A Phase II Trial of ADI-PEG 20 in Combination with Gemcitabine and Docetaxel for the Treatment of Soft Tissue Sarcoma, Osteosarcoma, Ewing's Sarcoma, and Small Cell Lung Cancer

Washington University School of Medicine
Division of Oncology
660 South Euclid Avenue, Campus Box 8056
St. Louis, MO 63110

Protocol #: 201912062 (previously 201803202)
Version Date: 11/04/2025

Coordinating Center: **Washington University School of Medicine**

Principal Investigator: **Mia Weiss, M.D.**
Phone: (314) 362-5737
E-mail: m.c.weiss@wustl.edu

Sub-Investigators	**Institution**	**Modality**
Doug Adkins, M.D.	Washington University	Medical Oncology
Angie Hirbe, M.D.	Washington University	Medical Oncology
Nam Bui, M.D.	Stanford Cancer Institute	Medical Oncology
Steven Wong, M.D.	Sarcoma Oncology Center	Medical Oncology
Jingqin (Rosy) Luo, Ph.D.	Washington University	Biostatistics

Pharmacy Co-Chair: Sasha Haarberg, PharmD
Phone: (314) 273-2918
E-mail: sasha.haarberg@wustl.edu

Study Drugs: ADI-PEG 20 (pegylated arginine deiminase)
Gemcitabine (Gemzar)
Docetaxel (Taxotere)

IND #: **138701**
ClinicalTrials.gov #: **NCT03449901**

A Phase II Trial of ADI-PEG 20 in Combination with Gemcitabine and Docetaxel for the Treatment of Soft Tissue Sarcoma, Osteosarcoma, Ewing's Sarcoma, and Small Cell Lung Cancer

Protocol Revision History

Initial Approval Version	**03/28/2018**
Amendment #1 Version	**05/07/2018**
Amendment #2 Version	**05/14/2018**
Amendment #3 Version	**06/20/2018**
Amendment #4 Version	**08/24/2018**
Amendment #5 Version	**03/15/2019**
Amendment #6 Version	**06/06/2019**
Amendment #7 Version	**08/25/2019**
Amendment #8 Version	**07/10/2020**
Amendment #9 Version	**08/10/2020**
Amendment #10 Version	**11/04/2025**

A Phase II Trial of ADI-PEG 20 in Combination with Gemcitabine and Docetaxel for the Treatment of Soft Tissue Sarcoma, Osteosarcoma, Ewing's Sarcoma, and Small Cell Lung Cancer

Principal Investigator Signature Page

Principal Investigator (printed):

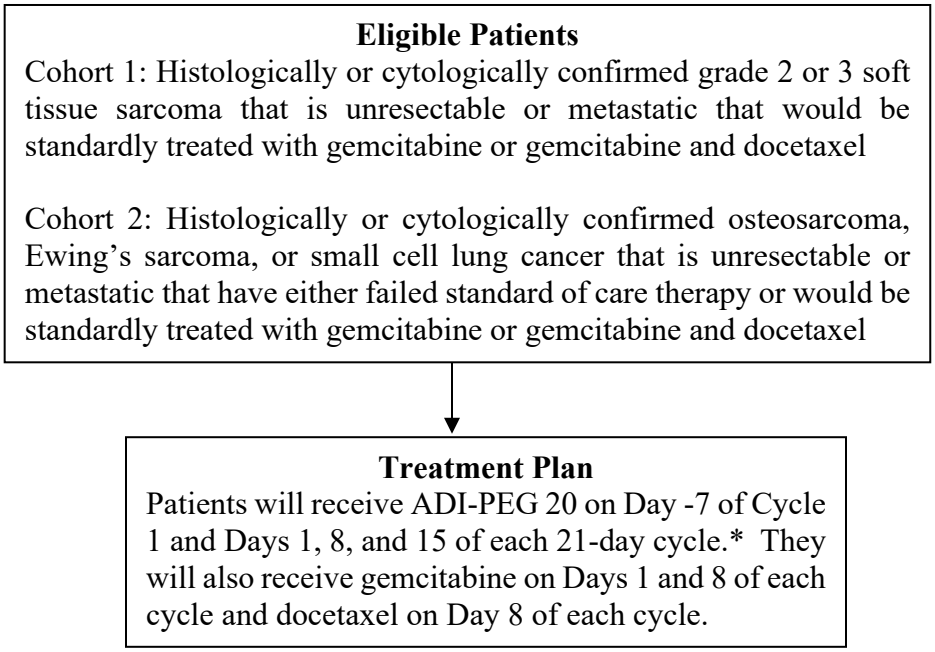
Name of Institution:

PI Signature *Date*

By my signature, I agree to personally supervise the conduct of this study and to ensure its conduct in compliance with the protocol, informed consent, IRB/HRPO procedures, the Declaration of Helsinki, ICH Good Clinical Practices guidelines, and the applicable parts of the United States Code of Federal Regulations or local regulations governing the conduct of clinical studies.

A Phase II Trial of ADI-PEG 20 in Combination with Gemcitabine and Docetaxel for the Treatment of Soft Tissue Sarcoma, Osteosarcoma, Ewing's Sarcoma, and Small Cell Lung Cancer

SCHEMA



*ADI-PEG 20 is to be given weekly regardless to Day 1 or Day 8 dose delays. This could cause cycles that extend beyond 21 days.

Table of Contents

SCHEMA 4
1.0 BACKGROUND AND RATIONALE 7
1.1 Sarcoma 7
1.2 Treatment of Metastatic Sarcoma 7
1.3 ADI-PEG 20 7
1.4 Gemcitabine 9
1.5 Docetaxel 10
1.6 Gemcitabine and Docetaxel Combination 10
1.7 Study Rationale 10
1.8 Correlative Studies Background 13
1.9 Rationale for Cohort 2 14
2.0 OBJECTIVES 14
2.1 Primary Objective 14
2.2 Secondary Objectives 14
2.3 Exploratory Objectives 14
3.0 PATIENT SELECTION 14
3.1 Inclusion Criteria 15
3.2 Exclusion Criteria 16
3.3 Inclusion of Women and Minorities 17
4.0 REGISTRATION PROCEDURES 17
4.1 Confirmation of Patient Eligibility 17
4.2 Patient Registration in the Siteman Cancer Center OnCore Database 18
4.3 Assignment of UPN 18
5.0 TREATMENT PLAN 18
5.1 Premedication Administration 18
5.2 Agent Administration 19
5.3 Evaluability 19
5.4 General Concomitant Medication and Supportive Care Guidelines 19
5.5 Women of Childbearing Potential 20
5.6 Duration of Therapy 20
5.7 Duration of Follow-up 21
5.8 Study Stopping Rule 21
6.0 DOSE DELAYS/DOSE MODIFICATIONS 21
6.1 Dose Modifications for ADI-PEG 20 21
6.2 Dose Modifications for Gemcitabine 21
6.3 Dose Modifications for Docetaxel 22
7.0 REGULATORY AND REPORTING REQUIREMENTS 23
7.1 Sponsor-Investigator Reporting Requirements 23
7.2 Secondary Site Reporting Requirements 25
7.3 Exceptions to Expedited Reporting 26
8.0 PHARMACEUTICAL INFORMATION 26
8.1 ADI-PEG 20 26
8.2 Gemcitabine (Gemzar) 28
8.3 Docetaxel (Taxotere) 29
9.0 CORRELATIVE STUDIES 31

9.1 Tumor Biopsy 31
9.2 Archival Tissue 32
9.3 Mandatory Peripheral Blood 32
10.0 STUDY CALENDAR 33
11.0 DATA SUBMISSION SCHEDULE 34
11.1 Adverse Event Collection in the Case Report Forms 34
12.0 MEASUREMENT OF EFFECT 34
12.1 RECIST 1.1 35
12.2 Choi Response Criteria 41
13.0 DATA AND SAFETY MONITORING 41
14.0 AUDITING 42
15.0 STATISTICAL CONSIDERATIONS 43
15.1 Study Design 43
15.2 Endpoints 43
15.3 Sample Size Justification 44
15.4 Accrual 44
15.5 Analysis Plan 44
15.6 Study Stopping Rules 44
15.7 Population for Analysis 45
16.0 MULTICENTER REGULATORY REQUIREMENTS 46
17.0 REFERENCES 47
APPENDIX A: ECOG Performance Status Scale 49
APPENDIX B: Definitions for Adverse Event Reporting 50
APPENDIX C: Reporting Timelines 52
APPENDIX D: Washington University Unanticipated Problem Reporting Cover Sheet 55

1.0 BACKGROUND AND RATIONALE

1.1 Sarcoma

Sarcomas are a heterogeneous group of rare cancers that share a common mesenchymal origin. Mesenchymal cells are derived from pluripotent embryonic connective tissue that differentiates into one or several lineages such as muscle, adipose, cartilage, nerves, vascular tissue, etc. They are broadly divided into bone sarcomas and soft tissue sarcomas. The incidence of bone and soft tissue sarcomas in 2016 was 12,110 and 3,010, respectively [1]. There are more than 50 histologic subtypes of soft tissue sarcomas, each with a distinctive clinical profile, response to treatment, and prognosis.

1.2 Treatment of Metastatic Sarcoma

Generally, localized sarcoma is treated with surgery when feasible and adjuvant radiation with or without chemotherapy. Like any other cancer, sarcomas have the ability for hematogenous spread to develop distant metastases. For the majority of patients with metastatic soft tissue sarcoma, their cancer is incurable. Therefore, the goal of treatment is to reduce cancer bulk, minimize cancer related symptoms, improve quality of life, and increase overall survival (OS). Despite our best efforts, the median survival of patients with distant metastases is 11 to 15 months with approximately 20-25% of patients being alive at three years [2].

Most cases of advanced soft tissue sarcoma are treated with conventional chemotherapy for palliation. Common first line treatment regimens are based on the agent doxorubicin. There have been many attempts to improve upon this treatment approach with dose intensification and combination therapies. A randomized trial by EORTC Soft Tissue and Bone Sarcoma Group compared doxorubicin alone, doxorubicin plus ifosfamide, and a four-drug regimen of cyclophosphamide, vincristine, doxorubicin, and dacarbazine. The combination treatment did not significantly improve OS, progression-free survival (PFS), or response rate (RR), and these combination regimens were significantly more toxic[3]. Another randomized phase III trial evaluated single agent doxorubicin compared to doxorubicin plus ifosfamide in advanced or metastatic soft tissue sarcoma. There was no significant difference in OS between the two treatment groups (median OS 12.8 months vs. 14.3 months, p=0.076). The combination chemotherapy group had a significantly higher PFS (7.4 months vs. 4.6 months, p 0.003) and there was a higher overall response rate in the combination treatment arm (26% vs. 14%)[3]. Olaratumab has demonstrated an overall survival benefit in a randomized phase II trial[4]. The combination of gemcitabine (900 mg/m^2 every day 1 and 8 of a 21 day cycle) with docetaxel (day 8) is also recommended as a second line treatment option in soft tissue sarcoma (NCCN Guidelines 2016) [5]. This is the treatment that will be tested in this protocol in combination with ADI-PEG 20.

