that colitis or enterocolitis of Grade 1 be evaluated for other non-immune mediated causes, then monitored closely and treated symptomatically without steroids, including a trial of loperamide may be used. For Grade ≥ 2 colitis or enterocolitis, recommendations include endoscopy. Even if colonoscopy does not reveal gross findings of colitis, biopsies should be performed and strong consideration should be given to upper endoscopy and biopsies. Patients with gross or biopsy proven colitis or enteritis should receive IV steroids (recommend 1 mg/kg methylprednisone daily x 7 days) followed by a minimum 30 day taper. In patients with Grade 3 or 4 enterocolitis that does not respond to high dose steroids after 7 days, further therapies should be administered as clinically indicated in consultation with gastroenterology subspecialists. Concern for immune-mediated liver toxicity may be elicited following hepatic enzyme (AST, ALT) elevation of 3-fold over baseline and/or right upper quadrant abdominal pain or unexplained nausea or vomiting. Other etiologies for transaminitis should be considered and evaluated and may include but are not limited to neoplastic, concurrent medications, viral hepatitis, and other toxic etiologies. Evaluation for autoimmune etiologies may be evaluated by ANA, pANC, and anti-smooth muscle antibody tests as well as hepatology consultation with possible biopsy. Pancreatitis has rarely been associated with checkpoint inhibitors and should be considered in cases of abdominal pain associated with elevations of amylase and lipase. Treatment of pancreatitis should be supportive and may include consultation with gastroenterology subspecialists. Grade 3 or 4 amylase or lipase abnormalities that are not associated with diabetes mellitus, associated liver or gallbladder inflammation, or clinical manifestations of pancreatitis and which decrease to ≤ Grade 2 within 7 days will not require discontinuation of avelumab. Any patient experiencing diarrhea (which may be defined as watery stool, or increase in the frequency stools above grade 1 with urgency or nocturnal bowel movement, or melena or hematochezia) should be further evaluated for etiology that should include a search for an infectious etiology, C. Difficile colitis and other alternative infections as clinically indicated. Consideration should be given to discontinuing medications known to exacerbate colitis.		
Severity of Diarrhea / Colitis (NCI-CTCAE	**Management**	**Follow-up**

v4.03)		
Grade 1 Diarrhea: < 4 stools/day over baseline Colitis: asymptomatic	Continue avelumab therapy Symptomatic treatment (e.g. loperamide)	Close monitoring for worsening symptoms Educate participant to report worsening immediately If worsens: treat as Grade 2 or 3 to 4.
Grade 2 Diarrhea: 4 to 6 stools/day over baseline; IV fluids indicated < 24 hours, not interfering with ADLs Colitis: abdominal pain, blood in stool	Delay avelumab therapy Symptomatic treatment	If improves to Grade 1 within 7 days, resume avelumab therapy If persists > 5 to 7 days or recur: 0.5–1.0 mg/kg/day methylprednisolone (or equivalent). When symptoms improve to Grade 1, taper steroids over at least 1 month. Consider prophylactic antibiotics for opportunistic infections, and resume avelumab therapy per protocol. If worsens or persists > 3 to 5 days with oral steroids: treat as grade 3 to 4
Grade 3 to 4 Diarrhea (Grade 3): ≥ 7 stools/day over baseline; incontinence; IV fluids ≥ 24 hours; interfering with ADLs Colitis (Grade 3): severe abdominal pain, medical intervention indicated, peritoneal signs Grade 4: life-threatening, perforation	Discontinue avelumab therapy per protocol Start 1.0–2.0 mg/kg/day methylprednisolone IV (or equivalent) Add prophylactic antibiotics for opportunistic infections Consider endoscopy	If improves: continue steroids until Grade 1, then taper over at least 1 month If persists > 3 to 5 days or recurs after improvement: Add infliximab 5 mg/kg unless otherwise contraindicated (note: infliximab should not be used in cases of perforation or sepsis)
Dermatological irAEs		
Grade of Rash (NCI-CTCAE v4)	**Management**	**Follow-up**
Grade 1 to 2 Covering ≤ 30% body surface area	Symptomatic therapy (for example, antihistamines, topical steroids)	If persists > 1 to 2 weeks or recurs: Consider skin biopsy

	Continue avelumab therapy	Delay avelumab therapy Consider 0.5 to 1.0 mg/kg/day methylprednisolone IV or oral equivalent. Once improving, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume avelumab therapy. If worsens: Treat as Grade 3 to 4
Grade 3 to 4 Covering > 30% body surface area; life-threatening consequences	Delay or discontinue avelumab therapy Consider skin biopsy Dermatology consult 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent	If improves to Grade 1: Taper steroids over at least 1 month and add prophylactic antibiotics for opportunistic infections. Resume avelumab therapy

Pulmonary irAEs		
Grade of Pneumonitis (NCI-CTCAE v4)	**Management**	**Follow-up**
Grade 1 (Radiographic changes only)	Consider delay of avelumab therapy Monitor for symptoms every 2 to 3 days Consider Pulmonary and Infectious Disease consults	Re-image at least every 3 weeks If worsens: Treat as Grade 2 or Grade 3 to 4
Grade 2 (Mild to moderate new symptoms)	Delay avelumab therapy Pulmonary and Infectious Disease consults Monitor symptoms daily, consider hospitalization 1 mg/kg/day methylprednisolone IV or oral equivalent Consider bronchoscopy, lung biopsy	Re-image every 1 to 3 days If improves: When symptoms return to near baseline, taper steroids over at least 1 month and then resume avelumab therapy and consider prophylactic antibiotics If not improving after 2 weeks or worsening:

		Treat as Grade 3 to 4
Grade 3 or 4 Severe new symptoms; new / worsening hypoxia; life-threatening	Discontinue avelumab therapy Hospitalize Pulmonary and ID consults 2 to 4 mg/kg/day methylprednisolone IV or equivalent Add prophylactic antibiotics for opportunistic infections Consider bronchoscopy, lung biopsy	If improves to baseline: Taper steroids over at least 6 weeks If not improving after 48 hours or worsening: Add additional immunosuppression (e.g., infliximab, cyclophosphamide, intravenous immunoglobulin, or mycophenolate mofetil)
Hepatic irAEs		
Grade of Liver Test Elevation (NCI-CTCAE v4)	**Management**	**Follow-up**
Grade 1 AST or ALT > IULN to 3.0 x IULN and/or total bilirubin >IULN to 1.5 x IULN	Continue avelumab therapy	Continue liver function monitoring If worsens: Treat as Grade 2 or 3-4
Grade 2 AST or ALT > 3.0 to ≤ 5 x IULN and/or total bilirubin >1.5 to ≤ 3 x IULN	Delay avelumab therapy Increase frequency of monitoring to every 3 days	If returns to Baseline: Resume routine monitoring, resume avelumab therapy If elevations persist > 5 to 7 days or worsen: 0.5 to 1 mg/kg/day methylprednisolone or oral equivalent and when LFT returns to Grade 1 or baseline, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume avelumab therapy
Grade 3 AST or ALT >5-20 x IULN and/or total bilirubin >3-10 x IULN Grade 4 AST or ALT >20 IULN Bilirubin >10 IULN	Discontinue avelumab therapy Increase frequency of monitoring to every 1-2 days 1 to 2 mg/kg/day methylprednisolone IV or	If returns to Grade 2: Taper steroids over at least 1 month If does not improve in > 3 to 5 days, worsens or rebounds: Add mycophenolate

