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**Title: Phase II Study of nab-Paclitaxel in Combination with Gemcitabine for Treatment of Recurrent/Refractory Sarcoma in Teenagers and Young Adults**


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**SCHEMA**

The combination of gemcitabine and docetaxel is widely used for recurrent pediatric bone and soft tissue sarcoma, although efficacy is suboptimal. Nab-Paclitaxel (Abraxane®) is a nanoparticle albumin-bound preparation of paclitaxel that is more active and less toxic than either paclitaxel or docetaxel in breast cancer patients. Compelling activity has been seen with nab-paclitaxel against the Pediatric Preclinical Testing Program panel of sarcoma xenografts, including maintained complete responses in multiple models of Ewing sarcoma and rhabdomyosarcoma. In addition, the combination of gemcitabine and nab-paclitaxel is now approved by the US Food and Drug Administration for treating pancreatic cancer, based on preclinical models and clinical trials showing potential synergy with these drugs. Taken together, these findings suggest nab-paclitaxel may be a better taxane for the treatment of pediatric sarcoma. To test this hypothesis, we will conduct a single-arm, multi-strata, open-label Phase II study to assess the activity of gemcitabine and nab-paclitaxel in teenagers and young adults with sarcoma. Following the schema below, patients age $\geq$12 years with recurrent or refractory Ewing sarcoma, osteosarcoma, rhabdomyosarcoma, or other soft tissue sarcoma will receive the previously established regimen of gemcitabine 1,000 mg/m$^2$ together with nab-paclitaxel 125 mg/m$^2$ on days 1, 8, and 15 of every 4-week cycle. A two-stage Simon design will be used, with up to 18 total patients enrolled in each of the four disease cohorts. The combination will be of sufficient interest for further study if the estimated rate of partial + complete responses is $\geq$ 35% for patients with Ewing and soft tissue sarcoma, and if the estimated 4-month progression-free survival is $\geq$ 35% for patients with osteosarcoma.

| | Nab-paclitaxel (125 mg/m$^2$ iv over 30 min) | Gemcitabine (1,000 mg/m$^2$ iv over 90 min) | Myeloid Growth Factor |
|---|---|---|---|
| Day 1 | X | X | |
| Day 8 | X | X | |
| Day 15 | X | X | |
| Day 16-18 | | | X |
| Day 16-28 | REST | | |

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# 1. OBJECTIVES

## 1.0 Primary Objectives

1. To assess the antitumor activity of nab-paclitaxel combined with gemcitabine in patients with relapsed or refractory osteosarcoma, Ewing sarcoma, rhabdomyosarcoma and other soft tissue sarcoma using RECIST 1.1 criteria and progression-free survival

## 1.1 Secondary Objectives

1. To describe the toxicities of the combination in adolescents and young adults with relapsed/refractory sarcoma
2. To assess the immunohistochemical expression of SPARC, CAV-1, and hENT1 in archival tumor tissue, and compare results with treatment response
3. To evaluate a set of radiomic biomarkers calculated from baseline and post-therapy CT scans, and compare results with treatment response.
4. To quantify circulating tumor cells (CTC) in patients with recurrent or refractory sarcomas, and determine if CTC changes reflect response to therapy or predict risk of progression.

# 2. BACKGROUND

## 2.0 Recurrent or Refractory Sarcomas in Adolescent and Young Adult Patients

Although many adolescent and young adults with localized bone or soft tissue sarcoma can become long-term survivors, more effective therapies are needed for patients who present with metastatic disease or whose tumors recur after completing therapy. For all the major subtypes of sarcoma seen in this age group, less than one-third of patients with metastatic or recurrent disease are cured, and new therapeutic strategies are clearly required. Attractive treatment options include those using commercially available drugs for which there is preclinical synergy and biologic rationale, as well as previously established well-tolerated outpatient regimens for administration.

Over the past decade, the combination of gemcitabine and docetaxel has been widely used as salvage therapy for relapsed or refractory pediatric-type sarcomas such as osteosarcoma, Ewing sarcoma, and rhabdomyosarcoma. As seen in Table 1 below, over 100 patients with such sarcomas have been treated in at least 6 published studies of this regimen in which gemcitabine is administered on days 1 and 8, and docetaxel is given on day 8 of the 3-week cycle. While single-institution retrospective series have reported complete or partial responses in as many as 24-50% of patients [1-3], the response rate in a prospective multi-institutional trial was < 15% [6]. These findings suggest that modifications to this treatment are necessary in order to improve activity to a sufficient level to justify investigation in clinical trials of newly-diagnosed patients.

Adolescents and young adults may also develop non-rhabdomyosarcomatous soft tissue sarcomas, which are termed adult-type sarcomas because of the frequency in older patients. Such histologies include synovial sarcoma, malignant peripheral nerve sheath tumor, high-grade undifferentiated sarcoma, and a wide variety of other sarcoma subtypes. Collectively these tumors make up 3-4% of all pediatric cancers. The combination of gemcitabine and docetaxel has been commonly used to treat adult-type soft tissue sarcoma, as shown in Table II. Though responses and prolonged event-free survival have routinely been observed in patients receiving this therapy, the issues of toxicity and suboptimal activity have kept this regimen from being used frontline in most adult sarcoma patients.

**Table 1. Key Trials Using Gemcitabine and Docetaxel for Recurrent Pediatric-Type Sarcoma**

| Author (ref) | Evaluable Patients | Population | Gemcitabine ($mg/m^2$) Days 1,8 | Docetaxel ($mg/m^2$) Day 8 | Response Rate (CR + PR) | Comments |
|---|---|---|---|---|---|---|
| Song (1) | 17 | OS | 675-900 | 100 | 24% | Retrospective analysis |
| Navid (2) | 14 | OS (10), ES (2), others | 675 | 75-100 | 29% all evaluable | RR 30% in OS |
| Mora (3) | 10 | ES (6), SS (2), OS (1) | 1,000 | 100 | 50% | RR 67% in ES; 50% in synovial sarcoma |
| Rapkin (4) | 18 | OS (6), RMS (5), ES (2), others | 675 | 75 | 11% for RMS | No objective responses in OS or ES |
| Qi (5) | 18 | OS | 675 | 75-100 | 6% | Retrospective analysis |
| Fox (6) | 28 | OS (14), ES (14) | 675 | 75 | 7% for OS 14% for ES | Prospective multi-institutional trial |

OS, osteosarcoma; ES Ewing sarcoma; SS, synovial sarcoma; RMS, rhabdomyosarcoma; RR, response rate

**Table II. Key Trials Using Gemcitabine and Docetaxel for Advanced/Metastatic Adult-Type Soft Tissue Sarcoma (STS)**

| Author (ref) | Evaluable Patients | Population | Gemcitabine ($mg/m^2$) Days 1,8 | Docetaxel ($mg/m^2$) Day 8 | Response Rate (CR + PR) | Comments |
|---|---|---|---|---|---|---|
| Maki (7) | 73 | Metastatic | 900 | 100 | 16% | Superior survival compared to GEM alone |
| Davis (8) | 29 | Localized, resectable | 900 | 100 | 3% | Longer disease-free survival compared to doxo/ifos ($p = 0.08$) |
| Seddon (9) | 128 | Advanced unresectable or metastatic | 675 | 75 | PFR at 24 weeks was 46% | Similar PFR-24 as doxorubicin, but more toxic |

PFR, progression-free rate; GEM, gemcitabine; doxo/ifos, doxorubicin/ifosfamide

## 2.1 Use of nab-Paclitaxel

Paclitaxel is an antimitotic agent that stabilizes tubulin by preventing depolymerization. Although paclitaxel is widely used in adult oncology to treat breast, lung, and ovarian cancer, toxicity can limit its use and require change to a different chemotherapy regimen. Conventional paclitaxel (Taxol®) is emulsified with the solvent Cremphor EL and ethanol, and this preparation is associated with hypersensitivity reactions and prolonged neuropathy related to the carrier vehicle. Another factor contributing to paclitaxel toxicity is its slow clearance rate, which leads to prolonged systemic exposure and resulting neutropenia.

Reformulation of paclitaxel appears to circumvent these drug delivery and pharmacokinetic limitations. Advances in nanotechnology have allowed such insoluble hydrophobic agents to become encapsulated using albumin nanoparticles. This new formulation, called nanoparticle albumin-bound paclitaxel (nab-paclitaxel, Abraxane®), is created through a proprietary high-pressure homogenization process in which nab-paclitaxel is non-covalently bound to albumin to create a 130 nM albumin particle form of paclitaxel [reviewed in 10]. The biochemical nature of this compound has important implications regarding its distribution into tissue, pharmacokinetics, and tolerance. For example, the albumin nanoparticle can bind to the endothelial cell receptor gp60, which induces caveolin-1 (CAV-1) to assist with internalization of

the albumin-substrate complex into caveolae and subsequently into the interstitial space. Once there, nab-paclitaxel gains entry into the tumor cell through the binding protein SPARC (secreted protein, acidic and rich in cysteine). This mechanism of drug transport into the tumor cell suggests that either CAV-1 and/or SPARC may be potential biomarkers to predict sensitivity to nab-paclitaxel, as discussed further in Section 2.3.4. Further, preclinical experiments suggest that nab-paclitaxel delivery to xenografted tumor cell is 33% greater compared to similar doses of paclitaxel [11], which contributes to the improved efficacy of this new agent as described below.

Because the carrier vehicle for nab-paclitaxel is albumin rather than Cremaphor XL, the incidence of hypersensitivity reactions is dramatically reduced, and obviates the need for the steroid and antihistamine premedication that is required with conventional paclitaxel. Nab-paclitaxel can also be given in a 30-minute infusion rather than the typical 180-minute infusion of paclitaxel, and no special tubing or filters are required for intravenous administration. The absence of Cremaphor and ethanol may also be related to the more rapid resolution of neuropathy when nab-paclitaxel is compared to carrier-based paclitaxel, as both Cremaphor and ethanol have been shown to have direct toxic effects on neurons such as axonal degeneration that can be long-lasting.

Finally, nab-paclitaxel has more predictable first-order pharmacokinetics, with rapid clearance and a large volume of distribution consistent with more widespread tissue penetration. This is in contrast to carrier-based paclitaxel, in which clearance is prolonged and may be related to myelosuppression caused by extended exposure of low-level paclitaxel [12]. The reduction in toxicity with this new formulation allows for 50% higher paclitaxel doses to be given, resulting in activity in preclinical models and patients whose tumors were resistant to paclitaxel [reviewed in 10, 11].

### 2.1.1 Clinical Experience with nab-Paclitaxel in Adults

In the initial phase I study of adults with melanoma or breast cancer, the maximum tolerated dose (MTD) of nab-paclitaxel was 300 mg/m2 when given as a 30-minute intravenous infusion every three weeks [13]. Dose-limiting toxicities (DLT) were sensory neuropathy, stomatitis, and superficial keratopathy, with only one patient having DLT (neuropathy) at the MTD. Other side effects included modest myelosuppression and nausea. Importantly, there were no hypersensitivity reactions reported when using a 30-minute infusion with no steroid or antihistamine premedication.

Linear pharmacokinetics were observed following nab-paclitaxel administration, with a rapid decrease in plasma levels as drug is distributed to peripheral tissues, followed by slower second phase of drug elimination. In a comparison study of nab-paclitaxel 260 mg/m2 over 30 min vs paclitaxel 175 mg/m2 over 180 min, the maximum exposure to unbound paclitaxel was nearly 3-fold higher for nab-paclitaxel [14]. Although the elimination half-lives were similar (approximately 21 hours), nab-paclitaxel had greater clearance from plasma and greater volume of distribution, suggesting improved tissue binding. The metabolism and excretion of nab-paclitaxel is the same as the conventional formulation, with drug metabolized by the liver into the primary metabolite 6-alpha-hydroxypaclitaxel.