1.3 ADI-PEG 20

ADI-PEG 20 is arginine deiminase (ADI) conjugated to polyethylene glycol (PEG) of

20,000 molecular weight (pegargiminase [United States Adopted Names Council Name]). It is a novel biological therapeutic agent currently being developed to treat unresectable hepatocellular carcinoma (HCC), metastatic melanoma and other cancers.

ADI is a microbial enzyme that degrades arginine, a crucial amino acid central to metabolism and biosynthesis and required for neoplastic growth and development. While normal human cells are able to synthesize arginine from metabolic precursors, some cancer cells such as melanoma and HCC are deficient in the necessary enzymatic pathway to make arginine due to loss of expression of argininosuccinate synthetase (ASS1) in the urea cycle and must instead obtain arginine from the blood for growth and survival. As such, these cancers are auxotrophic for arginine. Therefore, depleting arginine from the blood can control tumor growth and even eliminate arginine requiring cancers without damage to normal cells.

There is precedence for the use of an amino acid degrading enzyme as an effective treatment for specific forms of cancer which are auxotrophic for non-essential amino acids. Conjugation to PEG (20,000 mw) increases the circulating half-life and decreases the antigenicity of ADI. Similar pegylation technology has been used with a number of microbial derived therapeutic proteins for the same reasons. For example, a pegylated form of the microbial derived, asparagine-degrading enzyme asparaginase has been used as front-line therapy against childhood leukemia for over 30 years with few serious side effects.

ADI-PEG 20 has shown efficacy in mice implanted with tumors that require arginine for growth. ADI-PEG 20 also has been effective in treating dogs with spontaneous melanoma. A number of tumor cell lines from a variety of human tumors are auxotrophic for arginine, including melanomas and HCC. ADI-PEG 20 has been tested in Phase 1 & 2 trials in the USA and in Europe in metastatic melanoma and in unresectable HCC in the USA, Europe and Taiwan, and was tested in a global Phase 3 study in HCC. Results from several completed and ongoing clinical studies have shown that ADI-PEG 20 is well-tolerated in humans.

Since humans do not appear to require arginine for survival and growth, ADI-PEG 20 may have utility in the treatment of cancers that are auxotrophic for arginine. Arginine depletion with ADI-PEG 20 in cancers has recently been reviewed [6,7].

More specifically, ADI-PEG 20 is also being evaluated in multiple phase 1 combination studies with single and double chemotherapy agents, including docetaxel (Tomlinson 2015), and gemcitabine plus nab-paclitaxel in pancreatic carcinoma (ClinicalTrials.gov # NCT02101580). This combination has been well tolerated and has moved into the post-dose escalation cohort with the standard doses of gemcitabine and nab-paclitaxel used in pancreatic cancer (1000 mg/m^2 and 125 mg/m^2, respectively, on days 1, 8, and 15 every 4 weeks) and the highest dose of ADI-PEG 20 (36 mg/m^2) tested (Lowery 2017 Cancer-in press). Thus, in that study, patients received 9000 mg/m^2 of gemcitabine total every 12 weeks, in addition to nab-paclitaxel and weekly ADI-PEG 20 36 mg/m^2. All planned subjects have been accrued and treated. The triplet has been well tolerated and has resulted

in numerous objective responses (Lowery 2017 Cancer-in press). In this study, patients receive of gemcitabine every 12 weeks and weekly ADI-PEG 20 36 mg/m^2 along with nab-paclitaxel. As such, there is precedent for the combination of weekly ADI-PEG 20 36 mg/m^2 and gemcitabine days 1 and 8 of 21 and a taxane as proposed in this phase 2 study.

Thus this metabolic targeting of tumor cell metabolism as an anticancer strategy may potentially attack cancer's Achilles' heel. Therefore, anticancer PEG enzymes are a current approach to therapy of cancer [8].

Recent trials of ADI-PEG 20 looked at toxicity in combination with taxanes and gemcitabine and saw that ADI-PEG 20 did not increase toxicity. First, in a phase I trial of ADI-PEG 20, doses of 18 mg/m^2, 27 mg/m^2, or 36 mg/m^2 were given together with pemetrexed 500 mg/m^2 and cisplatin 75 mg/m^2; no dose-limiting toxicities of ADI-PEG20 were reported.[9] Second, in a phase I trial, patients received ADI-PEG 20 weekly via intramuscular injection at doses ranging from 4.5 to 36 mg/m^2 and up to 10 doses of docetaxel (75 mg/m^2) every 3 weeks; ADI-PEG 20 demonstrated reasonable toxicity in combination with docetaxel [10]. Finally, in a trial most similar to ours, Phase 1/1B Trial of ADI-PEG 20 Plus Nab-Paclitaxel and Gemcitabine in Patients with Advanced Pancreatic Adenocarcinoma, the RP2D for ADI-PEG 20 was 36 mg/m^2 weekly in combination with standard-dose gemcitabine and nab-paclitaxel. Overall, they concluded that ADI-PEG 20 was well tolerated in combination with gemcitabine and nab-paclitaxel [11]. This data provides the rationale for not performing a phase I safety lead in.

1.4 Gemcitabine

Gemcitabine is a nucleoside metabolic inhibitor indicated in combination with carboplatin for the treatment of advanced ovarian cancer that has relapsed at least 6 months after completion of platinum-based therapy; in combination with paclitaxel for first-line treatment of metastatic breast cancer after failure of prior anthracycline-containing adjuvant chemotherapy (unless anthracyclines were clinically contraindicated); in combination with cisplatin for the treatment of non-small cell lung cancer; and as a single agent for the treatment of pancreatic cancer. It is also used as a single agent or in combination with docetaxel for the treatment of soft tissue sarcoma [12, 5].

The most common (≥20%) adverse reactions of single-agent gemcitabine are nausea/vomiting, anemia, increased ALT, increased AST, neutropenia, increased alkaline phosphatase, proteinuria, fever, hematuria, rash, thrombocytopenia, dyspnea, and edema. The most common (≥5%) Grade 3 or 4 adverse reactions were neutropenia, nausea/vomiting; increased ALT, increase alkaline phosphatase, anemia, increased AST, and thrombocytopenia. Approximately 10% of the 979 patients discontinued gemcitabine due to adverse reactions. Adverse reactions resulting in discontinuation of gemcitabine in 2% of 979 patients were cardiovascular adverse events (myocardial infarction, cerebrovascular accident, arrhythmia, and hypertension) and adverse reactions resulting in discontinuation of gemcitabine in less than 1% of the 979 patients were anemia, thrombocytopenia, hepatic dysfunction, renal dysfunction, nausea/vomiting, fever, rash, dyspnea, hemorrhage, infection, stomatitis, somnolence, flu-like syndrome, and edema.

1.5 Docetaxel

Docetaxel is a microtubule inhibitor indicated for locally advanced or metastatic breast cancer after chemotherapy failure, operable node-positive breast cancer, locally advanced or metastatic non-small cell lung cancer after platinum therapy failure, unresectable locally advanced or metastatic untreated non-small cell lung cancer, hormone-refractory prostate cancer, untreated advanced gastric adenocarcinoma, and locally advanced squamous cell head and neck cancer.

The most common adverse reactions are infections, neutropenia, anemia, febrile neutropenia, hypersensitivity, thrombocytopenia, neuropathy, dysgeusia, dyspnea, constipation, anorexia, nail disorders, fluid retention, asthenia, pain, nausea, diarrhea, vomiting, mucositis, alopecia, skin reactions, and myalgia.

1.6 Gemcitabine and Docetaxel Combination

In 2007, Maki et al published SARC02, a randomized Phase II clinical trial comparing gemcitabine versus the combination of gemcitabine with docetaxel [12]. One hundred twenty-two patients were randomized using adaptive randomization; 73 received gemcitabine and docetaxel and 49 received gemcitabine alone. The progression-free survival was 6.2 months for gemcitabine and docetaxel versus 3.0 months for gemcitabine alone. The overall survival was 17.9 months for gemcitabine-docetaxel and 11.5 months for gemcitabine. The RECIST Response Rate was 16% (gemcitabine-docetaxel) and 8% (gemcitabine). In addition, median overall survival was 17.9 months for gemcitabine-docetaxel and 11.5 months for gemcitabine. This established the regimen of gemcitabine and docetaxel as a standard second-line therapy for most high grade soft tissue sarcomas [5]. More recently, the control arm of the Morphoteck clinical trial of gemcitabine, docetaxel, and Morb009 demonstrated a PFS of 5.6 months, which was not measurably different than the Maki trial. This left gemcitabine and docetaxel as a standard second-line therapy for most high grade soft tissue sarcomas.

1.7 Study Rationale

In a search for common metabolic alterations that could provide treatment opportunities for a broad range of sarcomas, we investigated the expression of metabolic genes that are commonly altered in other chemorefractory tumors. We immediately noted that ASS1 expression is commonly lost in multiple such tumors including hepatocellular carcinoma, renal cell carcinoma, and platinum refractory ovarian cancer[13]. Given the chemorefractory nature of sarcoma, we decided to perform an extensive analysis across 45 of the most common subtypes in 701 unique tumors (Figure 1A). In collaboration with a sarcoma pathologist, we initially planned to apply an intensity score for ASS1 expression (intensity score 0-3) similar to that used for other biomarkers such as ER and AR, and similar to that used for ASS1 in other tumors where heterogeneous expression is noted. Surprisingly, most patients 653/701 (93.2%) diagnosed with bone 34/39 (87.2%) or soft tissue sarcomas 619/662 (93.5%) tumors showed absolutely no signal for ASS1 expression by IHC. Thus

sarcomas harbor a metabolic defect, ASS1 deficiency, which would be anticipated to make them uniquely susceptible to arginine depletion therapy with ADI-PEG 20.

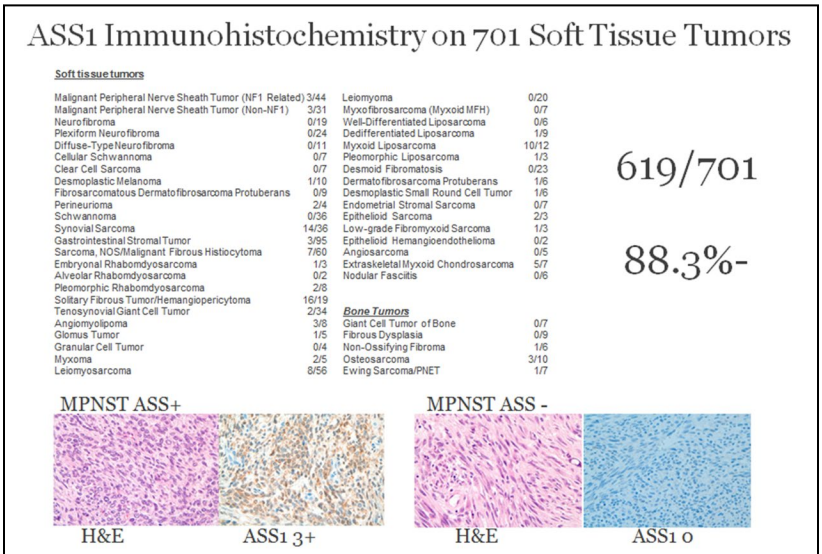


Figure 1 Top. In a panel of primary sarcoma tumors with anti-ASS immunohistochemistry, ASS1 lacks strong expression in 619/662 (93.5%) primary soft tissue and 34/39 (87.2%) bone sarcoma tumors representing 45 soft tissue and 5 bone sarcoma histological subtypes. Bottom. Immunohistochemistry of MPNST ASS1+ and ASS1- tumor samples stained with Hematoxylin & Eosin, and anti-ASS1.