	IV equivalent 2.0 mg/kg/day Add prophylactic antibiotics for opportunistic infections Consult gastroenterologist Consider obtaining MRI/CT scan of liver and liver biopsy if clinically warranted	mofetil 1 gram (g) twice daily If no response within an additional 3 to 5 days, consider other immunosuppressants per local guidelines
Endocrine irAEs		
Patients experiencing symptoms such as fatigue, myalgia, impotence, mental status changes, constipation, or other symptoms thought to be associated with endocrine abnormalities should be evaluated for thyroid, pituitary, or adrenal endocrinopathies and an endocrinologist should be consulted. Patients with Grade 2 hypothyroidism should be evaluated by an endocrinologist for further management. Patients with Grade 2 hypothyroidism adequately managed with thyroid hormone replacement may continue on protocol therapy. Patients with Grade 3 or greater hypothyroidism will be considered to have had a dose-limiting toxicity. These patients should be managed according to table below and evaluation by an endocrinologist is recommended for further management. Patients who enter the study on thyroid replacement should have their medication adjusted to maintain TSH in the normal range.		
Endocrine Disorder	**Management**	**Follow-up**
Asymptomatic TSH abnormality	Continue avelumab therapy If TSH < 0.5 x LLN, or TSH > 2x IULN, or consistently out of range in 2 subsequent measurements: include T4 at subsequent cycles as clinically indicated; consider endocrinology consult	
Symptomatic endocrinopathy	Evaluate endocrine function Consider pituitary scan Symptomatic with abnormal lab/pituitary scan: Delay avelumab therapy 1 to 2 mg/kg/day methylprednisolone IV or oral equivalent Initiate appropriate hormone therapy No abnormal lab/pituitary MRI scan but symptoms persist:	If improves (with or without hormone replacement): Taper steroids over at least 1 month and consider prophylactic antibiotics for opportunistic infections Resume avelumab therapy Participants with adrenal insufficiency may need to continue steroids with mineralocorticoid component

	Repeat labs in 1 to 3 weeks MRI in 1 month	
Suspicion of adrenal crisis (for example, severe dehydration, hypotension, shock out of proportion to current illness)	Delay or discontinue avelumab therapy Rule out sepsis Stress dose of IV steroids with mineralocorticoid activity IV fluids Consult endocrinologist If adrenal crisis ruled out, then treat as above for symptomatic endocrinopathy	
Cardiac irAEs		
Myocarditis	**Management**	**Follow-up**
New onset of cardiac signs or symptoms and / or new laboratory cardiac biomarker elevations (e.g. troponin, CK-MB, BNP) or cardiac imaging abnormalities suggestive of myocarditis.	Withhold avelumab therapy Hospitalize. In the presence of life threatening cardiac decompensation, consider transfer to a facility experienced in advanced heart failure and arrhythmia management. Cardiology consult to establish etiology and rule-out immune-mediated myocarditis. Guideline based supportive treatment as appropriate per cardiology consult.* Consider myocardial biopsy if recommended per cardiology consult.	If symptoms improve and immune-mediated etiology is ruled out, re-start avelumab therapy. If symptoms do not improve/worsen, viral myocarditis is excluded, and immune-mediated etiology is suspected or confirmed following cardiology consult, manage as immune-mediated myocarditis.
Immune-mediated myocarditis	Permanently discontinue avelumab. Guideline based supportive treatment as appropriate per cardiology	Once improving, taper steroids over at least 1 month and add prophylactic antibiotics for opportunistic infections.

	consult.* Methylprednisolone 1–2 mg/kg/day.	If no improvement or worsening, consider additional immunosuppressions (e.g. azathioprine, cyclosporine A)

5.4 Autoimmune or Immune System Disorders Affecting Other Organ Systems

Patients experiencing symptoms that may be associated with autoimmune or immune-mediated adverse events possibly, probably or definitely related to protocol therapy should be evaluated and monitored closely. These may include but are not limited to pneumonitis, sarcoid-like granuloma and neurologic events including hypophysitis, encephalitis, aseptic meningitis, and cranial neuropathy especially seventh cranial nerve (CNVII) palsy. Consideration should be given to subspecialty consultation particularly if systemic immune suppression is considered.

5.5 Central Venous Catheter

While not required for the purposes of this study, the insertion of a central venous catheter/line (CVL) is recommended for all patients for intravenous (IV) administration of study drug as well as other supportive medications and laboratory blood draws. Placement of the CVL may be performed by the treating institution based on local guidelines for best medical practice.

5.6 Concomitant Therapy

Concurrent cancer therapy, including chemotherapy, radiation therapy, immunotherapy or biologic therapy may NOT be administered to patients receiving study drug. If these treatments are administered the patient will be removed from protocol therapy. Immunotherapy and immunosuppressive drugs (i.e., systemic corticosteroids except for short term treatment of allergic reactions or for the treatment of immune-related adverse events) should not be administered during the study. For patients with adrenal insufficiency, physiological dosing of steroids is acceptable. Herbal remedies with immunostimulating properties (e.g., mistletoe extract) or interference with major organ function (e.g., hypericin) should not be used during the study. Vaccines should not be administered during treatment, with the exception of administration of the inactive influenza vaccine. No other investigational agents may be given while the patient is on study.

6.0 DRUG INFORMATION

6.1 Avelumab (MSB0010718C, anti-PD-L1)

Source and pharmacology: Avelumab is a fully human antibody of the immunoglobulin (IgG) 1 isotype that specifically targets and blocks PD-L1.

Formulation and stability: The product is a sterile, clear and colorless solution presented at concentration of 20mg/mL in United States Pharmacopeia (USP) type I glass vials closed with a rubber stopper and sealed with an aluminum Flip Off© crimp seal closure. Each single use 20mg/mL vial contains 200mg of avelumab as a preservative-free acetate-buffered solution (ph 5.2) containing mannitol and polysorbate 20 (Tween 20). Only excipients that conform to the current USP are used.

Storage: Avelumab vials must be stored at 2°C to 8°C until use. Vials that are stored at room or higher temperature for extended periods of time may be subject to degradation. The drug product must not be frozen. Rough shaking of solution must be avoided.

Solution preparation: Avelumab must be diluted with 0.9% saline solution (sodium chloride injection) supplied in an infusion bag to a total volume of 250mL. Prior to preparation of dilution, each vial should be allowed to equilibrate to room temperature. For mixing purposes, after dilution of avelumab in the infusion bag, the mixture should be gently inverted 10 times. Vial contents from different lots should not be mixed in the same infusion.

Stability: The chemical and physical in-use stability for the infusion solution of avelumab in 0.9% saline solution (sodium chloride injection) has been demonstrated for a total of 24 hours at room temperature. However, from a microbiological point of view, this solution should be used immediately and is not intended to be stored unless dilution has taken place in a controlled and validation aseptic condition

Supplier: Pfizer will supply avelumab to all participating institutions.

Toxicity: Most commonly reported adverse events are fatigue, influenza-like illness, pyrexia, lymphopenia. See Investigator's Brochure for full description.

Dosage and route of administration: Avelumab should be administered as an intravenous infusion over 60 minutes. Premedication is required; see treatment plan sections.

7.0 REQUIRED EVALUATIONS, TESTS, AND OBSERVATIONS

7.1 Evaluations Before and During Therapy

All participants enrolled at St. Jude Children's Research Hospital should be invited to participate in the St. Jude tissue banking protocol (TBANK) and pharmacogenetics protocol PGEN5 at the time of study entry. All clinical and laboratory studies to determine eligibility must be performed within 7 days prior to enrollment unless otherwise indicated. Imaging

studies must be obtained within 14 days prior to the start of protocol therapy (repeat tumor imaging if necessary).