Subsequent clinical trials have examined different administration schedules for nab-paclitaxel, and in direct comparison the greatest activity has been seen when it is given weekly for 3 weeks followed by one week of rest [15]. The MTD using this 3-of-4 schedule has ranged from 100 to 150 mg/m$^2$, depending on the extent of patient pretreatment [16]. The toxicity profile does not appear to be different with this schedule compared to administration once every three weeks. The grade 3-4 toxicities most commonly encountered with once-weekly nab-paclitaxel administration at the MTD are neutropenia (44%), and neuropathy (14%). When nab-paclitaxel was compared to single-agent docetaxel 100 mg/m$^2$ q 3 weeks in a phase III trial, docetaxel had much greater myelosuppression and similar neurotoxicity, with grade 3-4 neutropenia in 94% and grade 3 neuropathy in 12% of patients [15]. Of note, the grade 3 neuropathy encountered with nab-paclitaxel resolved to $\leq$ grade 2 faster than for patients receiving

docetaxel (22 vs. 37 days), and was readily managed with dose reduction. The incidence of febrile neutropenia and grade 3 fatigue in this study was 8% and 19% for docetaxel vs. 1% and 3% for nab-paclitaxel, respectively. Important for the consideration of heavily-pretreated sarcoma patients, grade 4 thrombocytopenia with single-agent ABX is unusual, and has been reported to occur in < 5% of adult patients [10].

**Table III. Comparison of toxicities in adults with metastatic breast cancer receiving either nab-paclitaxel 150 mg/m$^2$ weekly or docetaxel 100 mg/m$^2$ q 3 weeks**

| | Nab-paclitaxel | Docetaxel |
|---|---|---|
| Grade 4 neutropenia | 9% | 75% |
| Grade 3 neuropathy* | 14% | 12% |
| Grade 3 fatigue | 3% | 19% |
| Febrile neutropenia | 1% | 8% |
| Median progression-free survival | 12.9 mo | 7.5 mo (p=0.0065) |

*resolved to ≤ grade 2 in 22 days (nab) vs 37 days (paclitaxel)

In terms of efficacy assessed in randomized phase III trials, single-agent nab-paclitaxel showed superior response rates and disease control in breast cancer patients compared to either docetaxel [15] or paclitaxel [17]. When nab-paclitaxel was combined with carboplatin in a phase III trial of lung cancer patients, that combination showed greater efficacy and less toxicity than carboplatin + paclitaxel [18]. These studies led to FDA approval of nab-paclitaxel as a single agent for metastatic breast cancer, and in combination with carboplatin for non-small cell lung cancer.

### 2.1.2 Preclinical Experience with nab-Paclitaxel in the Treatment of Pediatric Tumors

Early preclinical studies using mouse models of adult cancers demonstrated that at equivalent doses, nab-paclitaxel had 33% greater intratumoral accumulation of drug compared to conventional paclitaxel [11]. As with humans, mice are able to tolerate higher doses of nab-paclitaxel compared to conventional paclitaxel, and studies comparing equitoxic doses show nab-paclitaxel has superior activity in mouse models of adult carcinomas [11] as well as in rhabdomyosarcoma [19] and osteosarcoma [20]. Also noted was tumor regression when nab-pacitaxel therapy was substituted in mice whose xenografted rhabdomyosarcoma tumors were progressing on paclitaxel [19].

The most comprehensive and impressive demonstration of activity of single-agent nab-paclitaxel has been reported by the National Cancer Institute's Pediatric Preclinical Testing Program. They demonstrated "high" activity in 5 of 8 Ewing sarcoma models tested, including 4 models with maintained complete responses [21]. Similar responses were seen in the rhabdomyosarcoma panel, in which 5 of 8 models also had "high" activity, with 5 maintained complete responses. To date, 4 different laboratories have now reported on the activity of nab-paclitaxel in a total of 9 models of Ewing sarcoma, 10 rhabdomyosarcoma, and 4 osteosarcoma, with notable activity being seen in the majority of these models [19-22]. These results have led to an ongoing pediatric Phase I trial of single-agent nab-paclitaxel in children with recurrent solid tumors [clinicaltrials.gov identifier NCT01962103].

### 2.1.3 Nab-Paclitaxel in Combination with Gemcitabine

The modest toxicity profile of nab-paclitaxel makes it an excellent drug to consider for combination chemotherapy, as demonstrated by the success seen when given together with carboplatin in lung cancer patients [18]. In this regard, gemcitabine may also be a suitable partner for nab-paclitaxel. Gemcitabine is a nucleoside analog which inhibits DNA replication by blocking DNA synthesis and disrupting usual repair mechanisms. Gemcitabine is FDA approved for the treatment of ovarian, breast, lung, and pancreatic cancer, and a wide variety of combination therapies have been investigated. In adults, the most common toxicities include mild-to-moderate myelosuppression, nausea, fever, rash, flu-like

symptoms, and swelling of the legs.

In a phase I trial of children with refractory solid tumors, gemcitabine was administered once weekly for three weeks followed by a one-week rest [23]. Seventeen evaluable patients were treated on this schedule, and the MTD was 1,200 mg/m2. At this dose, only one of eight patients experiencing DLT (grade 3 transaminitis that did not resolve within one week). At the higher dose of 1500 mg/m2, myelosuppression was dose-limiting. The pediatric MTD of 1,200 mg/m2 in heavily pretreated children compares with a MTD of 790 mg/m2 established in a phase I trial of heavily pretreated adults receiving a similar schedule [24].

In a phase II study of twenty pediatric patients (median age 15 years) with recurrent solid tumors who were treated for three consecutive weeks, myelosuppression was the prominent toxicity [25]. The study design called for dose reductions with any grade 3 or 4 toxicity, and approximately one-third of total doses were reduced. Only one patient had fever, one had grade 3 nausea, and none had grade 3-4 transaminitis. A second phase II study of relapsed sarcoma patients using gemcitabine 1,000 mg/m2 weekly for 7 weeks before transitioning to a weekly x3 schedule also demonstrated that gemcitabine is well tolerated in relapsed sarcoma patients, with no grade 4 hematologic toxicity and only 17% of patients experiencing grade 3 neutropenia or thrombocytopenia [26].

Preclinical studies suggest that gemcitabine may be synergistic with nab-paclitaxel. Possible mechanisms for this include increased intracellular concentrations of gemcitabine via changes in the tumor stroma and vasculature [27], and/or reduction of the primary gemcitabine metabolizing enzyme, cytidine deaminase [28]. These mechanisms may be responsible for the 2.8-fold increase in intracellular gemcitabine concentrations seen with this combination compared to treatment with gemcitabine alone [27]. Consistent with the synergy seen with adult tumors, combining nab-paclitaxel and gemcitabine showed improved activity in a mouse model of osteosarcoma when compared to treatment with each individual agent [22].

The combination of gemcitabine and nab-paclitaxel has been most thoroughly evaluated in pancreatic cancer. In a phase I/II trial of adults with untreated advanced pancreatic cancer, the MTD was 1,000 mg/m2 of gemcitabine plus 125 mg/m2 of nab-paclitaxel given once weekly for 3 weeks, followed by one week of rest [27]. For the 44 patients treated at this dose, grade 4 neutropenia was seen in 49% of patients, although only one patient experienced febrile neutropenia. Only 9% of patients had grade 4 thrombocytopenia, and 20% had grade 3 neuropathy.

In a larger phase III trial of adults with newly-diagnosed metastatic pancreatic cancer, the combination of gemcitabine and nab-paclitaxel on the above schedule was compared with gemcitabine alone weekly for 7 of 8 weeks [29]. Key toxicities and use of growth factor, done at the treating physician's discretion, are reported in Table IV. Although the rates of peripheral neuropathy and myelosuppression were somewhat higher with the combination, there was significant improvement in the progression-free and overall survival. These findings led to US FDA approval of the combination for this patient population.

**Table IV. Comparison of toxicities and growth factor use in adults with advanced pancreatic cancer receiving gemcitabine +/- nab-paclitaxel in a phase III trial**

| | Nab + Gem (n=421) | Gem Alone (n = 402) |
|---|---|---|
| Grade $\geq$ 3 neutropenia | 38% | 27% |
| Grade $\geq$ 3 thrombocytopenia | 13% | 9% |
| Febrile neutropenia | 3% | 1% |
| Grade $\geq$ 3 fatigue | 17% | 7% |
| Grade $\geq$ 3 neuropathy | 17% | 1% |

| | | |
|---|---|---|
| Received growth factor | 26% | 15% |
| Response rate | 23% | 7% ($p < .001$) |
| 1-year survival | 35% | 22% ($p < .001$) |

## 2.2 Rationale

Given the preclinical activity of nab-paclitaxel against pediatric sarcoma xenografts, as well as the tolerance and activity of gemcitabine and nab-paclitaxel seen in pancreatic cancer patients, nab-paclitaxel may be a superior partner for gemcitabine in treating adolescents and young adults with sarcoma. We hypothesize that this combination will be more tolerable and efficacious than the gemcitabine + docetaxel regimen widely being used, and that sufficient response rates and progression-free survival will be seen in these high-risk patients to justify further investigation in larger trials of newly-diagnosed patients. There currently is an ongoing phase I/II trial of single-agent nab-paclitaxel for pediatric patients with recurrent solid tumors. However, that trial is not expected to complete accrual until 2018, and experience would suggest that single-agent activity is unlikely to be as efficacious as combination cytotoxic chemotherapy for pediatric sarcoma. If the combination has the desired degree of activity, then additional studies could be performed to determine whether the gemcitabine is needed. If activity on this study is poor, it is unlikely that further studies with single-agent nab-paclitaxel will be performed for sarcoma. We therefore feel it is appropriate to move ahead with this phase II proposal, using the dosing and schedule of administration employed that has been previously approved by the FDA for pancreatic cancer.

## 2.3 Patient Population

Patients who have recurrent or progressive disease following frontline treatment for osteosarcoma or Ewing sarcoma have a dismal prognosis, with less than one-fifth of patients achieving long-term cure. Outcomes are also poor for those with recurrent or refractory rhabdomyosarcoma, especially in those with the aggressive alveolar subtype. The majority of patients with these particularly high-risk bone or soft tissue sarcomas are teenagers or young adults. Given the established tolerability of the proposed drug combination in adults, it seems reasonable to directly extend this treatment to patients of age $\geq$ 12 years with recurrent/refractory OS, ES, or RMS. A similar approach has been employed before by the Children's Oncology Group, in which the adult recommended Phase II dosage of the FDA-approved agent eribulin is being used in teenagers with recurrent osteosarcoma without first performing a phase I dose-finding trial (clinical trials.gov identifier number NCT02097238).

## 2.4 Dose and Schedule of Administration

We plan to use the standard doses (gemcitabine 1,000 mg/m$^2$; nab-paclitaxel 125 mg/m$^2$) and schedule of administration (both drugs weekly for 3 of 4 weeks) that are approved by the FDA for combination use. This scheduled is detailed below:

| | Nab-paclitaxel (125 mg/m$^2$ iv over 30 min) | Gemcitabine (1,000 mg/m$^2$ iv over 90 min) | **Myeloid Growth Factor** |
|---|---|---|---|
| Day 1 | X | X | |
| Day 8 | X | X | |
| Day 15 | X | X | |
| Day 16-18 | | | X |
| Day 16-28 | REST | | |

Maintaining dose intensity is reasonable given there is some suggestion of higher response rates when higher doses of gemcitabine and taxanes are used [1,3]. However, in order to balance the potential benefit of intensive therapy with safety concerns, the day 15 chemotherapy will be held in any patient who experiences grade 4 neutropenia (absolute neutrophil count < 500/mcL) on or before that day of the cycle. In that situation, patients will be subject to dose de-escalation with additional cycles as detailed in Section 6. Similarly, patients who experience grade 3 neuropathy will have nab-paclitaxel held for the remainder

of the cycle. Other dose modifications may be made based on toxicities, and are detailed in Section 6.