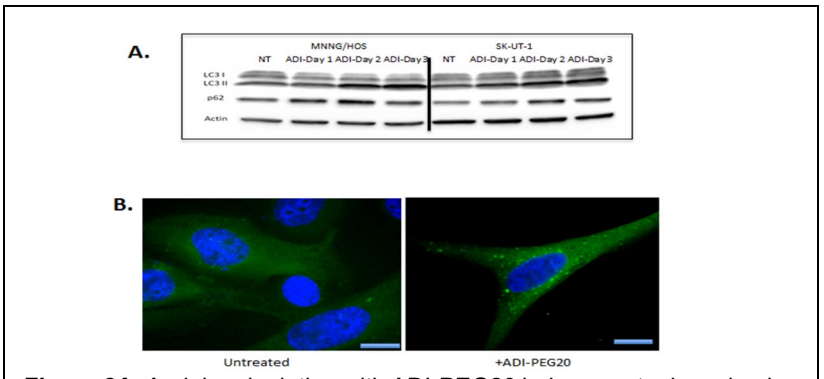


Figure 2A. Arginine depletion with ADI-PEG20 induces autophagy by day 2 as seen by increased LC3 cleavage and p62 alterations in ASS1 deficient MNNG/HOS and SKLMS-1 cell lines. B. Lentiviral expression of GFP-LC3 demonstrates an increase in the number of puncta after ADI-PEG20 treatment.

1.7.1 ADI-PEG 20 induces Autophagy

We analyzed markers of autophagy, including LC3 cleavage and p62 accumulation by immunoblotting. ADI-PEG 20-treated ASS1^{low} sarcoma cells demonstrated increased LC3 cleavage and increased p62 abundance, indicating the induction of autophagy after arginine deprivation (Fig. 2A). We then used lentiviral expression GFP-LC3[14] in leiomyosarcoma cell line SKLMS1 and treated with ADI-PEG20 for three days (Fig. 2B) and observed a dramatic increase in the number of GFP-LC3 puncta upon treatment. Western blots show this increase in the formation of LC3

cleavage, clearly implicating autophagy is a pro-survival mechanism for arginine depletion.

1.7.2 ADI-PEG 20 inhibits phospho-PKM2 expression

As part of a metabolic characterization of autophagic changes induced by arginine starvation, we treated the sarcoma cell lines SKLMS1 and SKUT1 with ADI-PEG20 for three days and immunoblotted for PKM2, phospho-PKM2, and PMK1 (Figure 3). A significant decrease in Phospho-PKM2 was seen using western blot. A decrease in phospho-PKM2 is important as it is the phosphorylated form of PKM2 that is responsible for the metabolic reprograming of cancer. The decrease of phospho-PKM2 would alter the flux of glucose, supporting the idea that prolonged ADI-PEG20 treatment decreases the predominant use of glycolysis for energy generation.

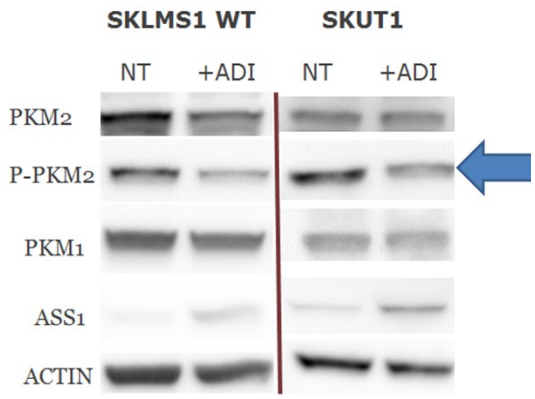


Figure 3: The sarcoma cell lines SKLMs1 and SKUT1 were treated for three days with ADI-PEG20. Loss of phospho-PKM2 espression is demonstated.

1.7.3 Loss of Phospho-PKM2 diverts glucose to serine biosynthesis for nucleotide biosynthesis

Finally, as we had discovered that loss of PKM2 led to glutamine anaplerosis for TCA substrates, we asked the question what the fate of glucose is in amino acid induced autophagy. Surprisingly, using Carbon 13 (C13) labeling of glucose at all 6 positions, we identified that glucose was not fluxing towards lactate, citrate or adenosine synthesis, but into serine biosynthesis (**Figure 4A**). Serine biosynthesis is part of the pyrimidine biosynthetic pathway. We then measured phosphoglycerate dehydrogenase (PHGDH), the first enzyme in the serine biosythetic pathway and found a dramatic upregulation. As PKM2 is known to downregulate serine biosynthesis as there is a feedback loop [15], this allows for an additional theraputic option. Indeed, gemcitibine was found to have a lower IC50 when combined with ADI-PEG20 (data not shown). As nucleotides are derived from glucose, targetting with nuceotide deivitive can also be considered a metabolic therapy.

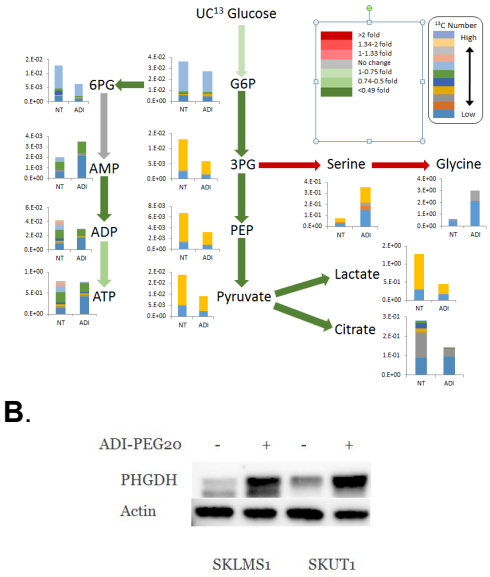


Figure 4: A. Carbon 13 tracing in SKLMS1 cells after treatment with ADI-PEG20 demonstrate that the carbons for glucose are diverted to the serine biosynthetic pathway. B. Phosphoglycerate dehydrogenase (PHGDH), the first enzyme in the serine biosyntetic pathway involved in the early steps of L-serine synthesis that are used to make pyrimidines, is upregulated in autophagy.

1.7.4 Summary

1) ASS1 is a common metabolic defect in most sarcomas across histologies making it the most common defect.
2) ADI-PEG 20 induces autophagy in ASS1 deficient sarcomas.
3) Glucose is diverted to the serine biosynthetic pathway for pyrimidine synthesis due to inhibition of PKM2 by ADI-PEG20.
4) Combination therapy driven by a biomarker using metabolism can lead to synthetic lethal combinations.

In summary, the compelling preliminary data presented in this proposal provide a strong rationale for the clinical development of ADI-PEG 20 with gemcitabine in sarcoma patients. Our further understanding of the alterations in metabolism is critical for developing a biomarker based multi-agent metabolic therapy

1.8 Correlative Studies Background

Biopsy will be obtained at Day -7 and Day -1 for metabolomic analysis. There is no day 0 associated with this protocol. Serum will be collected at Days -7 and -1 in addition to Days 1 and 8 of each cycle for pharmacodynamics and immunogenicity. We will be evaluating the metabolomics of ADI response in human tissue and performing IHC analyses for ASS1

and phospho-PKM2.

1.9 Rationale for Cohort 2

Recently published data[17] shows that priming ASS1-deficient tumors with ADI-PEG 20 and docetaxel improves the effect of gemcitabine. Therefore, a cohort of patients consisting of ten patients diagnosed with either osteosarcoma or Ewing's sarcoma (ideally five of each), and five patients diagnosed with small cell lung cancer will be included as an exploratory cohort. Enrollment to Cohort 2 will occur concurrently with Cohort 1.

2.0 OBJECTIVES

2.1 Primary Objective

To determine progression-free survival in patients with soft tissue sarcoma who are treated with ADI-PEG 20 in combination with gemcitabine and docetaxel.

2.2 Secondary Objectives

1. To determine overall survival in patients with soft tissue sarcoma who are treated with ADI-PEG 20 in combination with gemcitabine and docetaxel.
2. To determine the clinical benefit rate (CBR) of patients with soft tissue sarcoma receiving treatment with ADI-PEG 20 in combination with gemcitabine and docetaxel (CBR = the percentage of patients who have experienced CR+PR+SD).
3. To evaluate the safety and tolerability of the combination of ADI-PEG 20, gemcitabine, and docetaxel (by CTCAE v 5.0) in soft tissue sarcoma, osteosarcoma, Ewing's sarcoma and small cell lung cancer.
4. To determine cancer-related mortality in patients with soft tissue sarcoma who are treated with ADI-PEG 20 in combination with gemcitabine and docetaxel.

2.3 Exploratory Objectives

1. To determine the effects of treatment with ADI-PEG 20, gemcitabine, and docetaxel on arginine, citrulline, and glutamine plasma levels.
2. To determine the rate of immunogenicity against ADI-PEG 20.
3. To evaluate the metabolomics of paired biopsies taken before and after receipt of ADI-PEG 20.
4. To correlate response (CBR) to ADI-PEG 20 with phospho-PKM2 status.
5. To correlate response (CBR) to ADI-PEG 20 with ASS1 negativity in clinical samples.
6. To correlate response (CBR) to ADI-PEG 20 by Choi criteria[16].

3.0 PATIENT SELECTION

Each of the criteria in the checklist that follows must be met in order for a patient to be considered

eligible for this study. Use the checklist to confirm a patient's eligibility. For each patient, this checklist must be photocopied, completed and maintained in the patient's chart.

Patient's Initials (F,M,L)_____

NOTE: All questions regarding eligibility should be directed to the principal investigator, Mia Weiss, M.D. or the primary coordinator, Jacqui Toeniskoetter.

NOTE: Institutions may use the eligibility checklist as source documentation if it has been reviewed, signed, and dated prior to registration by the treating physician.

3.1 Inclusion Criteria

_____ 1. Cohort 1: Histologically or cytologically confirmed grade 2 or 3 soft tissue sarcoma that is unresectable or metastatic that would be standardly treated with gemcitabine or gemcitabine and docetaxel. For all others, please contact the principal investigator. Prior surgery for primary or metastatic disease after chemotherapy following a response is allowed.

Cohort 2: Histologically or cytologically confirmed osteosarcoma, Ewing's sarcoma, or small cell lung cancer that is unresectable or metastatic that have either failed standard of care therapy or would be standardly treated with gemcitabine or gemcitabine and docetaxel.

_____ 2. Measurable disease defined as lesions that can be accurately measured in at Least one dimension (longest diameter to be recorded) as ≥ 10 mm with CT scan, as ≥ 20 mm by chest x-ray, or ≥ 10 mm with calipers by clinical exam.