Evaluations, Tests and Observations

Studies to be Obtained	Baseline	During Cycle 1	Prior to Cycle 2	Prior to Subsequent Cycles	End of Therapy
History	X		X	X	X
Physical exam with vital signs	X	Weekly	X	X	X
Height, weight, BSA	X		X	X	X
Performance status	X		X	X	X
CBC, diff, platelets[1]	X	Weekly	X	X	X
Electrolytes, including CA^{++}, PO_4, Mg^{++}[1]	X		X	X	X
BUN/creatinine[1]	X		X	X	X
ALT (SGPT), AST and total bilirubin[1]	X		X	X	X
Total protein/albumin	X		X	X	X
Amylase, lipase, CRP	X		X	X	X
Pregnancy test (females)[2]	X		X	X	X
Free T4 and TSH[3]	X		X	X	X
Cortisol	X		X	X	X
Tumor disease evaluation[4]	X		End of cycle 2	End of Cycle 4, then every 3 cycles	X
CT Chest[5]	X		End of cycle 2	End of Cycle 4, then every 3 months	X
^{18}F-FDG PET/PET-CT or bone scan[6]	X		End of cycle 2	End of Cycle 4, then every 3 months	X
Pulse oximetry	X		X	with Tumor Disease Evaluation	X
Quality of Life	X			X[7]	X
Correlative research studies (see Section 8.0)					
Correlative biology (baseline and prior to cycle 3 only)	X			X	
Tumor tissue (if available)	X				

[1]Blood chemistry and hematology assessments: must be performed at baseline, prior to each avelumab dose, at end of treatment visit and at 30 days post-treatment safety follow-up.
[2]Women of childbearing potential require a negative pregnancy test prior to starting treatment and must be willing to adhere to effective contraceptive during and for 60 days after the last dose of avelumab. Males who are sexually active with women of childbearing potential must be willing to adhere to effective contraception during and for 60 days after the last dose of avelumab. Abstinence is an acceptable method of birth control.
[3]Free T4 and TSH must be performed at baseline and at least every 8 weeks during treatment and at end of treatment or 30 days post-treatment safety follow-up (if not performed in the previous 8 weeks)
[4] MRI of primary tumor and CT or MRI of all non-primary lesions meeting eligibility criteria. Use the same imaging modality for all disease evaluations. AP and lateral radiographs should be obtained of the primary tumor (if present) as well as any skeletal metastases.
[5]CT of the chest is required for all patients to assess for the development of progressive pulmonary disease.
[6] ^{18}F-FDG PET/PET-CT or bone scan is required with each disease evaluation. It is recommended to obtain MRI or CT scan on any positive bone lesion on ^{18}F-FDG PET/PET-CT or bone scan. Investigators are encouraged to use

the same modality used at baseline to follow positive lesions that are identified with [18]F-FDG PET/PET-CT or bone scan
[7]QOL questionnaires will be completed at baseline, at the end of cycle 2 (8 weeks ±1week), at the end of cycle 4 (16 weeks ±1week) and at the end of avelumab therapy.

7.2 Extended Safety Follow-up

Given the potential risk for delayed immune-related toxicities, safety follow-up must be performed up to 90 days after the last dose of avelumab administration.

The extended safety follow-up beyond 30 days after last study drug administration may be performed either via a site visit or via a telephone call with subsequent site visit requested in case any concerns noted during the telephone call.

8.0 CORRELATIVE RESEARCH STUDIES

8.1 Tumor Tissue

All patients enrolling on this protocol require institutional histological confirmation of osteosarcoma at the time of original diagnosis. Even though biopsy to confirm recurrence is strongly encouraged, histologic confirmation of recurrence/relapse is NOT required for inclusion in this study. For patients who have available tissue from diagnosis/recurrence, submission of tissue for biologic studies is strongly encouraged.

Tumor tissue that becomes available for biological studies will be processed into single cell suspensions. Purification of tumor-associated lymphocytes will be performed by Percoll gradient centrifugation and then frozen in appropriate freezing media or directly sorted for lymphocyte analyses (described below).

8.2 Immunologic Research – NCI Testing Battery

All patients 18 years of age and older will have 6 (10ml) green top tubes and 2 (7ml) SST and patients less than 18 years of age will have 2 (10ml) green top tubes and 1 (7ml) SST.
See Laboratory Manual for additional information on collection, processing and shipment of samples.

Timing: prior to start of therapy and prior to starting Cycle 3.

Blood samples may be used for other research studies, which may include phenotypic and functional analysis of immune cell subsets, circulating tumor cell evaluation, and analysis for cytokines, chemokines, antibodies, tumor-associated antigens and / or other markers.

The samples will be processed at The Clinical Support Laboratory at the Frederick National Laboratory for Cancer Research (see Appendix III):

Theresa Burks
Leido-Frederick
1050 Boyles Street
Bldg 469, Room 121
Frederick MD 21702
Phone 301-846-5125 or 301-846-1707

On days samples are drawn, Jen Bangh at the Clinical Support Laboratory should be notified (phone: (301) 846-5893; fax (301) 846-6536). She will arrange same-day courier delivery of the specimens.

The research samples will contain labels on the blood tubes that have the patient's initials, date of birth, the assigned protocol, and the date the samples were drawn. The transmittal forms accompanying the samples also contain the same information.

Once a patient's treatment schedule has been determined, it should be faxed to Caroline Jochems at the Laboratory of Tumor Immunology and Biology / NIH (Fax: (301) 496-2756; phone: (301) 402-6274) for planning purposes.

Parameters of immune activation

Immunologic testing will include:

- Peripheral blood mononuclear cells (PBMC)
  - Phenotypic and functional analysis of 123 immune cell subsets by flow cytometry (CD4, CD8, and regulatory T-lymphocytes, B-lymphocytes, NK cells, NKT cells, dendritic cells, myeloid derived suppressor cells and ratios of effector to suppressor cells).

- Serum
  - Analysis of cytokines (ifn-γ, IL-10, IL-12, IL-2, IL-4, TGF-β, etc.), chemokines, soluble CD27, soluble CD40L, antibodies, tumor-associated antigens and/or other markers

Flow-cytometry analysis of PBMC immune cell subsets: 27 markers, 123 subsets

1. **CD4**: Helper T lymphocytes (33 subsets)
2. **CD8**: Cytotoxic T lymphocytes (30 subsets)
   - **Maturation status of T lymphocytes (in CD4 and CD8)**:
     - **Naïve**: $CD45RA^{+}$ $CCR7^{+}$
     - **Central Memory**: $CD45RA^{-}$ $CCR7^{+}$
     - **Effector Memory**: $CD45RA^{-}$ $CCR7^{-}$
     - **Terminal (EMRA)**: $CD45RA^{+}$ $CCR7^{-}$
   - **T lymphocyte markers (in total and memory CD4 and CD8)**:
     - **CTLA-4:** inhibition
     - **PD-1:** activation/inhibition
     - **PD-L1:** activation/cross-inhibition
     - **TIM-3:** inhibition
     - **41BB:** co-stiactivation
     - **ICOS**: activation (only on CD4)
3. **Tregs**: Regulatory T lymphocytes ($CD4^{+}$ $CD25^{+}$ $FoxP3^{+}$ $CD127^{-}$) (7 subsets)
   - **CD45RA**: Tregs highly expandable *in vitro*
   - **CTLA-4**: Treg suppression
   - **CD49d:** "contaminating" effector lymphocytes (non-Tregs)
   - **ICOS**: Treg suppression
   - **PD-1:** activation/inhibition
   - **PD-L1:** cross-inhibition
4. **B lymphocytes**: $CD19^{+}$ (3 subsets)
   - **PD-1:** activation/inhibition
   - **PD-L1:** cross-inhibition
5. **NK**: Natural killer cells ($CD56^{+}$ $CD3^{-}$) (20 subsets)
   - **$CD16^{+}$ $CD56^{dim}$** : Mature NK, lytic
   - **$CD16^{+}$ $CD56^{br}$**: Functional intermediate, lytic, cytokine production
   - **$CD16^{-}$ $CD56^{br}$:** Immature, cytokine production, abundant in placenta
   - **$CD16^{-}$ $CD56^{dim}$**: non-lytic, non-cytokine production
   - **TIM-3:** activation
   - **PD-1:** activation/inhibition
   - **PD-L1:** cross-inhibition
6. **NK-T**: $CD56^{+}$ $CD3^{+}$ (4 subsets)
   - **TIM-3:** activation
   - **PD-1:** activation/inhibition
   - **PD-L1:** cross-inhibition
7. **cDCs (Conventional DCs):** $CD3^{-}CD56^{-}HLA\text{-}DR^{+}CD1c^{+}CD303^{-}$ (5 subsets)
8. **pDCs (plasmacytoid DCs):** $CD3^{-}CD56^{-}HLA\text{-}DR^{+}CD1c^{-}CD303^{+}$ (5 subsets)
   - **Markers of DC activation**
     - **CD83:** activation
     - **TIM-3:** inhibition
     - **PD-L1:** cross-inhibition
     - **PD1:** activation/inhibition
9. **MDSCs**: Myeloid-derived suppressor cells ($CD11b^{+}$ HLA-DR