Additional data collected from three patients treated on study suggest that removal from study because of the occurrence of dose-modifying hematologic toxicity as defined in Section 6 may not be necessary. These patients all experienced dose-modifying hematologic toxicity despite the planned use of pegfilgrastim and dose modifications. They were therefore removed from study, but since their physicians felt they were deriving clinical benefit from the treatment and the drugs are commercially available, they simply continued with therapy using gemcitabine and nab-paclitaxel. These patients tolerated continued therapy using reduced doses (gemcitabine 675 mg/m2/dose; nab-paclitaxel 100 mg/m2/dose) for 1-9 additional cycles, with no treatment delays or infectious toxicity noted. Importantly, one of the patients went on to experience a partial response with this extended off-protocol therapy. That patient used a modified schedule in which drugs are administered on days 1 and 8 in 21-day cycles, with pegfilgrastim given on day 9. This treatment regimen has been studied in a phase II trial for patients with metastatic breast cancer, with response rates similar to historical controls treated on the standard 28-day schedule. This 21-day schedule reduces the overall intensity by 11%, and is similar to the schedule of gemcitabine + docetaxel used previously for recurrent sarcoma [43]. Given this information, patients on this study with stable or responding disease who experience a dose-modifying hematologic toxicity after dose reduction may continue on study using the 21-day schedule described above and detailed in Section 6.

## 2.5 Study Design

This is a single-arm, multi-strata, open-label phase II study to assess the activity of nab-paclitaxel and gemcitabine in 4 disease types: osteosarcoma (OS), Ewing sarcoma (ES), rhabdomyosarcoma (RMS), and non-rhabdomyosarcoma soft tissue sarcoma (NRSTS). Osteosarcoma tumors are often composed of calcium, which historically in previous studies has confounded the assessment of response in some patients as calcified tumors are less likely to change in size even when anti-tumor therapy is effective. This particular feature of osteosarcoma has led the Children's Oncology Group (COG) to use progression-free survival (PFS) at 4 months as the preferred endpoint for phase II studies of relapsed osteosarcoma, as demonstrated in the most recent study [clinical trials.gov identifier number NCT02097238]. In a review of seven past COG phase II studies involving 95 evaluable patients, the median PFS was 12% at four months. Assessment of 4-month PFS therefore will be used in this study, with the threshold of $\geq$ 35% being the level of interest for further study.

For the other disease cohorts (ES, RMS, and NRSTS), the more traditional partial + complete response rate using RECIST 1.1 will be used. The threshold for further interest will also be a response rate of $\geq$ 35%, as used in previous COG studies for these tumor types [NCT01614795]. In contrast, the combination will be of insufficient interest if the 4-month PFS is <10% for OS patients, or the response rate is < 10% for patients with ES, RMS, or NRSTS.

Secondary endpoints include toxicity assessment using Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0, and immunohistochemical assessment of putative biomarker expression in archival tumor tissue for patients who consent to have this testing performed. We will also perform Radiomic analysis of the baseline and follow-up CT scans of patients.

A Simon two-stage design will be used to enroll patients to each of the four cohorts. As detailed in Section 13, eleven patients from each cohort will be enrolled in Stage 1, and seven additional patients may be added to the cohort if at least 2 responses are seen in the first 11 patients of that cohort. If the combination has a true response rate (or 4-month PFS for OS patients) of 10%, the two-stage design described above will identify it of sufficient activity for further study with probability 0.10 (type I error). If the combination has a true response rate (or 4-month PFS for OS patients) of 35%, the probability of identifying it of sufficient activity for further study with probability 0.91 (power against the alternative hypothesis $P$ = .35).

## 2.6 Correlative Studies

### 2.6.1 Immunohistochemical Expression of Putative Biomarkers in Archival Tumor Tissue

As described above, caveolin-1 (CAV-1) facilitates the passage of nab-paclitaxel across the endothelial cell membrane and into the tumor interstitium. From there, drug enters the tumor cell via the adhesion protein SPARC (secreted protein, acidic and rich in cysteine), which normally mediates the passage of albumin. In some tumor types, SPARC expression in either tumor cells or stroma correlates with the activity of nab-paclitaxel [27,30,31], although this association has not always been consistently identified [32]. SPARC expression is nearly universal in osteosarcoma [33], and high expression is correlated with worse prognosis [19]. SPARC expression has also been reported in tumor samples of patients with recurrent or metastatic Ewing sarcoma [22,34]. In addition, CAV-1 is expressed in both osteosarcoma as well as Ewing sarcoma, especially in patients with metastatic disease [33,34]. These findings suggest the possibility that expression of one or both of these proteins may be correlated with the activity of nab-paclitaxel regimens in pediatric sarcoma. In this study, protein expression of these putative markers will be assessed by immunohistochemistry analysis of archival tumor samples, using the most recent tumor sample already available. In addition, we will also assess the immunohistochemical expression of hENT1, which has been reported to be associated with improved outcomes in patients with pancreatic cancer [41] and sarcoma [42] receiving gemcitabine. This testing will be conducted by Dr. Hong Yin at Children's Healthcare of Atlanta, using previously validated immunohistochemical assays [22].

Please see lab manual for shipping instructions of correlative studies.

### 2.6.2 Radiomic Analysis of Diagnostic CT Images

Radiology has traditionally been a qualitative and subjective science. Advances in medical imaging and image analysis technologies now permit the high-throughput extraction of quantitative image features and conversion of digital medical images into mineable high-dimensional data. This process, known as radiomics, is motivated by the concept that biomedical images contain information that reflects underlying pathophysiology and that these relationships can be revealed via quantitative image analyses [35]. Quantitative radiomic features extracted from diagnostic CT scans show promise in predicting patient survival in NSCLC [36]. In a study of 1019 patients with lung or head-and-neck cancer, Aerts, Gillies, and colleagues analyzed 440 radiomic features to quantify tumor phenotypic differences based on image intensity, image texture and lesion shape, and identified a large number of features with prognostic power [37]. In a sub-study of 20 patients with histopathologically proven, measurable, primary STS or isolated local recurrence of STS after previous surgery, but without metastatic disease, who were treated with bevacizumab, radiotherapy and surgical resection, CT image texture features were associated with tumor angiogenesis and overall survival [38,39]. In this study, quantitative image features will be extracted from baseline and follow-up diagnostic CT scans, and the radiomic features at baseline, as well as changes in radiomic features between baseline and follow-up scans, will be compared with treatment response. Lesion delineation and RECIST 1.1 measurements will be performed by Dr. Rikesh Makanji at the Moffitt Cancer Center using the recently FDA-approved HealthMyne® PACS (www.healthmyne.com). Radiomic analysis of delineated lesions will be performed by Dr. Natarajan Raghunand and Dr. Olya Stringfield in the IRAT Core at the Moffitt Cancer Center, also using the HealthMyne platform to which radiomics capabilities have been added in an ongoing collaboration between HealthMyne and Dr. Gillies and other radiomics researchers at the Moffitt Cancer Center.

### 2.6.3 Minimal Residual Disease Studies

In addition, we will use this study population to gather preliminary data regarding biomarkers of Minimal Residual Disease (MRD) in sarcoma patients. MRD studies in sarcoma remain in their infancy, and it remains unclear if molecular studies, such as quantification of plasma tumor DNA (ptDNA) or circulating tumor cells (CTC), will provide superior results to imaging, including early evidence of

response and early hints of impending relapse. In this study, blood will be collected from subjects at the time of enrollment, after their first cycle of therapy, and in conjunction with each imaging event, and will be shipped to the laboratory of Dr. Masanori Hayashi at University of Colorado. CTC analysis will be performed in real time, while plasma will be cryopreserved in aliquots and analyzed in batches.

For plasma analysis, we will assess aberrations in genomic, epigenomic, and transcriptional profiles, as well as variations defined by future technologies. Circulating tumor profiling data at baseline and all timepoints will be compared. When available, we will utilize the same archival tissue as described in section 2.6.1 for additional comparisons to circulating tumor material.

Please see the study lab manual for instructions on submitting the following: archival tumor tissue, CT images, and biomarker specimens. Section 10.5 provides instructions for standardized diagnostic CT acquisition parameters.

## 3. PATIENT SELECTION

### 3.0 Eligibility Criteria

**3.0.1** Patients must be age ≥3 years and ≤30 years, and have had a histologic diagnosis of osteosarcoma, Ewing sarcoma, or rhabdomyosarcoma or non-rhabdomyosarcoma soft tissue sarcoma either at diagnosis or relapse.

PATIENTS MUST HAVE EXPERIENCED RELAPSE AFTER FRONT-LINE THERAPY, OR HAVE HAD DOCUMENTED DISEASE PROGRESSION DURING FRONT-LINE THERAPY.

**3.0.2** All patients must have measurable disease that can be assessed using RECIST 1.1 criteria, which is defined as the presence of at least one lesion on MRI or CT scan that can be accurately measured with the longest diameter of 10 mm in at least one dimension. For this phase II trial, patients with disease limited to bone or marrow metastases are NOT eligible, as disease at these sites cannot be assessed by RECIST 1.1 criteria.

**3.0.3** Patients must have relapsed or refractory cancers for which there is no known curative option.

**3.0.4** Prior Therapy for Treatment of Cancer: There is no limit to the number of prior therapies provided all eligibility criteria are met. However, patients must have recovered from the acute toxic effects of all prior treatment.

a. Patients must not have received prior therapy with either gemcitabine or nab-paclitaxel.
b. Myelosuppressive chemotherapy: Patients must not have received myelosuppressive chemotherapy within 3 weeks of protocol therapy on this study.
c. Hematopoietic growth factors: 7 days must have elapsed from the start of protocol therapy since the completion of therapy with filgrastim, and 14 days must have elapsed from the start of protocol therapy after receiving pegfilgrastim.
d. Biologic (anti-neoplastic agent): 7 day must have elapsed from the start of protocol therapy since the completion of therapy with a biologic agent.
e. Monoclonal Antibodies or Antibody Drug Conjugates: 3 half-lives or 28 days from the last dose, whichever is shorter, provided all toxicities have resolved, must have elapsed prior to the start of protocol therapy.

f. Radiotherapy: 2 weeks must have elapsed from the start of protocol therapy since local palliative XRT (small port); 3 months must have elapsed if 50% radiation of pelvis; 6 weeks must have elapsed if other substantial bone marrow irradiation was given.
g. Stem Cell Transplant or Rescue: No evidence of active graft vs. host disease and 2 months must have elapsed from the start of protocol therapy since transplant.

**3.0.5** Karnofsky performance score must be ≥ 60 (see Appendix A)

**3.0.6** Patients must have organ and marrow function as defined below:

- absolute neutrophil count ≥ 1,000/mcL
- platelets ≥ 100,000/mcL
- total bilirubin within normal institutional limits
- AST(SGOT)/ALT(SGPT) ≤ 2.5 × institutional upper limit of normal
- Serum creatinine within upper institutional limits

  OR
- creatinine clearance ≥ 60 mL/min/1.73 $m^2$ for patients with creatinine levels above institutional normal.

**3.0.7** Neuropathy: Patients must have ≤ grade 1 neuropathy at enrollment.

**3.0.8** CNS Metastases: Patients with known central nervous system metastases are excluded unless treated surgically or with radiotherapy and stable with no recurrent lesions for at least 3 months from the start of protocol therapy.

**3.0.9** Contraception: The effects of gemcitabine and nab-paclitaxel on the developing human fetus are unknown. For this reason and because these agents may be teratogenic, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception prior to the study, for the duration of study participation, and 4 months after completion of gemcitabine and nab-paclitaxel administration.

**3.0.10** Consent: Patients must have the ability to understand and the willingness to sign a written informed consent or assent document.

## 3.1 Exclusion Criteria

**3.1.1** Patients who are receiving any other investigational agents are ineligible.

**3.1.2** Patients must not be receiving any additional medicines being given for the specific purpose of treating cancer.

**3.1.3** Patients with a history of allergic reactions attributed to docetaxel or paclitaxel are ineligible.

3.1.4 Concomitant Medications: The metabolism of paclitaxel is catalyzed by CYP2C8 and CYP3A4. The following medicines should be avoided on this study because of their ability to inhibit or induce with CYP2C8 or CYP3A4:

Inhibitors: ketoconazole and other imidazole antifungals, erythromycin, fluoxetine, gemfibrozil, cimetidine, ritonavir, saquinavir, indinavir, and nelfinavir

Inducers: Rifampicin, carbamazepine, phenytoin, efavirenz, and nevirapine

Patients receiving any of the above medications are ineligible.