_____ 3. Treated with at least one previous line of systemic therapy. The allowable window between treatments is 21 days for chemotherapy or a TKI or 5 half-lives for a TKI (whichever is shorter), 21 days and progression by CT for immunotherapy, 21 days for RT, 21 days for surgery, or 28 days for an investigational agent.

_____ 4. Cohort 1: At least 16 years of age.

Cohort 2: Patients with osteosarcoma or Ewing's sarcoma must be at least 10 years of age. Patients with small cell lung cancer must be at least 18 years of age.

_____ 5. Cohort 2 (SCLC group ONLY): Must be amenable to biopsy

_____ 6. ECOG performance status ≤ 2 (see Appendix A)

_____ 7. Normal bone marrow and organ function as defined below:
_____ a. Leukocytes ≥ 3,000/mcL
_____ b. Absolute neutrophil count ≥ 1,500/mcl

_____ c. Platelets ≥ 100,000/mcl
_____ d. Total bilirubin ≤ 2 x IULN
_____ e. AST(SGOT)/ALT(SGPT) ≤ 3 x IULN (or ≤ 5 x IULN if liver metastases are present)
_____ f. Creatinine ≤ 1.5 x IULN OR Creatinine clearance ≥ 60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal
_____ g. Serum uric acid ≤ 8 mg/dL (with or without medication control)

_____ 8. Women of childbearing 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 participating in this study, she must inform her treating physician immediately.

_____ 9. Ability to understand and willingness to sign an IRB approved written informed consent document (or that of legally authorized representative, if applicable).

3.2 Exclusion Criteria

_____ 1. A history of other high grade malignancy ≤ 5 years previous. Exceptions include basal cell or squamous cell carcinoma of the skin which were treated with local resection only or carcinoma *in situ* of the cervix, or other tumors discussed with the study PI.

_____ 2. Currently receiving any other investigational agents.

_____ 3. Prior treatment with ADI-PEG 20, gemcitabine, or docetaxel. Patients treated > one year ago in the adjuvant/neoadjuvant setting with gemcitabine or docetaxel would be allowed to be enrolled on trial.

_____ 4. Known brain metastases. Patients with known brain metastases must be excluded from this clinical trial (except for patients with SCLC, see below) because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.

Patients with SCLC are allowed to enroll with brain metastases provided they are stable and they are at least 3 months post-treatment for brain metastases.

_____ 5. A history of allergic reactions attributed to compounds of similar chemical or biologic composition to ADI-PEG 20, gemcitabine, pegylated compounds, or other agents used in the study.

_____ 6. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris,

cardiac\arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

_____ 7. History of seizure disorder not related to underlying cancer.

_____ 8. Patients with Grade 2 or higher neuropathy

_____ 9. Pregnant and/or breastfeeding. Women of childbearing potential must have a negative pregnancy test within 14 days of study entry.

_____ 10. Known HIV-positivity on combination antiretroviral therapy because of the potential for pharmacokinetic interactions with the study treatment. 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.

_____
Physician Name

_____ _____
Physician Signature Date

3.3 Inclusion of Women and Minorities

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

4.0 REGISTRATION PROCEDURES

Patients must not start any protocol intervention prior to registration through the Siteman Cancer Center.

The following steps must be taken before registering patients to this study:

1. Confirmation of patient eligibility by Washington University
2. Registration of patient in the Siteman Cancer Center database
3. Assignment of unique patient number (UPN)

Once the patient has been entered in the Siteman Cancer Center OnCore database, the WUSM coordinator will forward verification of enrollment and the UPN via email.

4.1 Confirmation of Patient Eligibility

Confirm patient eligibility by collecting the information listed below and scanning and emailing it to the research coordinator listed in the *Siteman Cancer Center Clinical Trials*

Core Protocol Procedures for Secondary Sites packet at least one business day prior to registering patient:

1. Name and contact information (telephone number, fax number, and email address)
2. Site PI's name, the registering MD's name, and the institution name
3. Patient's race, sex, and DOB
4. Three letters (or two letters and a dash) for the patient's initials
5. Currently approved protocol version date
6. Copy of signed consent form (patient name may be blacked out)
7. Planned date of enrollment
8. Completed eligibility checklist, signed and dated by a member of the study team
9. Copy of appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center OnCore Database

Registrations may be submitted Monday through Friday between 8am and 5pm CT. Urgent late afternoon or early morning enrollments should be planned in advance and coordinated with the Washington University research coordinator. Registration will be confirmed by the research coordinator or his/her delegate by email within one business day. Verification of eligibility and registration should be kept in the patient chart.

All patients at all sites must be registered through the Siteman Cancer Center OnCore database at Washington University.

4.3 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. Patients will also be identified by first, middle, and last initials. If the patient has no middle initial, a dash will be used on the case report forms (CRFs). All data will be recorded with this identification number on the appropriate CRFs.

5.0 TREATMENT PLAN

5.1 Premedication Administration

It is recommended that patients be pre-medicated for docetaxel with dexamethasone 8 mg PO BID (or per institutional standards) the day before docetaxel administration and the day after docetaxel administration. It is also recommended that patients receive IV dexamethasone (10 mg recommended or per institutional standards) on day of docetaxel administration in order to reduce the incidence and severity of fluid retention as well as the severity of hypersensitivity reactions for both gemcitabine and docetaxel. Modifications to the recommended premedication regimen may be made after discussion with the PI.

All patients should be pre-medicated for gemcitabine on Days 1 and 8 for anti-emesis per institutional practice.

There is no recommended premedication for ADI-PEG 20 except for Diphenhydramine 50mg PO on Days 1, 8, +15, if prior reaction.

5.2 Agent Administration

ADI-PEG 20 will be given on Day -7 of Cycle 1 and then on Days 1, 8, and 15 of each subsequent cycle. Cycles are 21 days. ADI-PEG 20 is to be given weekly regardless to Day 1 or Day 8 dose delays. This could cause cycles that extend beyond 21 days.

ADI-PEG 20 will be given on an outpatient basis at a dose of 36 mg/m^2 via intramuscular injection into either the deltoid or gluteal muscle.

Gemcitabine will be given intravenously at a dose of 600 mg/m^2 over 90 (± 10 minutes) minutes on Days 1 and 8 of each cycle. Docetaxel will be given intravenously at a dose of 60 mg/m^2 over 60 minutes (± 10 minutes) on Day 8 of each cycle.

Patients started on gemcitabine at a dose of 900 mg/m^2 or 750 mg/m^2 or docetaxel at a dose of 75 mg/m^2 per previous protocol version will be allowed to continue at that dose level provided they are not experiencing unacceptable toxicity and the treating physician feels it is in their best interests to continue at that dose level.

On days of doublet or triplet dosing, ADI-PEG 20 is to be given first, followed by gemcitabine and then, if indicated, by docetaxel.

Growth factor support to be initiated on Day 9 is recommended with either pegfilgrastim, Neupogen or equivalent.

After Cycle 8, patients may continue on ADI-PEG 20 alone (without gemcitabine and docetaxel) upon request.

5.3 Evaluability

All patients who receive any study treatment are evaluable for toxicity. Patients are evaluated from first receiving study treatment until a 30-day follow up after the conclusion of treatment or death.

All Cohort 1 patients who receive gemcitabine are evaluable for disease response unless they discontinue treatment due to treatment related adverse events(s) prior to completion of Cycle 2 and have not had any disease assessment. Any Cohort 1 patient who does not receive gemcitabine will be replaced. Cohort 2 patients will not be replaced.

5.4 General Concomitant Medication and Supportive Care Guidelines

It is advised that uric acid levels should be routinely tested and that prompt treatment, including the administration of fluids, allopurinol, and/or urate oxidase, should be provided as clinically indicated to treat suspected tumor lysis syndrome.

5.5 Women of Childbearing Potential

Women of childbearing potential (defined as women with regular menses, women with amenorrhea, women with irregular cycles, women using a contraceptive method that precludes withdrawal bleeding, and women who have had a tubal ligation) are required to have a negative pregnancy test within 14 days prior to the first dose of ADI-PEG 20.

Female and male patients (along with their female partners) are required to use two forms of acceptable contraception, including one barrier method, during participation in the study and for 1 month following the last dose of any study agent.

If a patient is suspected to be pregnant, treatment should be immediately discontinued. In addition, a positive urine test must be confirmed by a serum pregnancy test. If it is confirmed that the patient is not pregnant, the patient may resume dosing.

If a female patient or female partner of a male patient becomes pregnant during therapy or within 1 months after the last dose of any study agent, the investigator must be notified in order to facilitate outcome follow-up, and the investigator will be responsible for reporting to Polaris per Section 7.6.

5.6 Duration of Therapy

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the protocol therapy should be discontinued and the reason(s) for discontinuation documented in the case report forms.

In the absence of treatment delays due to adverse events, treatment may continue for up to 34 cycles or 103 weeks or until one of the following criteria applies:

- Documented and confirmed disease progression (by RECIST 1.1)
- Death
- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of study drug
- General or specific changes in the patient's condition render the patient unable to receive further treatment in the judgment of the investigator
- Suspected pregnancy
- Serious non-compliance with the study protocol
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- The Siteman Cancer Center decides to close the study

- At the request of Polaris Pharmaceuticals

Patients who prematurely discontinue treatment for any reason will be followed as indicated in the study calendar for survival.

5.7 Duration of Follow-up

Patients will be followed as per standard of care. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. Data will be collected on progression, at the time of reporting, and survival at 5 years after the off treatment date by review of the medical record.

5.8 Study Stopping Rule

Accrual will be paused and the event will be reviewed by the Data and Safety Monitoring Board if a grade 5 non-hematological toxicity is observed that is deemed probably or definitely related to ADI-PEG 20, gemcitabine, docetaxel, or combination. Note that sepsis is considered a hematological toxicity.

6.0 DOSE DELAYS/DOSE MODIFICATIONS

Dosing adjustments beyond what is specified below may be made to the gemcitabine and docetaxel chemotherapy regimen for tolerance after discussion with the PI.

6.1 Dose Modifications for ADI-PEG 20

There will be no dose modifications for ADI-PEG 20. ADI-PEG 20 is to be given weekly regardless to day 1 or day 8 dose delays. In the event of an allergic reaction or anaphylaxis, treat with antihistamines and glucocorticoids. Treatment with ADI-PEG 20 should be immediately discontinued for grade 3 or greater allergic reaction. Patients who discontinue ADI-PEG 20 may continue to receive gem/tax on protocol.

6.2 Dose Modifications for Gemcitabine

Recommended dose modifications for myelosuppression are described in the table below.

Absolute granulocyte count ($\times 10^6$/L)		**Platelet count ($\times 10^6$/L)**	**% of full dose**
≥ 1000	And	≥ 100,000	100
500-999	Or	50,000-99,999	Reduce by 1 dose level
< 500	Or	< 50,000	Hold

For patients continuing on gemcitabine 900 mg/m^2, if toxicity develops, reduce dose to 750 mg/m^2 and then follow current protocol dose modification guidelines. For

patients continuing on gemcitabine 750 mg/m^2, if toxicity develops, reduce dose to 600 mg/m^2 and then follow current protocol dose modification guidelines.