Samples sent to the Clinical Support Laboratory at NCI

All data associated with patient samples are protected by using a secure database. All samples drawn at the NIH Clinical Center will be transported to the Clinical Support Laboratory at the Frederick National Laboratory for Cancer Research by couriers.
Samples will be tracked and managed by the Central Repository database. All samples will be stored in liquid nitrogen. These freezers are located at NCI Frederick Central Repository in Frederick, Maryland.

Fisher BioServices manages the NCI Frederick Central Repositories under subcontract to Leidos Biomedical, Inc. NCI Frederick Central Repositories store, among other things, biological specimens in support of NIH clinical studies. All specimens are stored in secure, limited access facilities with sufficient security, back-up and emergency support capability and monitoring to ensure long-term integrity of the specimens for research.

Specimens are stored in accordance will applicable HHS and FDA Protection of Human Subjects Regulations in accordance with Fisher BioServices Federal-wide Assurance. Fisher BioServices' role is limited to clinical research databases and repositories containing patient specimens. Fisher BioServices neither conducts nor has any vested interest in research on human subjects, but does provide services and supports the efforts of its customers, many of which are involved in research on human subjects. The Fisher BioServices IRB reviews policies and procedures for labeling, data collection and storage, access, and security. The IRB will review protection of privacy issues prior to acceptance of any new work and in the event of change impacting privacy issues in existing work.

It is the intent and purpose of Fisher BioServices to accept only de-identified samples and sample information. To the best of our ability, every effort will be made to ensure that protected information is not sent electronically or by hard copy or on vial labels.
Sample data are stored in the BioSpecimen Inventory (BSI) System II. This inventory tracking system is used to manage the storage and retrieval of specimens as well as maintain specimen data. BSI is designed for controlled, concurrent access. It provides a real-time, multi-user environment for tracking millions of specimens. The system controls how and in what order database updates and searches are performed. This control prevents deadlocks and race conditions. For security, BSI has user password access, 3 types of user access levels, and 36 user permissions (levels of access) that can be set to control access to the system functions. BSI provides audit tracking for processes that are done to specimens including shipping, returning to inventory, aliquoting, thawing, additives, and other processes. BSI tracks the ancestry of specimens as they are aliquoted, as well as discrepancies and discrepancy

resolution for specimens received by the repository. If a specimen goes out of the inventory, the system maintains data associated with the withdrawal request. Vials are labeled with a unique BSI ID, which is printed in both eye-readable and bar-coded format. No patient-specific information is encoded in this ID.

Investigators are granted view, input and withdrawal authority only for their specimens. They may not view specimen data or access specimens for which they have not been authorized. Access to specimen storage is confined to repository staff. Visitors to the repositories are escorted by repository staff at all times.

NCI Protocol Completion/Sample Destruction

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described above. The study will remain open so long as sample or data analysis continues. Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed. Once primary research objectives for the protocol are achieved, intramural researchers can request access to remaining samples providing they have an IRB approved protocol and patient consent.

The PI will report any loss or destruction of samples to the NCI IRB as soon as he is made aware of such loss. The PI will report destroyed samples to the IRB if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container) or if a patient withdraws consent. Samples will also be reported as lost if they are lost in transit between facilities or misplaced by a researcher. Freezer problems, lost samples or other problems associated with samples will also be reported to the IRB, the NCI Clinical Director, and the office of the CCR, NCI.

8.3 Immunologic Research – St. Jude Testing Battery

Three aliquots of peripheral blood mononuclear cells (PBMCs) (representing a 10 mL draw in Blue/Black CPT tubes) will be obtained for testing for TCR repertoire, T cell phenotype, transcriptional profile, and epigenetic landscape. Samples may be frozen for the purposes of shipping and handling. Should tumor tissue be available from resection of the patient's primary tumor AND/OR metastatic sites of disease, single cell suspensions of tumor cells should also be obtained for analysis.

See Laboratory Manual for additional information on collection, processing and shipment of samples

Timing: prior to start of therapy and prior to cycle 3. Samples should be sent via FedEx overnight services to:

Paul G. Thomas, PhD
Integrated Research Center, Room E7054
St. Jude Children's Research Hospital
262 Danny Thomas Place
Memphis, TN 38105

On the day of sample acquisition, please contact Paul Thomas or Ben Youngblood at (901) 595-6749 to arrange for pickup of materials.

Samples will be stained with fluorescently labeled antibodies and sorted for single cell analysis (1/3 of the sample) with the remaining sample sorted on specific cell phenotypes for transcriptional and epigenetic analysis.
Preparation of single cell suspension: Tumor sample (from freshly-resected surgical specimen) is weighed and sequentially minced into small pieces (approx. 3 mm in size) using scissors or scalpel. Tumor tissue is digested at 37^{o}C in RPMI medium supplemented with 5% FBS, Collagenase 4, and DNase I. Digested tumors are homogenized by pipetting, and filtered through a 70 um cell strainer. The remaining undigested pieces are forced to pass through the strainer using a syringe plunger. The single cell suspension is washed 3 times with ice cold PBS, and RBC are lysed (if needed) using an ammonium chloride isotonic solution. The number of live cells is determined using a hemocytometer after exclusion of dead cells by Trypan blue dye.

Polyparametric immunophenotypic analysis: Cells are labeled with fluorescent conjugated antibodies against the listed human epitopes, and subsequently analyzed in a flow cytometer (BD LSRII). We will evaluate PD-L1 expression in cell surface of infiltrating leukocytes (CD45+ fraction) as well as in CD45- fraction, which primary consists of tumor cells.

Specificity	Fluorophore	Clone
CCR7	FITC	G043H7
PD1	PE	EH12.2H7
TIM3	PECY7	F38-2E2
CD45RO	APC	UCHL1
CD3	APCCY7	SK7
CD8a	PB (421)	RPA-T8

CD45	BV605	HI30
PDL1	**BV786**	**MIH1**
7AAD	n/a	(dead cell exclusion dye)

Samples sent to St. Jude

All data associated with patient samples are protected by using a secure database. All samples will be stored in liquid nitrogen. These freezers are located at St. Jude in a secure facility. All specimens are stored in secure, limited access facilities with sufficient security, back-up and emergency support capability and monitoring to ensure long-term integrity of the specimens for research. Specimens are stored in accordance will applicable HHS and FDA Protection of Human Subjects Regulations in accordance with SJCRH Federal-wide Assurance. The St. Jude IRB reviews policies and procedures for labeling, data collection and storage, access, and security. The IRB will review protection of privacy issues prior to acceptance of any new work and in the event of change impacting privacy issues in existing work.