**3.1.5** Patients are ineligible if they have uncontrolled intercurrent illness including, but not limited to:

- ongoing or active infection
- symptomatic congestive heart failure
- unstable angina pectoris
- cardiac arrhythmia
- psychiatric illness/social situations that would limit compliance with study requirements

**3.1.6** Pregnancy and Breastfeeding: Pregnant women are excluded from this study because nab-paclitaxel and gemcitabine are agents with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with these drugs, breastfeeding should be discontinued if the mother is treated on this study.

**3.1.7** HIV Infection: HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with the study medications. In addition, these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate studies will be undertaken in patients receiving combination antiretroviral therapy when indicated.

**3.1.8** Patients who in the opinion of the investigator may not be able to comply with the safety monitoring requirements of the study are not eligible.

## 3.2 Inclusion of Women and Minorities

NIH policy requires that women and members of minority groups and their subpopulations be included in all NIH-supported biomedical and behavioral research projects involving NIH-defined clinical research unless a clear and compelling rationale and justification establishes to the satisfaction of the funding Institute & Center (IC) Director that inclusion is inappropriate with respect to the health of the subjects or the purpose of the research. Exclusion under other circumstances must be designated by the Director, NIH, upon the recommendation of an IC Director based on a compelling rationale and justification. Cost is not an acceptable reason for exclusion except when the study would duplicate data from other sources. Women of childbearing potential should not be routinely excluded from participation in clinical research. Please see http://grants.nih.gov/grants/funding/phs398/phs398.pdf.

## 3.3 Informed Consent Process

Eligible patients will be evaluated by a multi-disciplinary team at the site. During their visit, either a physician or a research coordinator will explain the study to the patient. Informed consent form will be provided to the patient, who will be given time to read over and ask questions of the study team. If the patient agrees to participate, a signed consent form will be obtained prior to participation and a copy of the signed consent will be provided to the patient.

## 4. REGISTRATION PROCEDURES

### 4.0 Site and Investigator Registration with Moffitt Cancer Center

Only those members of the Sunshine Project Consortium registered with Moffitt Cancer Center may participate and enroll patients on this trial. Sunshine Project Consortium Member sites are defined as those centers who have established financial agreements with Moffitt Cancer Center and who have designated a local Principal Investigator who will assume the responsibility of conduct of the trial according to MCRN procedure and Federal and State regulations (Policy MRI-P.CRO. 41). Additionally, Consortium member sites are those sites that have agreed to, and signed, the Site Responsibility page located in the Sunshine Project Handbook.

All patients intended to be enrolled on this trial must be registered with the Moffitt Cancer Center Clinical Research Network office and the Sunshine Project Coordinator (Coordinator) prior to being enrolled at the local level. Patients may not be treated with any agent without registration with Moffitt staff and an associated sequence number confirmed in the Moffitt Cancer Center electronic database OnCore.

### 4.1 Patient Registration

When a potential patient presents to a participating site, the local investigator will confirm that the patient is a candidate for screening by reviewing known inclusion and exclusion criteria. The participating site must notify the coordinating center to confirm slots are available for participation, and then discuss the protocol with the patient.

Before any patient can be enrolled, it is the responsibility of the institutional investigator to verify, sign, and date a completed Moffitt Cancer Center Eligibility Checklist and Moffitt Cancer Center Registration sheet.

The local investigator will confirm all eligibility criteria are met. A signed copy of the Checklist and Registration sheet must be faxed, with supporting source documentation verifying inclusion and exclusion criteria and signed consent form, to the Sunshine Project Coordinator prior to a patient being enrolled and receiving drug.

The source documentation and registration sheets must be faxed to the Sunshine Project Coordinator at Moffitt Cancer Center via email at SunshineProject@moffitt.org .

Please note: No study therapy may be initiated before confirmation that the subject has been enrolled on therapy.

### 4.2 General Guidelines

Following registration, patient information must be placed in the OnCore system within 24 hours of receipt of Subject Sequence Number (OnCore CRF pages: demographics, consent, eligibility, on-study, and treatment).

Additionally, treatment should begin within 5 days of registration. Issues that would cause treatment delays must be discussed with the Principal Investigator and Coordinator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled.

## 5. TREATMENT PLAN

### 5.0 Agent Administration

Treatment will be administered in 4-week cycles, in which patients will receive both nab-paclitaxel and gemcitabine on days 1, 8, and 15. Treatment may be given either in the outpatient clinic or in the hospital. For holidays and other schedule flexibility, doses can be moved +/- 2 days.

Following premedication with an antiemetic agent such as ondansetron or related agent, nab-paclitaxel will be given as a 30-minute infusion at the dose of 125 mg/m$^2$.

This will be immediately followed by gemcitabine 1,000 mg/m$^2$, given as a 90-minute infusion.

The following treatment schema will be used:

| | Nab-paclitaxel (125 mg/m$^2$ iv over 30 min) | Gemcitabine (1,000 mg/m$^2$ iv over 90 min) | Myeloid Growth Factor |
|---|---|---|---|
| Day 1 | X | X | |
| Day 8 | X | X | |
| Day 15 | X | X | |
| Day 16-18 | | | X |
| Day 16-28 | Myeloid growth factor, REST | | |

Patients will receive a course of myeloid growth factor beginning on day 16-18 of each cycle. Both chemotherapy agents will be held on day 15 if the patient experiences grade 4 neutropenia (absolute neutrophil count < 500/mcL) during the cycle on or prior to that day and be subject to dose de-escalation with additional cycles as detailed in Section 6. If grade 4 neutropenia is present at the day 8 visit, patients will still receive therapy on day 8.

Myeloid growth factor may consist of either a single dose of pegfilgrastim, or daily doses of filgrastim until the absolute neutrophil count is at least > 1,000/mcL.

Patients who experience grade 3 neuropathy will have nab-paclitaxel held for the remainder of the cycle. Held or missed doses will not be made up.

A complete list of dose modifications is included in Section 6. Patients with stable or responding disease who experience a dose-modifying hematologic toxicity despite dose reductions and pegfilgrastim, may continue treatment on study using an altered schedule of administration described in Section 6.

Reported adverse events and potential risks are described in Section 7.

Although antiemetic medications such as ondansetron or a related agent should be given prior to chemotherapy administration, antihistamine or steroid premedication is not required with nab-paclitaxel, nor is a special filter for intravenous administration.

### 5.0.1 Criteria for Starting Subsequent Cycles

A cycle may be repeated every 28 days if the patient has no clinical or radiographic evidence of progressive disease, and has again met laboratory parameters as defined by the eligibility section. Specifically, this means that platelets must be $\geq$ 100,000/mcL unsupported, and the absolute neutrophil count $\geq$ 1,000/mcL. In addition, other toxicities must have resolved to eligibility criteria prior to starting subsequent cycles.

## 5.1 General Concomitant Medication and Supportive Care Guidelines

Standard institutional guidelines should be followed for febrile illness, use of blood products, antiemetics, fluids and general supportive care.

This therapy is expected to cause lymphopenia and put patients at risk of developing *Pneumocystis* pneumonia. Therefore, prophylaxis with trimethoprim-sulfamethoxazole, pentamadine, or some other appropriate medication is indicated. Because there is a potential for interaction of nab-paclitaxel and/or gemcitabine with other concomitantly administered drugs, all concomitant medications will undergo a standard concomitant medication review at each study visit without data capture in the OnCore system. Please note: sites must be able to provide appropriate documentation for monitoring and review of all medications given while on study.

The Principal Investigator should be alerted if the patient is taking any agent known to affect or with the potential to affect selected CYP450 isoenzymes as described in Section 3.2.4.

## 5.2 Duration of Therapy

Patients may receive up to 12 cycles of protocol therapy (=52 weeks, 1 year) provided none of the following occur:

- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Patient decides to withdraw from the study
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Patient non-compliance with treatment or trial requirements: defined as any deviation from the protocol without prior agreement of the principal investigator
- Investigator non-compliance: defined as any significant medical or non-medical deviation from the protocol without agreement of the principal investigator
- Loss to follow-up

### 5.2.1 Patient Withdrawal

Patients may withdraw at any time or be dropped from the study at the discretion of investigator should untoward effects occur. In addition, a patient may be withdrawn by the investigator if he/she violates the study plan or for administrative and/or other safety reasons. The investigator or study coordinator must notify Moffitt immediately when a patient has been discontinued/withdrawn due to a serious adverse experience. All trial treatment-related toxicities and SAEs at the time of discontinuation/withdrawal should be followed until resolution or stabilization. Patients who are discontinued from the study will still be followed for disease progression.

### 5.2.2 Patient Death

All deaths that occur within the trial period or within 30 days after administration of the last dose of trial drug, regardless of relatedness, must be reported to Moffitt via the OnCore system for the purposes of serious adverse event (SAE) reporting.

Patients who discontinue from the study for reasons unrelated to the study (e.g., personal reasons, or adverse events after registration but prior to receiving study therapy) may be replaced as required for the study to meet its objective. The replacement will generally receive the same treatment or treatment sequence (as appropriate) as the allocation number replaced.

### 5.2.3 Off Protocol Treatment Criteria

For the purposes of this trial, “Off protocol treatment” and “Off Arm” date will be the **day the decision was made** to remove the patient from protocol therapy. The End of Therapy visit will be conducted at the time the decision was made to remove the patient from protocol therapy +/- 3 days.

### 5.2.4 Off Study Criteria

A patient is considered "Off study" after the follow period is completed. The "off study" date is considered the last day of Follow Up.

## 5.3 Duration of Follow Up

Patients that come off treatment due to progression of disease will be followed for 30 days (+/- 3 days) after end of treatment or until death, whichever occurs first. Patients that are removed due to an adverse event or they have completed all cycles of treatment, will be followed for progression free survival every 3 months until they have disease progression or they start a new treatment.

Patients that are removed due to an adverse event or they have completed all cycles of treatment, will be followed for survival every 3 months for 2 years or until death, whichever occurs first. Additionally, if the patients remain on gemcitabine and nabpaclitaxel therapy but off protocol, disease (SD, PR, CR, or PD) and survival status will be collected at these 3 month timepoints. Additional systemic therapies will not be collected. Any surgical resections or radiation therapy will also be collected qualitatively.

Additional testing may be performed as clinically necessary. All ongoing toxicities and adverse events will be followed until resolution, return to baseline or death within the 30 day follow up period. If the subject begins another line of therapy or is enrolled on another therapeutic trial, no new adverse events that may be attributed to that line of therapy will be collected during the 30 day follow-up period.

Serious adverse events are to be collected from the time the patient initiates protocol therapy until 30 days following discontinuation of protocol therapy

Follow-up data will be required unless consent is withdrawn. In all cases, the reason for withdrawal must be recorded in the CRF and in the subject's medical records.

## 5.4 Criteria for Removal from Study

Patients will be removed from study when any of the criteria listed in Section 5.3 applies. The reason and date for the removal from treatment and study must be captured in the OnCore eCRF.

## 5.5 Criteria for Being Considered Evaluable

### 5.5.1 Toxicity

Any patient who receives at least one dose of nab-paclitaxel or gemcitabine will be considered evaluable for toxicity.

### 5.5.2 Response

Any patient who is enrolled and receives at least one dose of nab-paclitaxel and gemcitabine will be considered evaluable for response provided: (1) the patient demonstrates progressive disease or death while on protocol therapy; (2) the patient is observed on protocol therapy for at least one cycle and the tumor is not removed surgically prior to the time complete response or partial response is confirmed; or (3) the patient demonstrates a complete or partial response according to protocol criteria. This definition excludes patients who stop therapy because of toxicity before the first disease evaluation, and such patients will be replaced for the purpose of assessing the primary objective of the study.

The evaluation period for determination of the best response will be 6 treatment cycles. All other patients will be considered non-responders.