For intolerance or prolonged cytopenias requiring dosing delays of more than 2 weeks, reduce gemcitabine to 500 mg/m^2 the first time, to 400 mg/m^2 the second time, and to 300 mg/m^2 the third time.

Permanently discontinue gemcitabine for any of the following:

- Unexplained dyspnea or other evidence of severe pulmonary toxicity
- Severe hepatic toxicity
- Hemolytic-uremic syndrome
- Capillary leak syndrome
- Posterior reversible encephalopathy syndrome

Withhold gemcitabine or reduce dose to 500 mg/m^2 for other severe (Grade 3 or 4) non-hematologic adverse drug reaction until resolved. No dose modifications are recommended for alopecia nausea, or vomiting. At the discretion of the investigator, the dose may be increased for future cycles.

If gemcitabine or docetaxel is held on Day 8, dose reduce gemcitabine one dose level at next treatment. If dose reduction would result in the patient coming off study, it should be done at the principal investigator's discretion.

6.3 Dose Modifications for Docetaxel

For patients continuing on docetaxel 75 mg/m^2, if toxicity develops, reduce dose to 60 mg/m^2, and then follow current protocol dose modification guidelines. Patients who are dosed initially at 60 mg/m^2 and who experience either febrile neutropenia, neutrophils < 500 cells/mm^3 for more than 1 week, or severe or cumulative cutaneous reactions during docetaxel should have the dosage adjusted from 60 mg/m^2 to 50 mg/m^2. If the patient continues to experience these reactions, the dosage should either be decreased from 50 mg/m^2 to 40 mg/m^2 or their treatment should be discontinued. Docetaxel should not be given if neutrophil counts are < 1500 cells/mm^3.

Docetaxel should not be given to patients with bilirubin > ULN or to patients with AST and/or ALT > 1.5 x ULN concomitant with alkaline phosphatase > 2.5 x ULN.

Severe hypersensitivity and anaphylaxis require immediate discontinuation of docetaxel.

If grade ≥ 3 peripheral neuropathy occurs, hold docetaxel until symptoms ≤ grade 1, then restart at a reduced dose level (if 75 mg/m^2, reduce to 60 mg/m^2; if 60 mg/m^2, reduce to 50 mg/m^2; if 50 mg/m^2, reduce to 40 mg/m^2; if 40 mg/m^2, discontinue docetaxel). Docetaxel can be discontinued independently of gemcitabine for neuropathy.

Docetaxel should not be given to patients with platelets less than 100 K/mm^3

Docetaxel should not be given to patients with hemoglobin < 7.0g/dL

Docetaxel should be dose reduced in discussion with the principal investigator when gemcitabine is reduced. After discussion with the principal investigator, reduce docetaxel to 50 mg/m^2 the first time, 40 mg/m^2 the second time, and 30 mg/m^2 the third time.

Any other dose modifications or delays should be discussed with the principal investigator before continuing treatment.

7.0 REGULATORY AND REPORTING REQUIREMENTS

The entities providing oversight of safety and compliance with the protocol require reporting as outlined below. Please refer to Appendix B for definitions and Appendix C for a grid of reporting timelines.

Adverse events will be tracked from start of treatment through 30 days following the last day of study treatment. All adverse events must be recorded on the toxicity tracking case report form.

Refer to the data submission schedule in Section 11 for instructions on the collection of AEs in the EDC.

Reporting requirements for Washington University study team may be found in Section 7.1. Reporting requirements for secondary site study teams participating in Washington University-coordinated research may be found in Section 7.2.

7.1 Sponsor-Investigator Reporting Requirements

7.1.1 Reporting to the Human Research Protection Office (HRPO) at Washington University

Reporting will be conducted in accordance with Washington University IRB Policies.

Pre-approval of all protocol exceptions must be obtained prior to implementing the change.

7.1.2 Reporting to the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University

The Washington University Sponsor-Investigator (or designee) is required to notify the QASMC of any unanticipated problems involving risks to participants or others occurring at WU or any BJH or SLCH institution that has been reported to and acknowledged by HRPO. (Unanticipated problems reported to HRPO and withdrawn during the review process need not be reported to QASMC.)

QASMC must be notified within **10 days** of receipt of IRB acknowledgment via email to a qasmc@wustl.edu. Submission to QASMC must include the myIRB form and any supporting documentation sent with the form.

For events that occur at secondary sites, the Washington University Sponsor Investigator (or designee) is required to notify the QASMC within 10 days of Washington University notification via email to qasmc@wustl.edu. Submission to QASMC must include either the myIRB form and supporting documentation or (if not submitted to myIRB) the date of occurrence, description of the event, whether the event is described in the currently IRB approved materials, the event outcome, determination of relatedness, whether currently enrolled participants will be notified, and whether the informed consent document and/or any study procedures will be modified as a result of this event.

7.1.3 Reporting to Polaris Pharmaceuticals

Polaris will be notified via email of all initial and follow up serious unexpected suspect adverse reactions (SUSARs) in real time (within 24 hours of study PI notification) and include copy of MedWatch(s).

Polaris will be notified of all AEs and SAEs monthly via AE notification or cross-reporting reports/documents per usual institutional practice. Report or document item requirements/format to be discussed. The reports should be sent via email to agreed upon personnel and or Polaris safety email – safety@polarispharma.com.

Polaris will be notified of any study drug overdoses and pregnancies that occur on study, as they occur. This will include initial reporting as well as follow up (resolution) data.

7.1.4 Reporting to the FDA

The conduct of the study will comply with all FDA safety reporting requirements. **PLEASE NOTE THAT REPORTING REQUIREMENTS FOR THE FDA DIFFER FROM REPORTING REQUIREMENTS FOR HRPO/QASMC.** It is the responsibility of the Washington University Sponsor-Investigator to report to the FDA as follows:

- Report any unexpected fatal or life-threatening suspected adverse reaction (refer to Appendix B for definitions) no later than **7 calendar days** after initial receipt of the information.
- Report a suspected adverse reaction that is both serious and unexpected (SUSAR, refer to Appendix B) no later than **15 calendar days** after it is determined that the information qualifies for reporting. Report an adverse event (refer to Appendix B) as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event, such as:

    - A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure
    - One or more occurrences of an event that is not commonly associated with drug exposure but is otherwise uncommon in the population exposed to the drug
    - An aggregate analysis of specific events observed in a clinical trial that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group
- Report any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies that suggest a significant risk in humans exposed to the drug no later than **15 calendar days** after it is determined that the information qualifies for reporting.
- Report any findings from animal or in vitro testing that suggest significant risk in humans exposed to the drug no later than **15 calendar days** after it is determined that the information qualifies for reporting.
- Report any clinically important increase in the rate of a serious suspected adverse reaction of that listed in the protocol or IB within **15 calendar days** after it is determined that the information qualifies for reporting.

Submit each report as an IND safety report in a narrative format or on FDA Form 3500A or in an electronic format that FDA can process, review, and archive. Study teams must notify the Siteman Cancer Center Protocol Development team of each potentially reportable event within 1 business day after initial receipt of the information, and must bring the signed 1571 and FDA Form 3500A to the Siteman Cancer Center Protocol Development team no later than 1 business day prior to the due date for reporting to the FDA.

Each notification to FDA must bear prominent identification of its contents ("IND Safety Report") and must be transmitted to the review division in the Center for Drug Evaluation and Research (CDER) or in the Center for Biologics Evaluation and Research (CBER) that has responsibility for review of the IND. Relevant follow-up information to an IND safety report must be submitted as soon as the information is available and must be identified as such ("Follow-up IND Safety Report").

7.1.5 Reporting to Secondary Sites

The Washington University Sponsor-Investigator (or designee) will notify the research team at each secondary site of all unanticipated problems involving risks to participants or others that have occurred at other sites within **10 working days** of the occurrence of the event or notification of the Sponsor-Investigator (or designee) of the event. This includes events that take place both at Washington University and at other secondary sites, if applicable. Refer to Section 16.0 (Multicenter Management) for more information

7.2 Secondary Site Reporting Requirements

The research team at each secondary site is required to promptly notify the Washington University Sponsor-Investigator and designee of all serious adverse events (refer to Appendix B, Section D) within **1 working day** of the occurrence of the event or notification of the secondary site's PI of the event. This notification may take place via email if there is not yet enough information for a formal written report (using FDA Form 3500a (MedWatch) and Washington University's cover sheet (Appendix D)). A formal written report must be sent to the Washington University Sponsor-Investigator and designee within **4 calendar days** (for fatal or life-threatening suspected adverse reactions) or **11 calendar days** (for serious unexpected adverse reactions) of the occurrence of the event or notification of the secondary site's PI of the event.

The research team at a secondary site is responsible for following its site's guidelines for reporting applicable events to its site's IRB according to its own institutional guidelines. The research team at Washington University is responsible for reporting all applicable events to the FDA and Polaris Pharmaceuticals as needed.

Washington University pre-approval of all protocol exceptions must be obtained prior to implementing the change. Local IRB approval must be obtained as per local guidelines. Washington University IRB approval is not required for protocol exceptions occurring at secondary sites.

7.3 Exceptions to Expedited Reporting

Events that do not require expedited reporting as described in Section 1.1 include:

- planned hospitalizations
- hospitalizations < 24 hours
- respite care
- events related to disease progression

Events that do not require expedited reporting must still be captured in the EDC.

8.0 PHARMACEUTICAL INFORMATION

8.1 ADI-PEG 20

8.1.1 ADI-PEG 20 Description

Arginine deiminase (ADI) is a recombinant protein cloned from M. hominis, produced in E. coli, and conjugated with PEG of 20,000 mw using a succinimidyl succinate linker. Thus ADI-PEG 20 is an arginine degrading enzyme, ADI, coupled to PEG.

8.1.2 Clinical Pharmacology

The primary mechanism of action of ADI is arginine depletion.

ADI catalyzes the hydrolysis of L-arginine into L-citrulline and ammonia. For tumors that are auxotrophic for arginine due to lack of ASS, the depletion of arginine via ADI treatment may lead to inhibition of protein synthesis and eventual cell death. Conversely, normal human cells will be able to utilize L-citrulline to synthesize L-arginine via the Krebs urea cycle and therefore should not be affected by the ADI treatment.

8.1.3 Pharmacokinetics and Drug Metabolism

Analyses for pharmacodynamics and pharmacokinetics after multiple dosing of ADI-PEG 20 were performed in multiple studies. The pharmacodynamic data demonstrate that in most humans, repetitive dosing of ADI-PEG 20 results in sustained decreases in peripheral blood arginine, with a concomitant rise in citrulline in humans with metastatic melanoma or HCC.

8.1.4 Supplier(s)

ADI-PEG 20 will be provided free of charge by Polaris Pharmaceuticals, Inc.

8.1.5 Dosage Form and Preparation

ADI-PEG 20 is ready for injection without reconstitution. All vials should be used only once and any remaining material present in the vial should be discarded.

The preparation does not need a laminar flow unit.