It is the intent and purpose of the investigators at St. Jude to accept only de-identified samples and sample information. To the best of our ability, every effort will be made to ensure that protected information is not sent electronically or by hard copy or on vial labels.

9.0 EVALUATION CRITERIA

9.1 Response Definitions and Criteria for Solid Tumors

See the table in section 7.1 for the schedule of tumor evaluations. In addition to the scheduled scans, a confirmatory scan should be obtained 28 days following initial documentation of objective response.

Response and progression will be evaluated in this study using the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1)[90]. Key points are that up to 5 target lesions may be identified and that changes in the *largest* diameter (unidimensional

measurement) of the tumor lesions but the *shortest* diameter of malignant lymph nodes are used in the RECIST v 1.1 criteria.

9.1.1 Definitions

Evaluable for objective response: Patients who exhibit objective disease progression prior to the end of cycle 1 will be considered evaluable for response. For all other patients, only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response.

Evaluable Non-Target Disease Response: Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

9.1.2 Disease Parameters

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).

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable. If the investigator thinks it appropriate to include them, the conditions under which such lesions should be considered must be defined in the database.

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 no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

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.

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 that 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.

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.

9.1.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. 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.

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

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.
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 (e.g. for body scans). Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans.

PET-CT: At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Tumor markers: Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Cytology, histology: These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain). Cytology should be obtained if an effusion appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease.

^{18}F-FDG PET/PET-CT: While ^{18}F-FDG PET/PET-CT response assessments need additional study, it is sometimes reasonable to incorporate the use of ^{18}F-FDG PET/PET-CT scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease).

New lesions on the basis of ^{18}F-FDG PET/PET-CT imaging can be identified according to the following algorithm:

a. Negative ^{18}F-FDG PET/PET-CT at baseline, with a positive ^{18}F-FDG PET/PET-CT at follow-up is a sign of PD based on a new lesion.
b. No ^{18}F-FDG PET/PET-CT at baseline and a positive ^{18}F-FDG PET/PET-CT at follow-up: If the positive ^{18}F-FDG PET/PET-CT at follow-up corresponds to a new site of disease confirmed by CT,

this is PD. If the positive ^{18}F-FDG PET/PET-CT at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal ^{18}F-FDG PET/PET-CT scan). If the positive ^{18}F-FDG PET/PET-CT at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

Note: A 'positive' ^{18}F-FDG PET/PET-CT scan lesion means one that is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

9.2 Response Criteria for Patients with Solid Tumor and Measurable Disease

9.2.1 Evaluation of Target Lesions

Complete response (CR): Disappearance of all target and non-target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm. If immunocytology is available, no disease must be detected by that methodology.

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 progressions). Note: in presence of SD or PR in target disease but unequivocal progression in non-target or non-measurable disease, the patient has PD if there is an overall level of substantial worsening in non-target disease such that the overall tumor burden has increased sufficiently to merit discontinuation of therapy.

Stable disease (SD): Insufficient shrinkage to qualify for PR or insufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

9.2.2 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)

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

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.

Overall best response assessment: Each patient will be classified according to his "best response" for the purposes of analysis of treatment effect. Best response is determined as outlined in Section 9.4 from a sequence of overall response assessments.

9.3 Response Criteria for Evaluable Disease

Note: all participants must have measurable disease, but they can also have evaluable disease in addition to measurable disease.

Evaluable disease: The presence of at least one lesion, with no lesion that can be accurately measured in at least one dimension. Such lesions may be evaluable by nuclear medicine techniques, immunocytochemistry techniques, tumor markers or other reliable measures.

Complete response (CR): Disappearance of all evaluable disease.

Partial response (PR): Partial responses cannot be determined in patients with evaluable disease.

Stable disease (SD): That which does not qualify as Complete Response (CR), Partial Response (PR), or Progressive Disease.

Progressive disease (PD): The appearance of one or more new lesions or evidence of laboratory, clinical, or radiographic progression.

Overall best response assessment: Each patient will be classified according to his "best response" for the purposes of analysis of treatment effect. Best response is determined as outlined in Section 9.4 from a sequence of overall response assessments.

9.4 Best Response

9.4.1 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.

Table 1: Patients with Measurable Disease (i.e., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Non-CR/Non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-CR/Non-PD/not evaluated	No	PR
SD	Non-CR/Non-PD/not evaluated	No	SD
PD	Any	Yes or No	PD
Any	PD*	Yes or No	PD
Any	Any	Yes	PD

** 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 any 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.

Table 2: Patients with Non-Measurable Disease (i.e., 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.*

Table 3: Sequences of overall response assessments with corresponding best response

1st Assessment	2nd Assessment	Best Response
Progression		Progressive disease
Stable, PR, CR	Progression	Progressive disease
Stable	Stable	Stable
Stable	PR, CR	Stable
Stable	Not done	Not RECIST classifiable
PR	PR	PR
PR	CR	PR
PR, CR	Not done	Not RECIST classifiable
CR	CR	CR

9.5 Duration of Response

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.

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.

9.6 Toxicity Evaluation Criteria

This study will utilize the CTCAE v4.03 for toxicity and performance reporting. A copy of the CTCAE v4.03 can be downloaded from the CTEP home page (http://ctep.info.nih.gov). Additionally, the toxicities are to be reported on the appropriate data collection forms.

10.0 OFF TREATMENT AND OFF STUDY CRITERIA

A genuine effort must be made to determine the reason(s) why a participant fails to return for the necessary visits or is discontinued from the trial. Information regarding the reason for not completing the trial will be recorded on the appropriate case report forms.

It will be documented whether or not each patient completed the clinical study. If for any patient study treatment or observations were discontinued, the reason will be recorded on the appropriate case report form. Reasons that a patient may discontinue participation in a clinical study are listed below. All patients will be followed for survival until they meet the criteria for off-study.

10.1 Off Treatment Criteria

- Clinical or radiographic evidence of progressive disease of greater than 20% increase baseline target lesions selected according to RECIST criteria
- Adverse events/toxicity requiring removal from protocol therapy
- Refusal of further protocol therapy by patient/parent/guardian
- Non-compliance that in the opinion of the investigator does not allow for ongoing participation

- Physician determines it is not in the patient's best interest to continue on therapy
- Repeated eligibility laboratory studies are outside the parameters required for eligibility prior to the start of protocol therapy
- Study is terminated by the sponsor
- Pregnancy
- Development of a second malignancy

10.2 Off Study Criteria

- Death
- Lost to follow-up
- Patient subsequent enrollment on another study with tumor therapeutic intent (e.g. at recurrence) or additional non-protocol anti-tumor therapy.
- Withdrawal of consent for any further data submission
- Patient did not receive protocol treatment after study enrollment
- Withdrawal of consent

11.0 SAFETY AND ADVERSE EVENT REPORTING REQUIREMENTS

11.1 Reporting Adverse Experiences and Deaths to St. Jude IRB

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

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

Unrelated, expected deaths that occur after patient has completed protocol treatment do not require reporting to the IRB. Though death is "serious", the event must meet the other two requirements of "related or possibly related" and "unexpected/unanticipated" to be considered reportable. However, all deaths while on active protocol treatment (or within 30 days of last protocol treatment) will be require reporting to the IRB.