### 5.5.3 Progression-Free Interval

Eligible patients who receive at least one dose of nab-paclitaxel and gemcitabine will be included in

analysis of progression-free survival. Progression-free interval (PFI) will be calculated as the date of enrollment until the end PFI date, where that date is calculated as the date of disease progression, date of death, date of removal of all tumor by surgery or last patient contact, whichever occurs first. Patients whose end PFI date is disease progression or death will be considered to have experienced an event. Patients whose end PFI date is date of removal of all tumor by surgery and this occurs prior to the 4 month time point will be considered removed from the analytic set at that time by a competing event and replaced. Patients whose end PFI date is date of removal of all tumor by surgery and this occurs after the 4 month time point will be considered to have met the primary endpoint. Patients whose end PFI date is the date of last patient contact will be considered censored for PFI analysis. The probability of remaining progression-free as a function of days since enrollment will be calculated according to the method of Gray, accounting for censoring and the competing events. The data from the osteosarcoma, Ewing sarcoma, rhabdomyosarcoma and non- rhabdomyosarcoma soft tissue sarcoma strata obtained from this study will be compared with a dataset of patients with the appropriate diagnosis from the cohort of patients treated on various previously reported studies for similar patients, using the method of Gray.

### 5.6 Replacement

If a patient is withdrawn from protocol therapy prior to receiving at least one dose of nab-paclitaxel and gemcitabine, a replacement patient will be enrolled in that cohort.

## 6. DOSING DELAYS/DOSE MODIFICATIONS

**All toxicities should be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0, which is located on the CTEP website at http://ctep.cancer.gov. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0.**

Hematologic and non-hematologic dose-modifying toxicities will be defined differently and will be based on events that occur any time during the study period following initiation of chemotherapy. In order to be declared as a dose-modifying toxicity, an adverse event must be possibly, probably, or definitely attributed to study therapy.

Dose-modifying toxicities and their required modifications are described in the table below:

| Toxicity | Gemcitabine Dose Change | Nab-paclitaxel Dose Change |
|---|---|---|
| Febrile neutropenia | Reduce to 675 mg/m$^2$ | Reduce to 100 mg/m$^2$ |
| ANC < 1,000/mcL by day 29 of cycle | Reduce to 675 mg/m$^2$ | Reduce to 100 mg/m$^2$ |
| ANC <500/mcL by day 15 | Hold treatment on day 15, AND reduce to 675 mg/m$^2$ for all subsequent cycles | Hold treatment on day 15, AND reduce to 100 mg/m$^2$ for all subsequent cycles |
| Platelet count < 20,000/mcL on 2 separate days during a cycle, **OR** requiring platelet transfusions on 2 separate days within a 7-day period | 675 mg/m$^2$ | 100 mg/m$^2$ |
| Platelet count < 100,000/mcL by day 29 of cycle | 675 mg/m$^2$ | 100 mg/m$^2$ |
| Grade 3 or greater neuropathy | No change | Immediately hold for duration of cycle, then resume next cycle at 100 mg/m$^2$ |
| Other grade 3-4 non-hematologic toxicities (*excluding those listed below) | 675 mg/m$^2$ | 100 mg/m$^2$ |

*excludes grade 3 nausea or vomiting of < 3 days duration, grade 3 ALT/AST that resolves to eligibility criteria by the start of the next cycle and does not recur, grade 3 fatigue < 3 days, or grade 3 electrolyte deficiencies that respond to supplementation

Patients who experience the same dose-modifying toxicities defined above even **AFTER** the appropriate dose reductions will be withdrawn from protocol therapy, with the **EXCEPTION** OF patients who have stable or responding disease and the dose-modifying toxicity is hematologic. Patient's meeting this criteria may continue to receive treatment on study using the following dose schedule [43].

| | **Nab-paclitaxel (100 mg/m$^2$ iv over 30 min)** | **Gemcitabine (675 mg/m$^2$ iv over 90 min)** | **Myeloid Growth Factor** |
|---|---|---|---|
| Day 1 | X | X | |
| Day 8 | X | X | |
| Days 9-21 | | | X |
| Days 9-21 | REST | | |

Patients treated on the 28-day schedule who do not meet eligibility criteria to start subsequent cycles by Day 43 will be removed from protocol therapy. Similarly, patients treated on the 21-day schedule who do not meet eligibility criteria by Day 36 will be removed from protocol therapy.

## 7. ADVERSE EVENTS: DEFINITIONS AND REPORTING REQUIREMENTS

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial (See section

7.3).

Additionally, certain adverse events must be reported in an expedited manner for more timely monitoring of patient safety and care. The following sections provide information about expedited reporting. The characteristics of an observed AE will determine whether the event requires expedited reporting via a Medwatch form and OnCore SAE eCRF to the Coordinating center **in addition** to routine reporting.

## 7.0 Adverse Event Characteristics

Characteristics of an Adverse Event must be characterized as follows:

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **Attribution** of the AE:
- Definite – The AE *is clearly related* to the study treatment.
- Probable – The AE *is likely related* to the study treatment.
- Possible – The AE *may be related* to the study treatment.
- Unlikely – The AE *is doubtfully related* to the study treatment.
- Unrelated – The AE *is clearly NOT related* to the study treatment.

## 7.1 Expedited Adverse Event Reporting (Serious Adverse Event)

### 7.1.1 Determination of Reporting Requirements:

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

### 7.1.2 Steps to Determine if an Adverse Event is to be Reported in an Expedited Manner

Step 1: Identify the type of event: The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

Step 2: Grade the event using the NCI CTCAE version 4.0.

Step 3: Determine whether the adverse event is related to the protocol therapy (investigational or commercial). Attribution can only be determined by the Investigator. Attribution categories are as follows: Unrelated, Unlikely, Possible, Probable, and Definite.

Step 4: Determine the prior experience of the adverse event. Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered unexpected, for expedited reporting purposes only, when either the type of event or the severity of the event is NOT listed in the investigational brochure or package insert for the investigational agent.

**If any of the following criteria are met, the Adverse Event should be reported in an expedited manner and will qualify as a Serious Adverse Event on this trial.**

A Serious Adverse Event is defined as any untoward medical occurrence that, at any dose, meets any of the following criteria:

- Results in death.
- Is life-threatening: The term 'life-threatening' in the definition refers to an event in which the patient was at risk of death at the time of the event, it does not refer to an event which hypothetically might have caused death if it were more severe.
- Requires inpatient hospitalization or prolongation of existing hospitalization: A hospitalization or prolongation of hospitalization will not be regarded as an SAE if at least one of the following exceptions is met:
    - The admission results in a hospital stay of less than 24 hours.
    - The admission is pre-planned. (i.e. elective or scheduled surgery arranged prior to the start of the study)
- The admission is not associated with an AE. (e.g. social hospitalization for purposes of respite care).
- Disability or Permanent Damage
- Congenital Anomaly/Birth Defect
- Other Serious (Important Medical Events)

Please see the FDA website: http://www.fda.gov/Safety/MedWatch/HowToReport/ucm053087.htm for more information regarding the definition of a Serious Adverse Event

### 7.1.3 Serious Adverse Event Reporting

Serious Adverse Event (SAE) reporting for this study must follow a two-step reporting process to comply with Moffitt AE reporting guidelines (Policy standard CTO.65).

Step 1: All SAEs will be reported to the MCRN Coordinating office in 24 hours of learning of the event via the OnCore eCRF. In addition, please email the Sunshine Project coordinator (SunshineProject@moffitt.org) that an SAE has been placed in the system.

The OnCore reporting system will require the following information:

- Event start date
- Event end date
- Reported date
- Date of death (if applicable)
- Event Narrative
- Treating Physician comments
- PI comments
- Attribution
- SAE Classification
- Adverse Event Details (CTCAE v. 4.0 category, grade, expectedness, attributions to all therapies administered, and any follow up action or therapy taken as a result of the SAE)

Step 2: Within 24 hours of learning of the event, download a Medwatch form: Form FDA 3500A – Mandatory Reporting from the FDA website: http://www.fda.gov/safety/medwatch/howtoreport/downloadforms/default.htm.

- Fill out required information
- Email Medwatch form to SunshineProject@moffitt.org.
- A follow up Medwatch form will be required to update all information captured on the original form.

Serious Adverse Event Reporting at Site Level:
All SAE's should be reported to the treating institutions IRB or Ethics board per local IRB policy. The Moffitt Cancer Center will also report all SAE's to the Moffitt IRB Supporting documentation for serious adverse events will be monitored and the following
should be available to support reported information:

- Laboratory reports
- Emergency room notes
- Hospital discharge summary
- Death certificate (if available)
- Autopsy report (if available)

REMINDER: **All Deaths** that occur while the subject is receiving a test article or intervention and/or a minimum of 30 days post administration of the test article or intervention will be reported as a Serious Adverse Event.

### 7.1.4 Distribution of Adverse Event and Serious Adverse Event Reports

Routine Adverse Events for each patient will be discussed on the Bi-Weekly calls held by the Coordinating center. The calls are a consistent bi-weekly check-in with PIs and site coordinators to ensure the continued safety of all patients receiving treatment, and for the distribution of information regarding dosing and drug toxicity seen in real time with enrolled patients.

The Sunshine Project Coordinators are responsible for the distribution of Serious Adverse Event reports (via Medwatch forms) to the enrolling sites participating on the trial. Sites are responsible for the reporting of those Serious Adverse Event reports to the local PI and IRB according to the site's reporting policy.

### 7.1.5 Expedited Reporting Guidelines- Death

**A death on study requires <u>both</u> routine and expedited reporting, regardless of causality. Attribution to treatment or other cause must be provided.**

Death due to progressive disease should be reported as **Grade 5 "Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (Progressive Disease)"** under the system organ class (SOC) of the same name. Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

## 7.2 Routine Adverse Event Reporting

Adverse events (AE) will be monitored throughout the course of the study. The National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v 4.0 will be utilized for AE reporting.

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. AEs are reported in a routine manner and must be collected at every assigned or unintended study visit and/or contact with the patient (phone call, email, etc.)

An adverse experience is defined as:

- Any unfavorable and unintended sign, symptom, or disease temporally associated with the use of an investigational product, whether or not related to the investigational product.

- An adverse event can result from use of the drug as stipulated in the protocol or labeling, as well as from accidental or intentional overdose, drug abuse, or drug withdrawal.
- Any worsening of a pre-existing condition or illness is considered an adverse event.
- Laboratory abnormalities and changes in vital signs are considered adverse events only if they result in discontinuation from the study, necessitate therapeutic medical intervention, be considered a dose limiting toxicity, and/or if the investigator considers them to be clinically significant.
- The investigator will evaluate all adverse experiences as to their severity and relationship to the investigational agent.

For this study, routine reporting will be done through study data submissions via the OnCore system. Routine reporting will include:

- All MedWatch reportable events
- Any toxicity considered dose-modifying (as per Section 6)
- Grade 2 or higher neuropathy
- Any Grade 3 or higher adverse events

A follow-up visit will occur 30 days post day of final treatment. All ongoing toxicities and adverse events will be followed until resolution, return to baseline or death for the 30 follow up period. If the subject begins another line of therapy or is enrolled on another therapeutic trial, no new adverse events that may be attributed to that line of therapy will be collected during the 30 day follow-up period.

Please note: All adverse events must be entered into the OnCore system **within 14 days** of the study visit. All AEs that qualify as SAEs must be reported on the OnCore system Adverse Event CRF log, listed as (Serious = Yes).

### 7.2 Second Malignancy

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

## 8. PHARMACEUTICAL INFORMATION

### 8.0 Nab-Paclitaxel (Abraxane®)

**8.0.1** Chemistry: Nab-paclitaxel is a Cremophor EL-free, albumin-bound paclitaxel particle with a mean size of approximately 130 nm. The chemical name is 5β,20-Epoxy-1,2α,4,7β,10β,13αhexahydroxytax-11- en-9-one 4,10-diacetate 2-benzoate 13-ester with (2R,3S)-N-benzoyl- 3phenylisoserin.

**8.0.2** Mechanism of Action – Nab-paclitaxel is an antimicrotubule agent that promotes the assembly of microtubules from tubulin dimers and stabilizes microtubules by preventing depolymerization. This stability results in the inhibition of the normal dynamic reorganization of the microtubule network that is essential for vital interphase and mitotic cellular functions. Paclitaxel induces abnormal arrays or "bundles" of microtubules throughout the cell cycle and multiple asters of microtubules during mitosis.