The drug may be drawn up into a syringe by the pharmacy or the vials may be dispensed to a nurse who can draw up the required amount “at the bedside.”

8.1.6 Storage and Stability

ADI-PEG 20 is shipped on dry ice. Histidine-buffered ADI-PEG 20 must be stored frozen at -20°C. The drug can be thawed by one of two methods.

Immediately prior to use each study medication vial may be taken out of the freezer and left at room temperature to thaw. This may take approximately 1-2 hours. Once thawed and brought to room temperature ADI-PEG 20 is ready for use. If there is delay in administering the study medication, the thawed vial may be stored in a refrigerator until ready for use. If stored in a refrigerator allow the vial to stand at room temperature for at least 10 minutes prior to administration.

Alternatively, each vial may be taken out of the freezer and thawed overnight in the refrigerator at 2-8°C prior to the day of administration. Allow the vial to come to room temperature for ~10 minutes prior to administration. Once thawed and

brought to room temperature ADI-PEG 20 is ready for use.

The study medication vial must be used within 24 hours after removal from the freezer. The study medication is stable at 2-8°C for up to 24 hours.

8.1.7 Administration

ADI-PEG 20 will be given on an outpatient basis as a single intramuscular injection into either the deltoid or gluteal muscle.

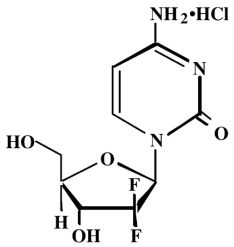
8.1.8 Special Handling Instructions

No special handling instructions.

8.2 Gemcitabine (Gemzar)

8.2.1 Gemcitabine Description

Gemcitabine is a nucleoside metabolic inhibitor that exhibits antitumor activity. Gemcitabine HCl is 2'-deoxy-2',2'-difluorocytidine monohydrochloride (β-isomer). The structural formula is as follows:



The empirical formula for gemcitabine HCl is $C_9H_{11}F_2N_3O_4 \cdot HCl$. It has a molecular weight of 299.66.

8.2.2 Clinical Pharmacology

Gemcitabine kills cells undergoing DNA synthesis and blocks the progression of cells through the G1/S-phase boundary. Gemcitabine is metabolized by nucleoside kinases to diphosphate (dFdCDP) and triphosphate (dFdCTP) nucleosides. Gemcitabine diphosphate inhibits ribonucleotide reductase, an enzyme responsible for catalyzing the reactions that generate deoxynucleoside triphosphates for DNA synthesis, resulting in reductions in deoxynucleotide concentrations, including dCTP. Gemcitabine triphosphate competes with dCTP for incorporation into DNA. The reduction in the intracellular concentration of dCTP by the action of the diphosphate enhances the incorporation of gemcitabine triphosphate into DNA (self-potentiation). After the gemcitabine nucleotide is incorporated into DNA, only one additional nucleotide is added to the growing DNA strands, which eventually results in the initiation of apoptotic cell death.

8.2.3 Pharmacokinetics and Drug Metabolism

Gemcitabine disposition was studied in 5 patients who received a single 1000 mg/m^2/30-minute infusion of radiolabeled drug. Within one (1) week, 92% to 98% of the dose was recovered, almost entirely in the urine. Gemcitabine (<10%) and the inactive uracil metabolite, 2'-deoxy-2',2'-difluorouridine (dFdU), accounted for 99% of the excreted dose. The metabolite dFdU is also found in plasma.

The active metabolite, gemcitabine triphosphate, can be extracted from peripheral blood mononuclear cells. The half-life of the terminal phase for gemcitabine triphosphate from mononuclear cells ranges from 1.7 to 19.4 hours.

8.2.4 Supplier(s)

Gemcitabine will be given as per routine care from commercial supply.

8.2.5 Dosage Form and Preparation

Gemcitabine for injection, USP, is available in sterile single-use vials individually packaged in a carton containing: 200 mg white to off-white lyophilized powder in a 10-mL size sterile single use vial or 1 g white to off-white lyophilized powder in a 50-mL size sterile single use vial.

8.2.6 Storage and Stability

Unopened vials of gemcitabine are stable until the expiration date indicated on the package when stored at controlled room temperature 20°C to 25°C and that allows for excursions between 15°C and 30°C.

8.2.7 Administration

Gemcitabine should be given as an intravenous infusion over the course of 90 minutes (± 10 minutes).

8.2.8 Special Handling Instructions

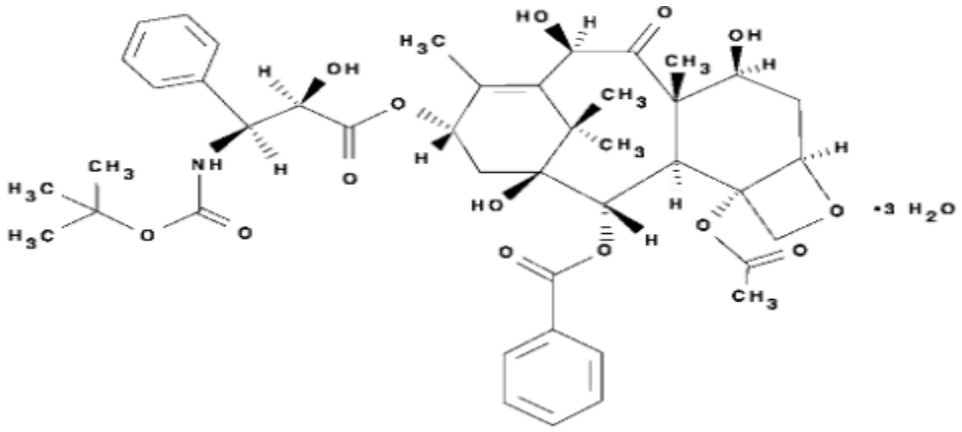
No special handling instructions.

8.3 Docetaxel (Taxotere)

8.3.1 Docetaxel Description

Docetaxel is an antineoplastic agent belonging to the taxoid family. It is prepared by semisynthesis beginning with a precursor extracted from the renewable needle biomass of yew plants. The chemical name for docetaxel is (2R,3S)-N-carboxy-3-

phenyllisoserine,N-*tert*-butyl ester, 13-ester with 5β-20-epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4-acetate 2-benzoate, trihydrate. Docetaxel has the following structural formula:



Docetaxel is a white to almost-white powder with an empirical formula of $C_{43}H_{53}NO_{14} \cdot 3H_2O$ and a molecular weight of 861.9.

8.3.2 Clinical Pharmacology

Docetaxel is an antineoplastic agent that acts by disrupting the microtubular network in cells that is essential for mitotic and interphase cellular functions. Docetaxel binds to free tubulin and promotes the assembly of tubulin into stable microtubules while simultaneously inhibiting their disassembly. This leads to the production of microtubule bundles without normal function and to the stabilization of microtubules, which results in the inhibition of mitosis in cells. Docetaxel's binding to microtubules does not alter the number of protofilaments in the bound microtubules, a feature which differs from most spindle poisons currently in use.

8.3.3 Pharmacokinetics and Drug Metabolism

The AUC is dose proportional following doses of 70 mg/m^2 to 115 mg/m^2 with infusions times of 1 to 2 hours. Docetaxel's pharmacokinetic profile is consistent with a three-compartment pharmacokinetic model, with half-lives for the α, β, and γ phases of 4 min, 36 min, and 11.1 hr, respectively. Mean total body clearance was 21 L/h/m2.

It is metabolized by the CYP3A4 isoenzyme. It is eliminated in both the urine and feces following oxidative metabolism of the *tert*-butyl ester group, but fecal excretion was the main elimination route.

8.3.4 Supplier(s)

Docetaxel will be given as per routine care from commercial supply.

8.3.5 Dosage Form and Preparation

Docetaxel is supplied in a single use vial as a sterile, pyrogen-free, non-aqueous solution. One-vial (injection concentrate) docetaxel requires NO prior dilution with a diluent and is ready to add to the infusion solution. Use only a 21 gauge needle to withdraw from the vial because larger bore needles may result in stopper coring and rubber particulates. Inject via a single injection into a 250 mL infusion bag or bottle of either 0.9% Sodium Chloride solution or 5% Dextrose solution to produce a final concentration of 0.3 mg/mL to 0.74 mg/mL. Thoroughly mix the infusion by gentle manual rotation.

8.3.6 Storage and Stability

Store between 2°C and 25°C. Retain in the original package to protect from light.

8.3.7 Administration

Docetaxel should be given as an intravenous infusion over the course of 60 minutes (± 10 minutes).

8.3.8 Special Handling Instructions

No special handling instructions.

9.0 CORRELATIVE STUDIES

9.1 Tumor Biopsy

9.1.1 Collection of Specimens

Tumor biopsies (10 cores by needle biopsies) will be performed up to 21 days prior to initiation of ADI-PEG 20 and Day -1 (± 1 day as long as performed prior to initiation of gemcitabine). Tumor biopsies are mandatory for the first 20 patients amenable to biopsy enrolled at Washington University (completed as of 05/14/19) and for all patients enrolled to the SCLC cohort.

9.1.2 Handling of Specimens

Samples should be snap frozen in liquid nitrogen or an equivalent process such as placing in All Protect Tissue Reagent and placed on dry ice.

9.1.3 Specimen Storage

Samples will be stored in the Van Tine laboratory at -80°C.

9.2 Archival Tissue

Five to 10 slides 5 to 10 microns thick of archival tissue will be collected on all patients. Ship to:

Dr. Brian Van Tine – Research Lab
4515 McKinley, 3rd Floor
4523 Clayton Avenue
St. Louis, MO 63110

9.3 Mandatory Peripheral Blood

9.3.1 Collection of Specimens

Eight mL of blood will be drawn into 2 red top SST (Tiger) tubes at the following time points:

- Day -7 (pre-treatment)
- Day -1
- Days 1 and 8 of each cycle

9.3.2 Handling of Specimens

Invert the tubes gently 10 times. Let the tubes stand at room temperature to coagulate for 30-60 minutes. Centrifuge within one hour of collection at 1600 G for 15 minutes. Draw off serum from one red top SST tube and fill two 1.8ml. cryovials labeled "AAA" (amino acid analysis), ADI-PEG, subject ID, time point and date. Draw off serum from the second red top SST tube and fill two 1.8ml cryovials labeled "immun" immunogenicity), ADI-PEG, subject ID, time point, and date. Store tubes at -70° C or cooler until shipment. Please complete requisition and batch ship all frozen specimens per subject at the end of their treatment. Ship on dry ice to:

Dr. Brian Van Tine – Research Lab
4515 McKinley, 3rd Floor
4523 Clayton Avenue
St. Louis, MO 63110

9.3.3 Specimen Storage

Samples will be stored in the Van Tine laboratory at -80°C.

10.0 STUDY CALENDAR

Screening/baseline evaluations are to be conducted within 14 days prior to start of protocol therapy. Scans and x-rays must be done no more than 28 days prior to the start of the protocol therapy. There is a +/- 2 day window for all trial-related activities including treatment.