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

The following definitions apply with respect to reporting adverse experiences:

Serious adverse event (SAE): Any adverse event temporally associated with the subject's participation in research that meets any of the following criteria:

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

Unexpected adverse event:

- Any adverse event for which the specificity or severity is not consistent with the protocol-related documents, including the applicable investigator brochure, IRB approved consent form, Investigational New Drug (IND) or Investigational Device Exemption (IDE) application, or other relevant sources of information, such as

product labeling and package inserts; or if it does appear in such documents, an event in which the specificity, severity or duration is not consistent with the risk information included therein; or

- The observed rate of occurrence is a clinically significant increase in the expected rate (based on a credible baseline rate for comparison); or
- The occurrence is not consistent with the expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the adverse event and the subject's predisposing risk factor profile for the adverse event.

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

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

Unanticipated problem involving risks to subjects or others: An unanticipated problem involving risks to subjects or others is an event, which was not expected to occur and which increases the degree of risk posed to research participants.

Such events, in general, meet all of the following criteria:

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

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

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

Examples of unanticipated problems involving risks to subjects or others include:

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

The principal investigator has the obligation to report all serious adverse events to the FDA and IRB.

11.2 Reporting to St Jude IRB

This is an investigator-initiated study. The principal investigator, Michael W. Bishop and St. Jude are conducting the study and acting as the sponsor. Therefore, the legal/ethical obligations of the principal investigator include both those of a sponsor and those of an investigator.

11.3 Reporting from St. Jude to Pfizer (Drug Manufacturer)

The investigator primary responsibilities in the safety reporting are to identify and follow-up on Serious Adverse Events (SAEs) experienced by participants in the study and to forward the information to the local regulatory authorities and Pfizer, as required by local regulations (for regulatory reporting) and IIR agreement (for reporting to Pfizer).

The following reportable events must be submitted to Pfizer within 24 hours (or immediately for death or life-threatening events) using the provided *Investigator-Initiated Research Serious Adverse Event Form (IIR SAE)* with *the Pfizer Reportable Events Fax Cover Sheet* with each SAE submission.

- Adverse Events that are serious AND unexpected
- Exposure during Pregnancy or Breastfeeding (even if not associated with an adverse event)
- Occupational exposure (even if not associated with an adverse event)

- Potential drug-induced liver injury (Hy's Law cases): These events are considered important medical events and should be reported as SAEs.

Detailed guidance on the safety reporting is provided in the *Safety Reporting Reference Manual*.

Contact information for submission of reportable events to Pfizer:

Fax: Pfizer U.S. Clinical Trial Department, Fax 1-866-997-8322.
or
E-mail: CTPGrants@pfizer.com, specifying:

- Protocol
- Research Participant ID
- Site PI
- SAE/Onset

11.4 Reporting to St. Jude Regulatory Affairs Office and FDA

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

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

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

11.5 Recording Adverse Events and Serious Adverse Events

Adverse events (AEs) will be evaluated and documented by the clinical staff and investigators throughout inpatient hospitalizations and each outpatient visit. CRAs are responsible for reviewing documentation

related to AEs and entering directly into CRIS protocol-specific database for all adverse events grade 3 or higher. Peripheral neuropathy will be captured if Grade 2 or higher. The data to be recorded are 1) the event description, 2) the NCI CTCAE v4.0 code and grade, 3) the onset date, 4) the resolution date (or ongoing if it has not resolved at time of off study), 4) action taken for event, 5) patient outcome 6) relationship of AE to protocol treatment/interventions, 7) if AE was expected or unexpected, and 8) comments, if applicable. AEs that are classified as serious, unexpected, and at least possibly related will be notated as such in the database as "SAEs". These events will be reported expeditiously to the St. Jude IRB within the timeframes as described above.

Cumulative summary of Grade 2 clinically relevant events and all Grades 3-5 events will be reported as part of the progress reports to IRB at the time of continuing review. Specific data entry instructions for AEs and other protocol-related data will be documented in protocol-specific data entry guidelines, which will be developed and maintained by study team and clinical research informatics.

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

11.6 Process for Reporting Adverse Events from and to Collaborating Sites

Adverse events from collaborating sites will also be reviewed by the PI and discussed in study team meetings as described above. SAE reports from collaborating sites for AEs that are serious, unexpected, and at least possibly related to protocol treatment or interventions will be reported to site IRB and the St. Jude IRB within the reporting requirements described above. The PI will determine if this is an event that will need to be reported expeditiously to all participating sites, considering the following criteria:

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

With the submission of the "Reportable Event" in St. Jude TRACKS application, the PI will indicate if all sites should be notified to report to their IRBs, and if the protocol and/or consent should be amended (consent will be amended if event is information that should be communicated to currently enrolled subjects). Generally, only events that warrant an amendment to the protocol and/or consent will be reported expeditiously to all sites. However, any event may be reported expeditiously to all sites at the discretion of the PI. A cumulative summary of Grade 2-5 AEs and expected/unrelated deaths that occur more than 30 days after protocol treatment will be reported to all sites with study progress report at the time of continuing review.
For collaborating sites: Serious AND unexpected events are to be reported to the St. Jude PI (Dr. Michael Bishop) within 48-72 hours via fax or email.

Unexpected deaths must be reported to the St. Jude PI by email or phone call to Dr. Michael Bishop within 24 hours of the event. A written report must follow. The study team should be copied on all correspondence regarding the event. Sent report to:

Michael W. Bishop, MD, MS
Department of Oncology, Solid Tumor Division
St. Jude Children's Research Hospital
262 Danny Thomas Place
Memphis, TN 38105
Phone: 901-595-6407
Email: Michael.Bishop@stjude.org
OSTPDL1StudyTeam@stjude.org

12.0 DATA COLLECTION, MONITORING AND CONFIDENTIALITY

12.1 Data Collection

Electronic case report forms (e-CRFs) will be completed by the clinical trials staff from the Cancer Center Comprehensive Center, Developmental Therapeutics for Solid Tumor program. Data will be entered from record directly into a secure CRIS database, developed and maintained by St. Jude Clinical Research Informatics.

Data Management will be supervised by the Director of Clinical Trials Management, and Manager of Clinical Research Operations for the Developmental Biology for Solid Tumors Program working with Dr. Bishop or his designee. All protocol-specific data and all grade 2-5 adverse events will be recorded by the clinical research associates into the CRIS database, ideally within 2-4 weeks of completion of cycle. All questions will be directed to the attending physician and/or PI and reviewed at

regularly-scheduled working meetings. The attending physicians (or their designees) are responsible for keeping up-to-date roadmaps in the patient's primary SJCRH medical chart.

Regular (at least monthly) summaries of toxicity and protocol events will be generated for the PI and the department of Biostatistics to review.

12.2 Study Monitoring

Monitoring of this protocol is considered to be in the high-risk 3 category (HR-3). The study specific Monitoring Plan is a separate document from this protocol. The study team will meet at appropriate intervals to review case histories or quality summaries on participants. Highlights of the protocol monitoring plan are below:

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

The monitor will also verify 100% of all data points on the first two participants and on 15% of cases thereafter. Protocol compliance monitoring will include participant status, eligibility, the informed consent process, demographics, staging, study objectives, subgroup assignment, treatments, evaluations, responses, participant protocol status, off-study, and off-therapy criteria. The Monitor will generate a formal report which is shared with the Principal Investigator (PI), study team and the Internal Monitoring Committee (IMC). Monitoring may be conducted more frequently if deemed necessary by administration, the Institutional Review Board (IRB), or the IMC.

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

Source document verification of eligibility for all SJCRH cases will be performed within two weeks of completion of enrollment. This will include verification of appropriate documentation of consent. Monitoring of timeliness of serious adverse event reporting will be done as events are reported in TRACKS.

St. Jude affiliates and domestic collaborating study sites will be monitored on-site by a representative of St. Jude at intervals specified in the Data and Safety Monitoring Plan.