**8.0.3** Pharmacokinetics – Please see package insert for pharmacokinetics.

**8.0.4** Known Side Effects and Toxicities

The following side effects have occurred in adult patients taking nab-paclitaxel:

| Common (occurring in > 30% of patients) | Less Common (occurring in 20-29% of patients) | Uncommon (occurring in 10-19% of patients) | Rare (occurring in < 10% of patients) |
|---|---|---|---|
| • Anemia<br>• Neutropenia<br>• Thrombocytopenia<br>• Alopecia<br>• Peripheral neuropathy<br>• Fatigue<br>• Rash<br>• Nausea, vomiting<br>• Diarrhea<br>• Edema of hands and feet<br>• Poor appetite<br>• Epiphora<br>• Canalicular and nasolacrimal duct blockage | • Dehydration | • Myalgias<br>• Joint pain<br>• Weakness<br>• Mouth sores<br>• Dysgeusia<br>• Headache<br>• Hypokalemia<br>• Cough<br>• Depression | • Pneumonitis<br>• Hypersensitivity reactions, including anaphylaxis<br>• Hypertension |

**8.0.5** Pharmaceutical Data - Nab-paclitaxel is supplied as a white to yellow, sterile, lyophilized powder for reconstitution with 20 mL of 0.9% Sodium Chloride Injection, USP prior to intravenous infusion. Each single-use vial contains 100 mg of paclitaxel and approximately 900 mg of human albumin.

**8.0.6** Supply – Commercially available.

## 8.1 Gemcitabine

**8.1.1** Chemistry - Gemcitabine (2'-deoxy-2'2'-difluorocyti-dine monohydrochloride) is a purine analog structurally similar to cytarabine and an analog to deoxycytidine. Gemcitabine has two fluoride atoms in the geminal position of the second carbon of the ribose sugar.

**8.1.2** Mechanism of Action - Gemcitabine inhibits DNA synthesis in tumor cells by competing with deoxycytidine triphosphate for incorporation into DNA. Gemcitabine metabolites also inhibit enzymes in DNA synthesis. Finally, gemcitabine is masked from DNA repair enzymes with the addition of one additional nucleotide after gemcitabine is in the DNA chain.

**8.1.3** Pharmacokinetics - Gemcitabine is metabolized into active metabolites gemcitabine diphosphate and gemcitabine triphosphate. It is also metabolized to the inactive compound, gemcitabine difluorouridine. Ninety-nine percent of the dose is excreted in the urine and there is negligible protein binding. The serum half-life is significantly affected by decreases in creatinine clearance. However, there is no schedule for dose reduction in renal dysfunction.

**8.1.4** Known Side Effects and Toxicities - The primary dose limiting toxicity of gemcitabine is hematological including neutropenia, anemia and thrombocytopenia. Other toxicities include rash, constipation, diarrhea, fever, alopecia, pain, dyspnea and stomatitis. A summary of gemcitabine toxicities is listed below:

**Possible Side Effects of Gemcitabine:**

| | **Common**<br>Happens to 21-100 childrenout of every 100 | **Occasional**<br>Happens to 5-20 childrenout of every 100 | **Rare**<br>Happens to <5 childrenout of every 100 |
|---|---|---|---|
| **Immediate:** Within 1-2 days of receiving drug | • nausea<br>• vomiting<br>• fever<br>• rash[1]<br>• constipation<br>• pain<br>• dyspnea | • somnolence[1]<br>• diarrhea<br>• itching<br>• flu-like symptoms (fever,asthenia, anorexia, headache, cough, chills, myalgia)<br>• peripheral edema | • anaphylaxis<br>• bronchospasm<br>• phlebitis<br>• hypertension |
| **Prompt:** Within 2-3 weeks before next course of therapy | • myelosuppression<br>• elevated AST/ALT/alkali nephosphatase<br>• proteinuria<br>• hematuria | • mucositis/stomatitis<br>• elevated bilirubin<br>• elevated BUN/creatinine<br>• hemorrhage<br>• infection<br>• alopecia[1] | • hemolytic uremic syndrome<br>• renal failure<br>• thrombotic microangiopathy[1]<br>• cardiovascular events[1,2] (myocardial infarction, cerebrovascular accident,arrhythmia, CHF and hypertension)<br>• severe rashes[1] |
| **Delayed:** Anytime later during therapy, excluding the above conditions | | • paresthesias | • radiation recall reactions<br>• interstitial pneumonitis<br>• pulmonary fibrosis<br>• pulmonary edema<br>• ARDS<br>• sepsis<br>• liver failure |
| **Unknown Frequency andTiming:** | **Fetal and teratogenic toxicities**: Gemcitabine is embryotoxic causing fetal malformations (cleft palate, incomplete ossification) at doses of 1.5 mg/kg/day in mice (about 1/200 the recommended human dose on a mg/m2 basis). Gemcitabine is fetotoxic causing fetal malformations (fused pulmonary artery, absence of gall bladder) at doses of 0.1 mg/kg/day in rabbits (about 1/600 the recommended human dose on mg/m2 basis). Embryotoxicity was characterized by decreased fetal viability, reduced live litter sizes, and developmental delays. It is not known whether gemcitabine orits metabolites are excreted in human milk. | | |

1. Toxicity may also occur later
2. Primarily in patients with pre-existing cardiac disease

**8.1.5** Pharmaceutical Data – Gemcitabine is supplied in 200 mg and 1000 mg vials. Two hundred mg vials are reconstituted in 5 cc sodium chloride then diluted to a concentration of as low as 0.1 mg/ml if necessary for infusion. The dose is usually given over 30-90 minutes. One thousand mg vials are reconstituted with 25 cc sodium chloride. It is stored at room temperature until given.

**8.1.6** Supply – Commercially available.

## 9. BIOMARKER, CORRELATIVE AND SPECIAL STUDIES

### 9.0 Immunohistochemical Expression of SPARC and Cav-1 in Archival Tumor Tissue

The rationale for analysis of these biomarkers is detailed in Section 2.3.4. This testing will be conducted by Dr. Hong Yin at Children's Healthcare of Atlanta, using previously validated immunohistochemical assays [19].

Testing of tissue for research purposes is optional, and details regarding this testing will be included as a separate item in the informed consent document. Instructions for shipping tissue can be located in the lab manual.

### 9.1 Radiomic Analysis of Diagnostic CT Images

Standardized CT scan parameters are provided in section 10.5, to ensure consistent image data across patients and sites. Radiomic analysis will be performed on diagnostic CT scans obtained for RECIST 1.1 response assessment, and additional scans will not be required. The rationale for radiomic analysis is provided in section 2.3.4. This analysis will be performed by Dr. Natarajan Raghunand and Dr. Olya Stringfield in the Image Response Assessment Team (IRAT) Core of the Moffitt Cancer Center, using validated software. Images should be anonymized and submitted by subject number. The images should be submitted in DICOM format on CD with the image transmittal form to the IRAT Core as listed in the lab manual

### 9.2 Biomarkers of Minimal Residual Disease

The rationale for these studies is outlined in 2.6.3. Briefly, patients with recurrent sarcomas, even those who achieve a radiographic complete remission, are rarely cured, strongly suggesting the minimal residual disease (MRD) remains at the end of treatment. Preliminary studies have demonstrated the ability to detect circulating tumor cells (CTC) and plasma tumor DNA (ptDNA) in the blood of sarcoma patients [reviewed in 40]. There are several platforms for isolation of CTC in sarcoma patients, including the CellSieve™ filter, which is applicable to all sarcoma patients. CTC quantification and analysis will be performed in the laboratory of Dr. Masanori Hayashi. Plasma can also be processed for extraction of circulating nucleic acids, including ptDNA, and subsequent analyses, such as droplet digital PCR (ddPCR) and targeted sequencing, for quantification and profiling. For example, ptDNA can be utilized for assessment of patient-specific and tumor-specific genomic, epigenomic, and transcriptional variations that may be reflective of MRD. Plasma will be isolated, frozen, and banked in the Hayashi Laboratory for batched analysis and future studies. When available, remaining archival patient tumor samples collected for immunohistochemical expression of putative biomarkers (Dr. Hong Yin) will be sent to the Hayashi lab to aid circulating tumor test design.

At the time of enrollment, a package containing sufficient Cell Save Preservative tubes (for CTC analysis) and Streck Cell-free DNA BCT® tubes (for cfDNA analysis) will be sent from the Sarcoma research lab at the University of Colorado to the enrolling institution by overnight delivery service. At designated time points (**Refer to footnote 7**) 10 ml of blood will be drawn into a CellSave tube, and another 10 ml drawn into a Streck BCT® tube. Blood will be shipped at ambient temperature by overnight delivery service to the address found in the study specific lab manual

Blood for CTC analysis will be processed through CellSieve filters and evaluated per standard protocols. Plasma will be isolated by a double centrifugation technique and frozen at -80 ºC in 1 ml aliquots in a secure freezer with liquid nitrogen backup until needed for analysis.

## 10. STUDY CALENDAR

Baseline evaluations are to be conducted within 7 days (168 hours) prior to start of protocol therapy. Scans and x-rays must be done ≤ 28 days prior to the start of therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

| | | Cycle 1 | | | | Cycles 2-12 | | | | | EOT | Follow Up | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Baseline [1]** | **Day 1** | **Day 8** | **Day 15** | **Days 16-28** | **Day 1** | **Day 8** | **Days 9-21[11]** | **Day 15** | **Days 16-28** | **End of Protocol Therapy** | **30 Day Follow-up[8]** | **q3 Month Follow-up** |
| | | **(+/- 3 days)** | | | | | | | | | **(+/- 3 days)** | **+/- 7days** | **(+/- 1 month)** |
| History | X | | | | | | | | | | | | |
| Physical exam | X | X | X | X | | X | X | | X | | X | | |
| Height, weight, BSA | X | X | | | | X | | | | | X | | |
| Performance status | X | | | | | X | | | | | X | | |
| Adverse events | | X | X | X | X | X | X | X | X | X | X | X | |
| CBC with differential[2] | X | X | X | X | | X | | | X | | X[12] | | |
| Comprehensive Metabolic Panel (CMP) | X | X | X | X | | X | | | | | X[12] | | |
| Pregnancy test[3] | X | | | | | | | | | | | | |
| nab-Paclitaxel administration[10] | | X | X | X | | X | X | | X | | | | |
| Gemcitabine administration[10] | | X | X | X | | X | X | | X | | | | |
| Myeloid growth factor administration[4] | | | | | X | | | X | | X | | | |
| Archival tumor tissue[5] | X | | | | | | | | | | | | |
| Tumor disease evaluation[6] | X | | | | | | | X | | X | X[13] | | |
| MRD Biomarkers[7] | X | | | | | X | | X | | X | | | |
| Disease & Survival Status[9] | | | | | | | | | | | | | X |

Footnotes:

1 If baseline evaluations completed within 1 week prior to the start of protocol therapy, they do not need to be repeated.

2 If patients have Grade 4 neutropenia or thrombocytopenia, then CBCs should be checked every 3 to 4 days until recovery to Grade 3

3 Female patients of childbearing potential require a negative pregnancy test prior to starting treatment and must use an acceptable method of birth control. Abstinence is an acceptable method of birth control.

4 Myeloid growth factor (Neulasta, neupogen or similar agents) to be administered on day 16-18 of each cycle or on days 9-21 for patients on 21 day cycles

5 Submission of archival tumor tissue is strongly encouraged but NOT required for study enrollment. Samples should only be sent from patients who specifically consent for participation in this part of the study.

6 To be completed at the end of every other cycle. Tumor response will generally be followed by CT or MRI as detailed in Section 10.5. PET imaging may be used to complement these modalities, as discussed in Section 11.0.3.

7 To be collected at any time prior to the start of study therapy, on day 1 of cycle 2 prior to starting that cycle, and at the end of every even-numbered cycle +/- 3 days of when disease evaluation is performed through cycle 10. Sample for MRD will also be collected at time of disease progression.