	Screening	**D -7**	**D -1**	**D1 of each cycle**	**D8 of each cycle**	**D15 of cycle**	**Every 6 weeks +/- 1 week during treatment**	**30-Day F/U**	**5-Year F/U**
Informed consent	X								
H&P, ECOG PS	X	X		X					
CBC	X			X	X	X[5]			
CMP + uric acid	X			X					
β-hCG[1]	X								
CT C/A/P and tumor assessments	X						X		X[4]
ECG	X								
ADI-PEG 20		X		X	X	X			
Gemcitabine				X	X				
Docetaxel					X				
Archival tissue	X								
Tumor biopsy[6]		X	X						
Blood for amino acid analysis[2]		X	X	X	X				
Blood for immunogenicity[2]		X	X	X	X				
Adverse events		Monitor AEs continuously and during follow-up for 30 days after the last dose of study treatment.							
Review of medical record[3]									X

1. Women of childbearing potential only.
2. Obtained before drug treatment on days of drug treatment, and before tumor biopsy on days of tumor biopsy.
3. For progression and survival information
4. After discontinuation of treatment, scans will be performed every 6-12 weeks (for patients who discontinue for reasons other than progression). There will be a +/- 7 day window for the first year and a +/- 7 week window for Years 2-5. Scans will continue until progression. Patients who discontinue due to progression will not have any further scans mandated by this protocol.
5. Obtain CBC on day 15 of each cycle if patient is taking a blood thinner.
6. Tumor biopsies are mandatory for the first 20 patients amenable to biopsy enrolled at Washington University (completed as of 05/14/19) and for all patients enrolled to the SCLC cohort. Day -7 biopsy may occur up to 21 days prior to initiation of ADI-PEG 20. Day -1 biopsy may occur on Day -2 or Day 1 as long as it is performed prior to initiation of gemcitabine on Day 1.

11.0 DATA SUBMISSION SCHEDULE

Case report forms with appropriate source documentation will be completed according to the schedule listed in this section.

Case Report Form	Submission Schedule
Original Consent Form	Prior to registration
On-Study Form Cohort 2 On Study Form Archival Tissue Form	Prior to starting treatment
Treatment Form	Every cycle
Peripheral Blood Form	Day -7, Day -1, Day 1 of each cycle, Day 8 of each cycle
Tumor Biopsy Form	Day -7, Day -1
AE Form	Continuous
Treatment Summary Form	Completion of treatment
Follow Up Form	Day 30 after end of treatment, 5 years after end of treatment
RECIST Form Choi Response Form	Baseline, every 6 weeks while on treatment, every 6-12 weeks after treatment ends for 5 years or until time of progression.
Death Form	Time of death
MedWatch Form	See Section 7.0 for reporting requirements

Any queries generated by Washington University must be responded to within 28 days of receipt by the participating site. The Washington University research team will conduct a regular review of data status at all secondary sites, with appropriate corrective action to be requested as needed.

11.1 Adverse Event Collection in the Case Report Forms

All adverse events that occur beginning with start of treatment (minus exceptions defined in Section 7.0) must be captured in the Toxicity Form. Baseline AEs should be captured on the Toxicity Form.

Participant death due to disease progression should be reported on the Toxicity Form as grade 5 disease progression. If death is due to an AE (e.g. cardiac disorders: cardiac arrest), report as a grade 5 event under that AE. Participant death must also be recorded on the Death Form.

12.0 MEASUREMENT OF EFFECT

For the purposes of this study, patients should be re-evaluated for response every 6 weeks. In addition to a baseline scan, confirmatory scans should also be obtained 6 weeks following initial documentation of objective response.

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. Please refer to Section 12.1.

Choi response criteria will also be used to evaluate response in this study. Please refer to Section 12.2.

12.1 RECIST 1.1

12.1.1 Disease Parameters

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

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

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

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

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

Target lesions: All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion 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.

12.1.2 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 diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

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

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

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with

CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

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

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

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

Tumor markers: Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [JNCI 96:487-488, 2004; J Clin Oncol 17, 3461-3467, 1999; J Clin Oncol 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [JNCI 92:1534-1535, 2000].

Cytology, Histology: These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in

tumor types, such as germ cell tumors, where known residual benign tumors can remain).

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:

- Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
- 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.
- 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.

12.1.3 Response Criteria

12.1.3.1 Evaluation of Target Lesions

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

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

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

12.1.3.2 Evaluation of Non-Target Lesions

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

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

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

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

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).

12.1.3.3 Evaluation of Best Overall Response

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

For Patients with Measurable Disease (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 since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

12.1.3.4 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.

12.2 Choi Response Criteria

Using disease parameters and response assessment methods as outlined in Section 12.1, Choi response criteria are as follows[16]:

Complete Response (CR): Disappearance of all target lesions

Partial Response (PR): Decrease in tumor size ≥ 10% on CT or decrease in tumor density ≥ 15% on CT, no new lesions

Stable Disease (SD): Does not meet the criteria for CR, PR, or PD

Progressive Disease (PD): Increase in tumor size ≥ 10% (sum of longest diameters of lesions) and does not meet PR criteria by tumor density, or new intratumoral nodules or disease or an increase in the size of the existing intratumoral nodules

13.0 DATA AND SAFETY MONITORING

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, an independent Data and Safety Monitoring Board (DSMB) will be specifically convened for this trial to review toxicity data. A DSMB will consist of no fewer than 3 members including 2 clinical investigators and a biostatistician. DSMB members must be employed by Washington University, Barnes-Jewish Hospital, or St. Louis Children's Hospital. Like investigators, DSMB members are subject to the Washington University School of Medicine policies regarding standards of conduct. Individuals invited to serve on the DSMB will disclose any potential conflicts of interest to the trial principal investigator and/or appropriate university officials, in accordance with institution policies. Potential conflicts that develop during a trial or a member's tenure on a DSMB must also be disclosed.

Until such a time as the first secondary site enrolls its first patient, a semi-annual DSM report to be prepared by the study team will be submitted to the Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning 6 months after study activation at Washington University (if at least one patient has been enrolled) or one year after study activation (if no patients have been enrolled at the 6-month mark).

The DSM report for the DSMB will be prepared by the study team with assistance from the study statistician, will be reviewed by the DSMB, and will be submitted to the QASM Committee. The

DSMB must meet beginning six months after enrollment of the first patient at a secondary site, no more than one month prior to the due date of the DSM report to QASMC. This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual including numbers from participating sites
- Protocol activation date at each participating site
- Average rate of accrual observed in year 1, year 2, and subsequent years at each participating site
- Expected accrual end date and accrual by site
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities at all participating sites
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

Further DSMB responsibilities are described in the DSMB charter.

The study principal investigator and coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines (please refer to Section 7.0).

Refer to the Washington University Quality Assurance and Data Safety Monitoring Committee Policies and Procedures for full details on the responsibilities of the DSMB. This is located on the QASMC website at https://siteman.wustl.edu/research/clinical-research-resources/protocol-office-prmcqasmc/.

14.0 AUDITING

As coordinating center of this trial, Washington University (via the Quality Assurance and Safety Monitoring Committee (QASMC) will monitor each participating site to ensure that all protocol requirements are being met; that applicable federal regulations are being followed; and that best practices for patient safety and data collection are being followed per protocol. Participating sites will be asked to send copies of all audit materials, including source documentation. The audit notification will be sent to the Washington University Research Patient Coordinator, who will

obtain the audit materials from the participating institution.

Notification of an upcoming audit will be sent to the research team one month ahead of the audit. Once accrual numbers are confirmed, and approximately 30 days prior to the audit, a list of the cases selected for review (up to 10 for each site) will be sent to the research team. However, if during the audit the need arises to review cases not initially selected, the research team will be asked to provide the additional charts within two working days.

Items to be evaluated include:

- Subject screening and enrollment
- Reporting of adverse events
- Maintenance of HIPAA compliance
- Completeness of regulatory documentation
- Completeness of participant documentation
- Acquisition of informed consent
- IRB documentation
- Issues of protocol adherence

Additional details regarding the auditing policies and procedures can be found at https://siteman.wustl.edu/research/clinical-research-resources/protocol-office-prmcqasmc/.

15.0 STATISTICAL CONSIDERATIONS

15.1 Study Design

For Cohort 1, a single arm phase II trial will be conducted to evaluate effiacy of the combination regimen, where eligible grade 2 or 3 soft tissue sarcoma patients with unresectable or metastatic tumors will be treated by ADI-PEG20 in combination with gemcitabine and docetaxel.

For Cohort 2, 15 patients (10 with osteosarcoma or Ewing's sarcoma (ideally 5 of each) and 5 with SCLC) will be enrolled to evaluate safety and tolerability of the combination regimen.

15.2 Endpoints

The primary endpoint is progression-free survival (PFS) defined as time on study to time patients progressed on the drug combination or death or latest follow-up if progression/death is not observed yet.

The secondary endpoints include clinical benefit rate (CBR) defined as the proportion of patients with complete response (CR), partial response (PR), or stable disease (SD) lasting 24 weeks or longer by the RECIST standard, overall survival (OS) defined as time on study to time of death due to any reasons or latest follow up (whichever is earlier), and cancer-mortality rate.

15.3 Sample Size Justification

The median PFS of metastatic sarcoma patients receiving the standard gemcitabine and docetaxel treatment was estimated to be 6.2 months in a randomized phase II study (Maki et. al., 2007, JCO). With the addition of ADI-PEG 20, we target to improve the median PFS to 9 months, a 2.8 months or 12 weeks, or 45.2% improvement on patients treated on gemcitabine + docetaxel + ADI-PEG 20 against null hypothesis median PFS of 6.2 months. Assuming uniform accrual within a 12-month enrollment period and a minimum of 6-month follow up, the study will enroll 75 patients to Cohort 1 (with a per month accrual rate ~6.2), expecting 44 events (progression or death), to achieve 80% power to detect the improvement in PFS at a 5% alpha level.

Fifteen patients will be enrolled to Cohort 2: 10 with either osteosarcoma or Ewing's sarcoma (ideally 5 of each) and 5 with non-small cell lung cancer.

15.4 Accrual

The study accrual will continue until 44 events (progression or death) are observed, expected in 75 patients in Cohort 1. By the accrual rate of 6 to 8 patients per month, the study will take 10 to 13 months to enroll a total of 75 patients in Cohort 1. With a targeted minimum follow up of 6 months after enrollment period, the study will take 16 to 19 months to complete. Patient will be followed for 5 years for overall suvivial. Only patients who receive at least one dose of gemcitabine will be counted towards accrual and analysis.

15.5 Analysis Plan

Survival endpoints for Cohort 1 will be analyzed by the Kaplan-Meier product limit method and illustrated by the KM curves with 95% CI, overal and by groups of interest while survival probability will be estimated. Log-rank test will be applied to test difference in survival curves between groups of interest. The CBR and cancer-mortality rate will be estimated with 95% confidence interval (CI). Toxicity profiles will be decribed by descriptive statistics overall and by patient characterisics (age, gender etc) and response categories, by the two cohorts separately and in combination. For explorative analyses, arginine plasma level, phospho-PKM2 and metabolomics marker measured in baseline and a after-treatment sample will be compared by paired t-test or Wilcoxon signed rank test as proper while the measurements at each time point and the change after treatment relative to baseline will be comapred between patients showing CBR versus those not by two sample t-test or Wilcoxon rank sum test as approporate.