12.3 Confidentiality

Data abstracted from the subject's medical record and entered into CRFs will be entered into a secure, password protected, protocol-specific database maintained by St. Jude Clinical Research Informatics. The database will be stored in secure sites. All participants will be given a unique number and that will be their identifier in the trial. That number will be recorded on an enrollment log, linking the number to the participant's name, and that log will be stored securely in the trial master file. The privacy of the study subject and all confidentialities will be handled according to institutional and ICH guidelines. Any publication or presentation will not identify individual subjects by name.

The medical records of study participants may be reviewed by the St. Jude IRB, FDA, and clinical research monitors.

13.0 STATISTICAL CONSIDERATIONS

13.1 Study Design

13.1.1 Primary objectives

Primary objective 1: To estimate the response rate to four cycles of avelumab in patients with recurrent or progressive osteosarcoma.

Primary objective 2: To estimate the 12-week progression-free survival of patients with recurrent or progressive osteosarcoma after treatment with avelumab.

Responsible investigators: Michael Bishop, Alberto Pappo
Responsible Biostatisticians: Jianrong Wu
Estimated date for completion of data collection: 12/31/2021

The study is designed by treating RECIST response (CR+PR) after 4-cycle treatment of avelumab and the 16-week progression-free survival (PFS) as dual binary endpoints using the method proposed by Kocherginsky et al (software available upon request).[91] For each patient, two outcomes: RECIST response (CR+PR) and 16-week PFS will be recorded at the end of 4-cycle treatment of avelumab, here 16-week PFS is defined as a binary endpoint for patients who had response (CR+PR) or stable disease (SD) after 4-cycle treatment of avelumab. Patients who fail to be evaluated for response or PFS at the end of the 4-cycle will be counted as failure (no response and disease progression). The following table summarizes the sample size and the decision rule for the interim and final analyses based on 10% type I error and 90% power and using the method proposed by Kocherginsky et al. The proposed design below (Table 13.1) is used to

guard against futility and to facilitate the sample size calculation based on the following null and alternative hypotheses:

H0: pr≤5% and pnp ≤15%
vs. the alternative
Ha: pr>20% or pnp >35%

where pr is the true response rate and pnp is the true non-progression rate.

A total of 32 patients are needed for the study with one interim analysis after the first 18 patients had response and PFS evaluation. Accrual will be temporarily suspended at the time the interim sample size is reached (when the first 18 evaluable patients are enrolled) until the interim rules are evaluated. The study will be closed if the thresholds below at stage 1 are exceeded:

Table 13.1: Bivariate Binomial Two-stage Design

Stage	Sample Size	Decision rule
1	18	Continue trial if: # of response ≥ 2 or # of patients without PD after 4-cycle ≥ 3
2	32	Conclude therapy is promising if: # of response ≥ 4 or # of patients without PD after 4-cycle ≥ 9

This design yields 90% power to detect a true response rate of at least 20% or a true 16-week progression-free rate (CR+PR+SD) of at least 35%. The type I error is estimated as 9.2% if the true response rate is no more than 5% and the true 16-week progression-free rate is no more than 35%, with approximately 45% probability of early stopping in this case.

Patients who are removed from treatment due to toxicity will be also be treated as failures (non-response and disease progression). Thus, a total of 40 patients will insure that 32 patients are evaluable for the primary objectives.

It is anticipated that the study will enroll 10 patients per year, thus the study accrual duration will be approximately 4 years and the response and 16-week PFS data for all patients will be available in approximate 4.5 years.

13.2 Secondary Objectives

13.2.1 To describe the toxicities associated with the administration of avelumab in patients with recurrent or progressive osteosarcoma.

Responsible investigators: Michael Bishop, Alberto Pappo
Responsible Biostatisticians: Jianrong Wu
Estimated date for completion of data collection: 3/31/2023

Target toxicities for avelumab treatment are defined as any grade 3-5 dyspnea, pneumonitis, infusion-related reactions, or immune related adverse events at least possibly attributable to the agent observed anytime during the 26-cycle treatment period that a patient is on study (including the period between off treatment and off study). Due to a limited number of patients, following ad hoc stopping rule will be used to stop the trial accrual if 3 or more patients in the first 10 patients had target toxicities. Subsequently, the target toxicities will be monitored in a continuous fashion. That is at any time after 10^{th} patient, the trial accrual will be stopped if the rate of target toxicity exceeds 20%.

All toxicity will be carefully monitored in this trial. Any unexpected grade 3-4 toxicity will be reported to the IBR for review. Furthermore, in the event of a toxic death during the 4-cycle treatment, the accrual will be put on hold until a thorough review of the event take place and a deliberate decision can be made regarding resuming accrual.

13.3 Exploratory Objectives

13.3.1 To explore factors associated with response in patients treated with avelumab after recurrent or progressive osteosarcoma (e.g. tumor PD-L1 expression).

Responsible investigators: Michael Bishop, Alberto Pappo
Responsible Biostatisticians: Jianrong Wu
Estimated date for completion of data collection: 3/31/2023

Logistic regression analysis will be conducted to explore factors which may associate with response.

13.3.2 To measure parameters of immune activation including subsets of peripheral blood mononuclear cells (PBMCs) and serum markers of immune activation.

Responsible investigators: Michael Bishop, Benjamin Youngblood, Paul Thomas
Responsible Biostatisticians: Jianrong Wu, Guolian Kang
Estimated date for completion of data collection: 3/31/2023

Descriptive statistics (mean+/- SE or median, min and max) will be provided for the parameters of immune activation including subsets of PBMCs and serum markers of immune activation for each time point separately and changes between two time points. Box-plot will be provided for each time point to visually look at the trend of the parameters of immune activation over time of therapy.

13.3.3 To evaluate the role of T-cells in immune checkpoint blockade via measures of cell proliferation, co-inhibitory receptor expression on CD8 T cells, T cell repertoire, and epigenetic programming.

Responsible investigators: Michael Bishop, Benjamin Youngblood, Paul Thomas
Responsible Biostatisticians: Jianrong Wu, Guolian Kang
Estimated date for completion of data collection: 3/31/2023

For the analysis of T cell repertoire, we will compute statistics (such as diversity, entropy, richness) and characterize known receptor specificities of the immune repertoire for each time-point at which these studies are performed. Comparisons among PD1+ and PD1- and other inhibitory receptor-expressing subsets will be assessed for changes in repertoire associated with treatment. To assess the proliferation and activation phenotypes of memory CD8 T cell subsets will measure the percentage of naïve, central-memory (Tcm), effector-memory (Tem), and stem-cell (Tscm) memory CD8 T cell populations in the PBMC of treated individuals. The percentage of CD8 T cell subsets will be measured prior to treatment and following the start of therapy. Additionally, we will purify the phenotypically defined memory CD8 T cell subsets and measure subset-specific DNA methylation programs to determine if therapy results in epigenetic changes among the pool of naïve and memory T cell subsets. Overall, descriptive statistics (mean +/- SE or median, min and max) will be computed for the univariate data for each time point separately and changes between two time points and graphic presentations will be given for the longitudinal observations to demonstrate the changes across the courses of therapy (prior therapy and during therapy).

13.3.4 To assess the quality of life of patients with recurrent or progressive osteosarcoma undergoing treatment with avelumab, and to explore relationships between clinical factors and patient-reported HRQOL outcomes.

Responsible investigators: Michael Bishop
Responsible Biostatisticians: Jianrong Wu, Zhaohua Lu
Estimated date for completion of data collection: 3/31/2023

Patients will complete the PROMIS Pediatric or Adult Profile (based on their age at time of administration) at four time points during the study: prior to the start of treatment, at the end of cycle 2 (8 weeks ± 1 week), at the end of cycle 4 (16 weeks ± 1 week), and at the end of avelumab treatment. Patients less than 18 years old will completed the PROMIS-37 Profile v.1.1; patients age 18 years and older will complete the PROMIS-43 Profile v.2.0. Mixed effect models will be used to study the change patterns of the T-scores in each quality of life domain over time, and the association between clinical factors and the T-scores in each quality of life domain. Missing data points will be addressed by multiple imputation or full information maximum likelihood methods.