8 Patients that come off treatment due to progression of disease will be followed for 30 days after end of treatment or until death, whichever occurs first.

9 Patients that are removed from treatment due to an adverse event or they have completed all cycles of treatment, will be followed

every 3 months for survival for 2 years or until death, whichever occurs first. Patients that remain on gemcitabine and nabpaclitaxel therapy but off protocol will have disease status (SD, PR, CR, or PD) and survival data collected at these timepoints.

10 Patients on 21 day treatment schedule to receive gemcitabine and nab-Paclitaxel on days 1 and 8 only of each cycle

11 Patients with stable or responding disease who experience a dose-modifying hematologic toxicity despite dose reductions and pegfilgrastim, may continue treatment on study using an altered schedule of administration

12 If CBC and/or CMP completed within 1 week of EOT visit, they do not need to be repeated.

13 Required for patients coming off treatment due to disease progression only. If imaging completed within 2 weeks of EOT visit, it does not need to be repeated

## 10.0 Demographics

Information regarding the subject's gender, race, ethnicity and age will be collected at screening.

## 10.1 Medical History

Medical history findings (i.e. previous diagnoses, diseases or surgeries) meeting all criteria listed below will be collected at prior to the first dose of study drug:

- Previous disease treatments
- Not pertaining to the study indication (general Past Medical and Surgical History)
- Started before signing of the informed consent
- Considered relevant to the study (include planned interventions)

## 10.2 Physical Measurements

The screening examination should include physical measurements (height, weight, performance status). Height and weight should be recorded at the start of each cycle. The performance status should be recorded prior to starting protocol therapy.

## 10.3 Physical Exam

In addition to physical measurements, signs and symptoms should be assessed at each study visit by the appropriate staff.

## 10.4 Adverse Events

Adverse events will be collected starting with the first dose of study agent and until the patient is taken off study. Adverse events will be graded according to the NCI-CTCAE version 4.0.

## 10.5 Radiologic Evaluations

CT or MRI are the best currently available and reproducible methods to measure lesions selected for response assessment.

Study evaluations will take place in accordance with the table in section 10. All radiographic evaluations must be performed within 28 days of start of therapy utilizing CT/MRI. Unusual circumstances related to >28 day old scans not involving target lesions may be discussed with the study chair. Tumor response will be assessed by the investigator using the RECIST 1.1 (Response Evaluation Criteria in Solid Tumors). Use the table below (Table 1) to assist you in identifying landmarks for the various anatomic regions.

**Table 1: Anatomical Coverage Landmarks**

| Body Region | Anatomic Landmark | |
|---|---|---|
| | Superior | Inferior |
| Chest | Lung Apices | Adrenal Glands |
| Abdomen | Dome of the Liver | Iliac Crest |
| Pelvis | Iliac Crest | Pubic Symphysis |

| | | |
|---|---|---|
| Brain | Skull Vertex | Skull Base |
| Whole body | Base of the Skull | Mid-Thigh |

Subjects will receive standard of care diagnostic-quality CT/MRI scans of the chest, abdomen, pelvis and or extremities (Table 2). CT and MRI are the most commonly employed modality for the initial staging and the assessment of response in patients with solid tumors.

- Acquire scans according to RECIST 1.1 requirements:
  (a) Patients should be scanned on the same scanner across time points, whenever possible.
  (b) IV and Oral contrast should be administered as appropriate.
  (c) Please note that venous phase imaging is essential for lesion assessments by RECIST criteria; arterial phase, delayed phase, and non-contrast phase image acquisition are optional, but not required.
  (d) CT scan parameters provided in the following table should be used.

**Table 2: CT Acquisition and Reconstruction Parameters**

| **Parameter** | **Guideline** |
|---|---|
| IV Contrast | 80-150 cc tailored to meet needs/limitations of each subject |
| Oral Contrast | Oral contrast administered according to the institutional standard of care procedures |
| Superior Extent | Above Lung Apices |
| Inferior Extent | Pubic Symphysis |
| Tube Voltage | 120 kVp |
| Tube Current | Adjust as required (typically 150 – 200 mAs) |
| Convolution Kernel | GE – STANDARD; Siemens – B30f; |
| Pitch | 0.969 – 1 (higher is OK for children) |
| Field of View (FOV) | Ideally 40 cm x 40 cm, but adjustable based on patient size |
| Reconstructed image matrix size | 512 x 512 (Note: in conjunction with the 40 cm FOV, this will yield a pixel resolution of 0.781 mm x 0.781 mm) |
| Reconstructed Slice Thickness | 3 mm |
| Slice Interval | Contiguous |
| Bits Stored / Bits Allocated | 12 / 16 |

## 11. MEASUREMENT OF EFFECT

Although the clinical benefit of this drug combination for sarcoma has not yet been established, the intent of offering this treatment is to provide a possible therapeutic benefit, and thus the patient will be carefully monitored for tumor response and symptom relief in addition to safety and tolerability. Patients with measurable disease will be assessed by RECIST 1.1 criteria. For the purposes of this study, patients should be re-evaluated after every two cycles. In addition to a baseline scan, confirmatory scans will also be obtained at least 4 weeks following initial documentation of an objective response.

### 11.0 Antitumor Effect – Solid Tumors

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria. The criteria have been modified for this protocol to require a CT slice thickness of 3 mm (see section 10.7).

### 11.0.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity from the time of their first treatment with gemcitabine and nab-paclitaxel.

Evaluable for objective response. 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. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

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.

### 11.0.2 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 (≥2 cm) by chest x-ray or as ≥10 mm (≥1 cm) with CT scan, MRI, or 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 considered measurable only if there is progression after radiation.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm (≥1.5 cm) in short axis when assessed by CT. 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 [<1 cm] or pathological lymph nodes with ≥10 to <15 mm [≥1 to <1.5 cm] 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 this circumstance, the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters.

If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

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.

### 11.0.3 Methods for Evaluation of Measurable Disease

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

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

Clinical lesions Clinical lesions will only be considered measurable when they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and ≥10 mm (≥1 cm) 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 required.

Conventional CT and MRI CT acquisition and reconstruction guidelines are provided in section 10.7, including the recommended slice thickness of 3 mm

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

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 where known residual benign tumors can remain).

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

FDG-PET. While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified

according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET 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 FDG-PET scan). If the positive FDG-PET 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.
c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

   Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

**11.0.3.1 Response Criteria**

While all scans will be sent for central review, the determination of progressive disease and whether or not to continue therapy will be made by the enrolling site. Final determinations of partial and complete responses will be determined by central review for statistical endpoints.

**11.0.3.2 Evaluation of Target Lesions**

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm (<1 cm).

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 (0.5 cm). (Note: the appearance of one or more new lesions is also considered progressions).

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

**11.0.3.3 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 [<1 cm] 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.

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

**11.0.3.4 Evaluation of Best Overall Response**

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

**For Patients with Measurable Disease (*i.e.*, Target Disease)**

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

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

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

**For Patients with Non-Measurable Disease (*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 | | |

### 11.0.4 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.

### 11.0.5 Progression-Free Survival

Progression-free survival (PFS) is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

### 11.0.6 Response Review

All scans will be independently reviewed per RECIST V1.1 criteria by Rikesh Makanji, M.D., a board certified radiologist at Moffitt Cancer Center.

## 12. DATA REPORTING/REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: Definition and Reporting Requirements).

### 12.0 Data Reporting

Data collection for this study will be done exclusively through OnCore. All members of the study team are granted access to the trial in OnCore by the MCRN External Research Regulatory Coordinator. See instructions in Sunshine Project Handbook for OnCore access.

### 12.0.1 Method for Data Reporting and Monitoring

**Research Monitoring Core**

Data will be captured in OnCore, Moffitt's Clinical Trials Database. The Moffitt Research Monitoring Core will be responsible for monitoring data at external sites. The purpose of trial monitoring is to verify that the rights, wellbeing, and trial/data integrity of Human Subjects enrolled in Center (or other NCI Center) sponsored, Principal Investigator (PI) initiated clinical research trials are protected. Investigator-initiated clinical research therapeutic and other intervention trials utilizing investigational agents, devices or under an MCC held IND/IDE are monitored on-site (or via remote monitoring when appropriate) regularly to verify the reported trial data are accurate, complete, and verifiable from source documents; and the conduct of the trial is in compliance with the currently approved trial/amendments, data safety-monitoring plans, Good Clinical Practice (GCP) Guidelines, and applicable regulatory requirements.

Regulatory documents and case report forms will be reviewed routinely for accuracy, completeness and source verification of data entry, validation of appropriate informed consent process, adherence to study procedures, and reporting of SAEs and protocol deviations according to Moffitt's Monitoring Policies. Monitoring Visits will include a review of data entry to source documentation, regulatory documents, and when appropriate, investigational pharmacy or clinical laboratory.

**Audit and inspection**
Inspections by regulatory health authority representatives i.e. FDA and IEC(s)/IRB(s) are possible. Investigator responsibilities are set out in the ICH guideline for Good Clinical Practice (GCP) and in the US code of Federal regulations.

Investigators must enter study data into Moffitt's electronic database, OnCore. The investigator will permit study related audit visit by a sponsor or it's representatives, IRB/EC review, and regulatory inspection(s), providing direct access to the facilities where the study took place, to source documents, to CRF's and to other study documents.

Essential documents shall be archived safely and securely in such a way that ensures that they are readily available upon authorities' request.

Subject (hospital) files will be archived according to local regulations and in accordance with the maximum period of time permitted by the hospital, institution or private practice.

**Record Retention**
Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or as required by the State of Florida until 10 years have elapsed since the completion of the study, or whichever time period is longer. The informed consent documents (ICF) should be kept indefinitely within the electronic medical record. No records will be destroyed without the written consent of the funding source(s), if applicable. It is the responsibility of the funding source(s) to inform the investigator when there is no longer a need for these documents to be retained. Permission must be acquired from the State of Florida for document destruction after the 10-year minimum record-retention period described above has elapsed.

**Sunshine Project Clinical Trials Oversight Committee (CTOC)**
The CTOC is a group of pediatric oncologists who specialize in phase 1 clinical trials. This group of physicians review and evaluate the conduct of each trial the Sunshine Project conducts. Every quarter, the CTOC meets to ensure all clinical research conducted or coordinated by PCF is scientifically well designed, responsibly managed, appropriately reported, and protects the rights and welfare of human participants.

The CTOC provides an independent review of the interim results of each trial and offers objective guidance regarding unforeseen events that may occur during the course of a study. The CTOC will help to evaluate and ensure events that may have an impact on patient safety are appropriately addressed and managed on an on-going basis. At the quarterly meetings, discussions will include adverse events, accrual, and protocol deviations. Adverse event and deviation collection begins when a patient is first placed on trial.

All events are classified using the NCI Common Toxicity Criteria and are summarized and presented to all members at each safety meeting. The CTOC will also help to evaluate and ensure events that may have an impact on patient safety are appropriately addressed and managed. The CTOC will make recommendations regarding the conduct and safety of the trial and a public report will be made available to all participating member sites. The CTOC will be convened in the event of safety concerns brought to the attention of investigators or the study chair.

**Protocol Monitoring Committee**
The Moffitt Cancer Center reviews investigator initiated trials that take place at Moffitt through the

Protocol Support Office (PSO) at Protocol Monitoring Committee meeting. The PSO reviews all deviations, AEs, and SAEs on all Sunshine trials. Reports for phase I trials are created and reviewed when a trial increases a dose level or when a maximized tolerated dose is met.

**Sunshine Project Bi-Weekly Teleconference**
Starting with the first patient enrollment, a safety call consisting of principle investigators, a biostatistician, and study coordinators will occur biweekly. In addition, all AE's will be collected and shared with drug suppliers for input prior to escalating or de-escalating to the next dose level.

**Moffitt Cancer Center Scientific Review Committee and Institutional Review Board**
All Sunshine Project trials undergo an initial review at the center's Scientific Review Committee and Central Institutional Review Board.

### 12.0.2 Responsibility for Data Submission

It is the responsibility of the PI(s) at the site to ensure that all study team members at the site understand the procedures for data submission, and that protocol specified data is entered accurately and in a timely manner into the OnCore system.

All eCRF entries into OnCore will be verified via source documentation during on-site or remote monitoring visits. Data must be entered into the OnCore system **within 10 business days** of a subject visit or it will be considered delinquent.

## 12.1 Moffitt Cancer Center Guidelines

This protocol will adhere to the policies and requirements of the Moffitt Cancer Center Guidelines.

The Moffitt Cancer Center is responsible for distributing all IND Action Letters or Safety Reports received to all participating institutions for submission to their individual IRBs for action as required.

**Protocol modifications**
No modifications will be made to the protocol without the agreement of the investigators. Changes that significantly affect the safety of the patients, the scope of the investigation, or the scientific quality of the study will require Scientific Review Committee and Institutional Review Board approval prior to implementation, except where the modification is necessary to eliminate apparent immediate hazard to human subjects. Any departures from the protocol must be fully documented in the case report form and the source documentation.

**Monitoring of the study and regulatory compliance**
The Principal Investigator and the Clinical Research Coordinator assigned to the case will be primarily responsible for maintaining all study related documents including the clinical research forms. Oncore is the database of record for all CRF entries and will be verified with source documentation. The review of medical records within the health record will be done in a manner to assure that patient confidentiality is maintained.

**Suspension/Termination**
The PMC and/or the IRB may vote to suspend or terminate approval of a research study not being conducted in accordance with the IRB, the Cancer Center and/or regulatory requirements or that has been associated with unexpected problems or serious harm to subjects. The PMC/IRB will notify the PI in writing of such suspension or terminations. It is the responsibility of the PMC/IRB Chairperson to ensure prompt written notification of any suspensions or terminations of PMC/IRB approval to the relevant Federal Agencies, including OHRP, FDA, the study sponsor/funding source and if applicable, the Affiliate Program.

**Trial Discontinuation**
For reasonable cause the Investigator and/or sponsor may terminate this study prematurely. Conditions that may warrant termination include, but are not limited to: the discovery of an unexpected, significant, or unacceptable risk to the patients enrolled in the study or if the accrual goals are met. A written notification of termination will be issued.

## 13. STATISICAL CONSIDERATIONS

### 13.0 Study Design/Endpoints

**Assessment of Response:** Treatment response will be assessed with the most relevant imaging studies (e.g., CT or MRI) after every two cycles. Standard RECIST version 1.1 criteria will be used to assess responses.

**Assessment of Progression-Free Survival (PFS):** PFS will be calculated for all patients using KM curve analysis and Cox regression, and will be used to determine accrual for osteosarcoma patients as described below. PFS will be determined by the progression-free interval (PFI), which will be calculated as the date of enrollment until the date of disease progression, date of death, date of removal of all tumor by surgery or last patient contact, whichever occurs first. Patients whose end PFI date is disease progression or death will be considered to have experienced an event. Patients whose end PFI date is date of removal of all tumor by surgery will be considered removed from the analytic set at that time by a competing event. Patients whose end PFI date is the date of last patient contact will be considered censored for PFI analysis. The probability of remaining progression-free as a function of days since enrollment will be calculated according to the method of Gray, accounting for censoring and the competing events. The data from the osteosarcoma, Ewing sarcoma, rhabdomyosarcoma and non-rhabdomyosarcoma soft tissue sarcoma strata obtained from this study will be compared with a dataset of patients with the appropriate diagnosis from the cohort of patients treated on various previously reported studies for similar patients, using the method of Gray.

**Assessment of Toxicity:** Patients will undergo regular physical exams and laboratory monitoring as described in the Study Calendar in Section 10.

**Correlative studies:** For patients who consent to this optional part of the study, archival tumor tissue from the most recent available biopsy will be assessed for putative biomarker expression using immunohistochemistry as previously established [20]. At least five unstained slides containing tumor will be shipped to the laboratory of Dr. Julie Yin at Emory University for this batched analysis, and testing will be performed following completion of patient accrual. The extent of protein expression will be scored on a standard quantitative scale, and the relationship between protein expression and imaging response will be reported in a descriptive fashion.

**Statistical Considerations and Patient Accrual:** The best response of disease will be examined separately in each of the four disease strata. The following Simon's two stage design will be used for each stratum. For osteosarcoma patients, remaining progression-free after 4 cycles will be considered a response, as described in Section 2.3.3. For all other patients, RECIST criteria will be used and responding patients will be those with complete or partial responses.

The null hypothesis that the true response rate is 10% will be tested against a one-sided alternative response rate of 35% or higher. In the first stage, 11 patients will be accrued for each of the four disease strata. If there are one or fewer responses in these 11 patients, accrual to that strata will be stopped. If two or more responses are seen in the first 11 patients in a disease strata, 7 additional patients will be

accrued for a total of 18 in that strata. The null hypothesis will be rejected if 5 or more responses are observed in 18 patients. This design yields a type I error rate of 0.027 and power of 0.80 when the true response rate is 35%.

| | **Cumulative Number Of Patients with Response (ES, RMS, NRSTS) or Progression-Free at 4 Months (OS)** | **Decision** |
|---|---|---|
| **Stage 1:** Enter 11 patients per strata | 1 or less | Terminate strata: combination ineffective |
| | 2 of those 11 | Proceed to Stage 2 |
| **Stage 2:** Enter 7 additional patients per strata | 2 of those 7 or total of $\leq$ 4 responses among 18 cohort | Terminate strata: combination ineffective |
| | 3 of those 7 (or total of $\geq$ 5 responses among 18) | Recommend combination of interest for further study |

ES, Ewing sarcoma; RMS, rhabdomyosarcoma; NRSTS, non-rhabdomyosarcoma soft tissue sarcoma; OS osteosarcoma

Therefore, we will consider the combination not of sufficient interest for further evaluation in a disease category if the true response rate is 10% or less, and of sufficient activity if the true response rate is 35% or greater. If the combination has a true response rate of 10%, the two-stage design described above will identify it to be of insufficient activity for further study with one-sided significance 0.05 (type I error) and power 0.80. If the combination has a true response rate of 35%, the probability of identifying it of sufficient activity for further study is 0.80 (power against the alternative hypothesis $P = .35$).

Criteria for assessing which patients are evaluable for response and toxicity, determining which patients may be replaced, and how progression-free survival is defined are listed in Sections 5.6 and 5.7.

### 13.0.1 Safety Analysis

The population for the safety analysis will be comprised of all patients who received at least one dose of study medication. Patients will be monitored for adverse events using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. All treatment-emergent and baseline adverse events and hematological/biochemical toxicities based on laboratory measurements, as well as drug related AE's, will be summarized by NCI CTCAE v. 4.0 worst grade.

The incidence of deaths and treatment-emergent serious adverse events will be summarized. Also, the incidence of adverse events leading to discontinuation of investigational product and/or withdrawal from the study will be summarized and listed.

### 13.0.2 Statistical and Analytical Plans

General Statistical Approaches: Summary tabulations will be created that display the number of observations, mean, standard deviation, median, minimum, and maximum for continuous variables, and the number and percent per category for categorical data.

Demographic and clinical baseline characteristics will be descriptively summarized in an exploratory fashion. These include gender, age, race, weight, height, primary diagnosis, performance status, number of previous treatment regimens, time to progression (TTP) on most recent treatment regimen, size of measurable disease and other parameters, as appropriate.

## 13.1 Sample Size/Accrual Rate

A minimum of 44 eligible and evaluable patients will be enrolled, consisting of 11 in each of the 4 disease strata. Providing for possible ineligible and inevaluable patients, the minimum total number of patients will be 48.

Expansion will continue based on objective response (ES. RMS, and NRSTS patients) or 4-month PFS (OS patients), following the two-stage rule noted above. The maximum number of eligible and evaluable patients in a specific cohort is 18, with the maximum number of eligible and evaluable patients in the entire study being 72.

### 13.2 Analysis of Other Secondary Endpoints

SPARC, CAV-1, and hENT1 protein expression will be assessed using immunohistochemistry analysis of archival tumor tissue from the most recent surgery in which tissue is available, using previously validated assays [22]. Expression of these proteins will be scored as 0 = negative; 1+ = weak staining; 2+ = moderate staining; and 3+ = strong staining. The proportion of patients falling into each category (relative to the total number of patients evaluated) will be calculated. The response status of each patient will be cross-tabulated with expression of the proteins of interest. These protein expression data will be examined both with graphical tools (e.g., box plot, histogram, scatter plot, etc.) and descriptive statistics such as mean, median, variance, skewedness to understand their statistical distributions and characteristics and with univariate statistical tests such as chi-square test to find any significant expression differences of these proteins among patients with different responses.

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## APPENDIX A PERFORMANCE STATUS CRITERIA

| **ECOG Performance Status Scale** | | **Karnofsky Performance Scale** | |
|---|---|---|---|
| Grade | Descriptions | Percent | Description |
| 0 | Normal activity. Fully active, able to carry on all pre-disease performance without restriction. | 100 | Normal, no complaints, no evidence of disease. |
| | | 90 | Able to carry on normal activity; minor signs or symptoms of disease. |
| 1 | Symptoms, but ambulatory. Restricted in physically strenuous activity, but 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. |
| | | 70 | Cares for self, unable to carry on normal activity or to do active work. |
| 2 | In bed <50% of the time. Ambulatory and capable of all 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. |
| | | 50 | Requires considerable assistance and frequent medical care. |
| 3 | In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours. | 40 | Disabled, requires special care and assistance. |
| | | 30 | Severely disabled, hospitalization indicated. Death not imminent. |
| 4 | 100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair. | 20 | Very sick, hospitalization indicated. Death not imminent. |
| | | 10 | Moribund, fatal processes progressing rapidly. |
| 5 | Dead. | 0 | Dead. |

**APPENDIX B Sample Letter for Insurance Coverage for Gemcitabine and nab-Paclitaxel**

Re: Patient Name Here

DOB: --/--/--

To Whom It May Concern:

I am writing in support of the use of gemcitabine and nab-paclitaxel for the treatment of my patient (insert name here), who has (name of sarcoma) which has progressed following standard front-line therapy. Several previous studies have demonstrated the activity of combining gemcitabine and docetaxel for patients with relapsed/refractory bone and soft tissue sarcoma, and this has become a common salvage regimen for patients in this situation. The following evidence suggests that nab-paclitaxel may be a superior taxane to couple with gemcitabine:

1) Nab-paclitaxel has single-agent activity against preclinical models of pediatric sarcoma as assessed by the National Cancer Institute's Pediatric Preclinical Testing Program (see attached article by Houghton et al.). This data has led to an ongoing pediatric phase I/II trial of nab-paclitaxel which has shown encouraging responses in pediatric sarcoma patients (see attached 2016 ASCO abstract).

2) Nab-paclitaxel has superior anti-tumor effects and reduced toxicity when directly compared to docetaxel in patients with metastatic breast cancer (J Clin Oncol 2009;27:3611-9). Importantly, there is substantially less myelosuppression, which is an issue for our heavily-pretreated patients with recurrent sarcoma. Because of decreased toxicity with fewer hospitalizations and less need for myeloid growth factor, the use of nab-paclitaxel has shown superior cost-effectiveness when compared to docetaxel for the treatment of breast cancer patients (J Oncol Pharm Pract 2009;15:;67-78).

3) The combination of gemcitabine and nab-paclitaxel is now approved by the FDA for treatment of pancreatic cancer, based on the preclinical synergy, tolerability, and activity of this regimen.

4) A recent report presented at the 2016 American Society of Pediatric Hematology/Oncology has demonstrated this combination is well tolerated and demonstrates activity, building on preclinical evidence of synergy with this combination against models of pediatric bone sarcoma (see attached reports).

For these reasons, I feel that outpatient treatment with gemcitabine plus nab-paclitaxel using the FDA-approved dosing and treatment schedule represents the best available therapy for (insert patient's name). Please contact me directly at (insert contact information here) if I can provide additional information regarding this patient's past therapy or proposed treatment plans.

Sincerely,

(Insert Investigator/s name here)