13.4 Anticipated Completion Dates

Anticipated primary completion date: December 2020
Anticipated study completion date: December 2023

14.0 OBTAINING INFORMED CONSENT

14.1 Consent Prior to Research Interventions

Initially, informed consent for patients enrolling at St. Jude will be sought for the institutional banking protocol (TBANK research study), and for other procedures as necessary for standard medical care. During the screening process for eligibility, informed consent for the St. Jude pre-screening protocol (SCREEN) or for OSTPDL1 is required before any research tests are performed. Collaborating sites will follow local policies for screening and consent/assent.

14.2 Consent at Enrollment

The process of informed consent for OSTPDL1 will follow local institutional policy. The informed consent process is an ongoing one that begins at the time of diagnosis and ends after the completion of therapy. Informed consent should be obtained by the attending physician or his/her designee, in the presence of at least one non-physician witness. Initially, informed consent will be sought for the institutional banking protocol (research study), blood transfusion and other procedures as

necessary. After the diagnosis of relapsed or refractory solid tumor is established, we will invite the patient to participate in the OSTPDL1 protocol.

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

We will also obtain verbal assent from children 12 to 14 years old and written assent for all participants $\geq$14 years of age at St. Jude. Collaborating sites will follow institutional guidelines for assent.

14.3 Consent at Age of Majority

Participants who reach the age of majority while on study will be re-consented for continued participation on OSTPDL1 at the time of their next clinic visit after turning 18 year according to Cancer Center and local institutional policy.

14.4 Consent When English is Not the Primary Language

Obtaining consent for non-English speaking participants will follow local institutional policy. At St. Jude, when English is not the participant, parent, or legally authorized representative's primary language, the investigator or the Social Work department will determine the need for an interpreter. This information will be documented in the participant's medical record. Either a certified interpreter or the telephone interpreter's service will be used to translate the consent information. If the short form consent is available in the primary language of the participant, parent or legally authorized representative, this may be utilized to sign consent and joined with a full English consent signed by the research team. The process for obtaining an interpreter and for the appropriate use of an interpreter is outlined on the SJCRH Interpreter Services website, as well as OHSP and CPDMO websites.

14.5 Collection of Collaborating Institution Consent Forms

Signed collaborating institution's consent forms will be faxed to the St. Jude CPDMO Eligibility Office at 901-595-6265.

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APPENDIX I: KARNOFSKY AND LANSKY PERFORMANCE STATUS SCALES/SCORES

PERFORMANCE STATUS CRITERIA *Karnofsky and Lansky performance scores are intended to be multiples of 10*					
ECOG (Zubrod)		**Karnofsky**		**Lansky**	
Score	**Description**	**Score**	**Description**	**Score**	**Description**
0	Fully active, able to carry on all pre-disease performance without restriction	100	Normal, no complaints, no evidence of disease	100	Fully active, normal
		90	Able to carry on normal activity, minor signs or symptoms of disease	90	Minor restrictions in physically strenuous activity
1	Restricted in physically strenuous activity by ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work	80	Normal activity with effort; some signs or symptoms of disease	80	Active, but tires more quickly
		70	Cares for self; unable to carry on normal activity or do active work	70	Both greater restriction of and less time spent in play activity
2	Ambulatory and capable of self-care but unable to carry out any work activities; up and about more than 50% of waking hours	60	Requires occasional assistance, but is able to care for most of his/her needs	60	Up and around, but minimal active play; keeps busy with quieter activities
		50	Requires considerable assistance and frequent medical care	50	Gets dressed, but lies around much of the day; no active play, able to participate in all quiet play and activities
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours	40	Disabled, requires special care and assistance	40	Mostly in bed; participates in quiet activities
		30	Severely disabled, hospitalization indicated; death	30	In bed; needs assistance even for quiet play

			not imminent		
4	Completely disabled; cannot carry on any self-care; totally confined to bed or chair	20	Very sick, hospitalization indicated. Death not imminent	20	Often sleeping; play entirely limited to very passive activities
		10	Moribund, fatal processes progressing rapidly	10	No play; does not get out of bed

APPENDIX II: NEW YORK HEART ASSOCIATION FUNCTIONAL CLASSIFICATION

NYHA Class	Symptoms
I	Cardiac disease, but no symptoms and no limitation in ordinary physical activity, e.g. no shortness of breath when walking, climbing stairs etc.
II	Mild symptoms (mild shortness of breath and/or angina) and slight limitation during ordinary activity.
III	Marked limitation in activity due to symptoms, even during less-than-ordinary activity, e.g. walking short distances (20-100 m). Comfortable only at rest.
IV	Severe limitations. Experiences symptoms even while at rest. Mostly bedbound patients.

The Criteria Committee of the New York Heart Association. (1994). Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels. (9th ed.). Boston: Little, Brown & Co. pp. 253-256.

APPENDIX III: INSTRUCTIONS FOR COLLECTION AND SHIPPING OF RESEARCH BLOOD SAMPLES TO NCI

Collection:
PBMCS: green top (Na heparin, 10 mL) tubes, BD Vacutainer Ref #366480
Serum: red serum separator (8 mL) tubes, Greiner Bio-One Vacutainer Ref #455071

*Blood is collected at the following time points: Prior to Cycles 1 and 3.

Shipping:
PBMCs are to be sent on the day of draw; serum is to be frozen and batch-shipped at intervals. **Please note, PBMCs cannot be shipped within 72 hours after a bone scan injection due to the Technetium-99 radioisotope. If a bone scan is scheduled, PBMCs must be drawn prior to bone scan injection.

Materials:

- Pack blood vials in the EXAKT-PAK shipping kits described below or equivalent shipping materials:

  For ambient PBMCs:
  EXAKT-PAK for Vials Category B D-Pak MD8702V06 (Accommodates 6 vials)
  Includes Inner pack (Ambient) and Insulated Cooler
  With 2 cool packs per cooler, part #CP1003, room temperature or slightly cooled (not frozen) to keep samples between 18 and 30oC.

  For frozen serum:
  EXAKT-PAK Frozen Category B D-Pak MD8703V06
  Dry ice required (not included)

  EXAKT-PAK kits available at:
  https://www.exaktpak.com/store/
  EXAKT Technologies, Inc.
  Home office: 7002 N. Broadway Extension
  Oklahoma City, OK 73116-9006
  405-848-5800

Shipping Address:

- For Research Blood Samples, please FED-EX Overnight to the shipping address below:

  Leidos
  Biomedical Research
  Attn: Ludmila Krymskaya/Theresa Burks
  1050 Boyles Street

Bldg. 469/Room 121
Frederick, MD 21702
Phone 301-846-5125, or 301-846-1707

- Please notify the Frederick laboratory when specimens are being shipped. Please email Frederick prior to shipping to notify the lab.

- Emails should be sent to the following individuals:

  Ludmila Krymskaya, ludmila.krymskaya@nih.gov
  Theresa Burks, burkst@mail.nih.gov
  Caroline Jochems, jochemscm@mail.nih.gov

- Additionally, NO specimens should be shipped on Fridays or the day before a Federal holiday.

Labeling of Blood Samples:

- Please label research tubes with a coding mechanism. The following information should be contained on the label:

  - Site
  - Patient enrollment number and initials
  - Dose level or arm
  - Protocol number
  - Date drawn
  - Time point – such as 'baseline' or 'prior to cycle 3'