15.6 Study Stopping Rules

<u>Non-hematological toxicity and plans for data and safety monitoring:</u>
Early stopping of the Phase II phase of the trial will be based on unacceptable non-hematological toxicity. Approximately less than 25% of patients are expected to experience grade 3 and up non-hematological toxicity and a non-hematological toxicity rate of 40%

or more would definitely be unacceptable. Based on the sequential probability ratio test (SPRT) with 80% power and a 0.05 significance level, the study will be halted if 6 of the first 6, or 7 of the first 9, or 8 of the first 12, or 9 of the first 16, or 10 of the first 19, or 11 of the first 22, or 12 of the first 25, or 13/28, or 14/31, or 15/34, 16/37, 17/40, 18/43, 19/47, 20/50, 21/53, 22/56, 23/59, 24/62, 25/65, 26/68, 27/71, if the 28th non-hematological toxicity is observed before the last patient has completed the trial.

Accrual will be paused and the event will be reviewed by the Data and Safety Monitoring Board if a grade 5 non-hematological toxicity is observed that is deemed probably or definitely related to ADI-PEG 20, gemcitabine, docetaxel, or combination. Note that sepsis is considered a hematological toxicity.

For Cohort 2 with 15 patients in total, we similarly monitor its stopping due to over toxicities. Based on the sequential probability ratio test (SPRT) with 80% power and a 0.05 significance level, the enrollment to Cohort 2 will be halted if 6 of the first 6, or 7 of the first 9, or 8 of the first 12, or 9 before the last patient enrolled are observed with grade 4 or higher non-hematological toxicities not considered unrelated to study treatment and not considered related to disease progression.

Updated stopping rule as of Amendment 7:
Respecting the gemcitabine dosage reductions (from 900 mg/m^2 to 750 mg/m^2 to 600 mg/m^2), the stopping rule will be evaluated by dose cohort and sequential toxicity monitoring will be ensured for future trial conduct. Based on the redefined treatment-emergent serious adverse events (TESAEs, defined as any type of grade 4 or higher non-hematological treatment-emergent toxicity that is not considered unrelated to study treatment and is not considered related to disease progression) and evaluating TESAEs within each of the 3 starting dose cohorts separately, the safety boundary was crossed with gemcitabine at 900 mg/m^2 but stopped immediately after the boundary was crossed and the dose was reduced to 750 mg/m^2. The safety boundary was not crossed and should be safe by definition with gemcitabine at 750 mg/m^2 or 600 mg/m^2. Thus, the dose level of 600 mg/m^2 should be a safe starting dose level for future patients and the same stopping rule based on the sequential probability ratio test (SPRT) with 80% power and a 0.05 significance level will be **restarted** for evaluation on the trial patients enrolled to receive gemcitabine at 600 mg/m^2: the study will be halted if 6 of the first 6, or 7 of the first 9, or 8 of the first 12, or 9 of the first 16, or 10 of the first 19, or 11 of the first 22, or 12 of the first 25, or 13/28, or 14/31, or 15/34, 16/37, 17/40, 18/43, 19/47, 20/50, 21/53, 22/56, 23/59, 24/62, 25/65, 26/68, 27/71, or if the 28th non-hematological toxicity is observed before the last patient has completed the trial.

15.7 Population for Analysis

Safety Population: All subjects who receive at least one dose of ADI-PEG20 will be used for safety analyses. Cohort 1 and Cohort 2 will be analyzed separately and together for toxicities.

Efficacy Evaluable Population: All Cohort 1 subjects who complete the first 2 cycles of

treatments and receive at least 6 doses of ADI-PEG20 and have at least 1 post-baseline RECIST 1.1 assessment, or who discontinue treatment/study due to disease progression or death after having received at least 1 dose of the study drug ADI-PEG20.

16.0 MULTICENTER REGULATORY REQUIREMENTS

Washington University requires that each participating site sends its informed consent document to be reviewed and approved by the Washington University Regulatory Coordinator (or designee) prior to IRB/IEC submission.

Site activation is defined as when the secondary site has received official written documentation from the coordinating center that the site has been approved to begin enrollment. At a minimum, each participating institution must have the following documents on file at Washington University prior to study activation:

- Documentation of IRB approval of the study in the form of a letter or other official document from the participating institution's IRB. This documentation must show which version of the protocol was approved by the IRB.
- Documentation of IRB approval of an informed consent form. The consent must include a statement that data will be shared with Washington University, including the Quality Assurance and Safety Monitoring Committee (QASMC), the DSMC (if applicable), and the Washington University study team.
- Documentation of FWA, signed FDA Form 1572 (if applicable), and the CVs of all participating investigators.
- Protocol signature page signed and dated by the investigator at each participating site.

The coordinating center Principal Investigator (or designee) is responsible for disseminating to the participating sites all study updates, amendments, reportable adverse events, etc. Protocol/consent modifications and IB updates will be forwarded electronically to the secondary sites within 4 weeks of obtaining Washington University IRB approval. Activated secondary sites are expected to submit protocol/consent/IB modifications to their local IRBs within 4 weeks of receipt unless otherwise noted.

Upon the secondary sites obtaining local IRB approval, documentation of such shall be sent to the Washington University study team within 2 weeks of receipt of approval.

Documentation of participating sites' IRB approval of annual continuing reviews, protocol amendments or revisions, all SAE reports, and all protocol violations/deviations/exceptions must be kept on file at Washington University.

The investigator or a designee from each institution must participate in a regular conference call to update and inform regarding the progress of the trial.

17.0 REFERENCES

1 Siegel, R. L., Miller, K. D. & Jemal, A. Cancer statistics, 2016. *CA Cancer J Clin* **66**, 7-30, doi:10.3322/caac.21332 (2016).

2 Van Glabbeke, M., Verweij, J., Judson, I. & Nielsen, O. S. Progression-free rate as the principal end-point for phase II trials in soft-tissue sarcomas. *European journal of cancer* **38**, 543-549 (2002).

3 Santoro, A. *et al.* Doxorubicin versus CYVADIC versus doxorubicin plus ifosfamide in first-line treatment of advanced soft tissue sarcomas: a randomized study of the European Organization for Research and Treatment of Cancer Soft Tissue and Bone Sarcoma Group. *Journal of Clinical Oncology* **13**, 1537-1545, doi:10.1200/jco.1995.13.7.1537 (1995).

4 Tap D. W., L. J. L. R., Chmielowski B., et al. A randomized phase Ib/II study evaluating the safety and efficacy of olaratumab (IMC-3G3), a human anti-platelet-derived growth factor α (PDGFRα) monoclonal antibody, with or without doxorubicin (Dox), in advanced soft tissue sarcoma (STS). *J Clin Oncol 33, 2015 (suppl; abstr 10501)* (2015).

5 von Mehren, M. *et al.* Soft Tissue Sarcoma, Version 2.2016, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* **14**, 758-786 (2016).

6 Phillips, M. M., Sheaff, M. T. & Szlosarek, P. W. Targeting Arginine-Dependent Cancers with Arginine-Degrading Enzymes: Opportunities and Challenges. *Cancer research and treatment : official journal of Korean Cancer Association* **45**, 251-262, doi:10.4143/crt.2013.45.4.251 (2013).

7 Patil, M. D., Shinde, K. D., Patel, G., Chisti, Y. & Banerjee, U. C. Use of response surface method for maximizing the production of arginine deiminase by Pseudomonas putida. *Biotechnology Reports* **10**, 29-37, doi:10.1016/j.btre.2016.03.002 (2016).

8 Pasut, G., Sergi, M. & Veronese, F. M. Anti-cancer PEG-enzymes: 30 years old, but still a current approach. *Advanced Drug Delivery Reviews* **60**, 69-78, doi:https://doi.org/10.1016/j.addr.2007.04.018 (2008).

9 Beddowes, E. *et al.* Phase 1 Dose-Escalation Study of Pegylated Arginine Deiminase, Cisplatin, and Pemetrexed in Patients With Argininosuccinate Synthetase 1-Deficient Thoracic Cancers. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* **35**, 1778-1785, doi:10.1200/JCO.2016.71.3230 (2017).

10 Tomlinson, B. K. *et al.* Phase I Trial of Arginine Deprivation Therapy with ADI-PEG 20 Plus Docetaxel in Patients with Advanced Malignant Solid Tumors. *Clinical cancer research : an official journal of the American Association for Cancer Research* **21**, 2480-2486, doi:10.1158/1078-0432.CCR-14-2610 (2015).

11 Lowery, M. A. *et al.* A phase 1/1B trial of ADI-PEG 20 plus nab-paclitaxel and gemcitabine in patients with advanced pancreatic adenocarcinoma. *Cancer* **123**, 4556-4565, doi:10.1002/cncr.30897 (2017).

12 Maki, R. G. Gemcitabine and docetaxel in metastatic sarcoma: past, present, and future. *Oncologist* **12**, 999-1006, doi:12/8/999 [pii]
10.1634/theoncologist.12-8-999 (2007).

13 Delage, B. *et al.* Arginine deprivation and argininosuccinate synthetase expression in the treatment of cancer. *Int J Cancer* **126**, 2762-2772, doi:10.1002/ijc.25202 (2010).

14 Ju, J. S. *et al.* Valosin-containing protein (VCP) is required for autophagy and is disrupted in VCP disease. *The Journal of cell biology* **187**, 875-888, doi:10.1083/jcb.200908115 (2009).
15 Chaneton, B. *et al.* Serine is a natural ligand and allosteric activator of pyruvate kinase M2. *Nature* **491**, 458-462, doi:nature11540 [pii]
10.1038/nature11540.
16 Tirkes, T. *et al.* Response Criteria in Oncologic Imaging: Review of Traditional and New Criteria. *RadioGraphics* **33**, 1323-1341, doi:10.1148/rg.335125214 (2013).
17. Prudner BC, Rathore R, Robinson AM, et al. Arginine Starvation and Docetaxel Induce c-Myc-Driven hENT1 Surface Expression to Overcome Gemcitabine Resistance in ASS1-Negative Tumors. *Clin Cancer Res.* 2019 Aug 15;25(16):5122-5134. doi: 10.1158/1078-0432.CCR-19-0206.

We would like to thank the Alvin J. Siteman Cancer Center at Washington University School of Medicine and Barnes-Jewish Hospital in St. Louis, Missouri, for the use of the Clinical Trials Core which provided protocol development and study coordination services. The Siteman Cancer Center is supported in part by an NCI Cancer Center Support Grant #P30 CA91842.

APPENDIX A: ECOG Performance Status Scale

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
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).
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.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

APPENDIX B: Definitions for Adverse Event Reporting

A. Adverse Events (AEs)

As defined in 21 CFR 312.32:

Definition: any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug-related.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website:
http://www.hhs.gov/ohrp/policy/advevntguid.html

B. Suspected Adverse Reaction (SAR)

As defined in 21 CFR 312.32:

Definition: any adverse event for which there is a reasonable possibility that the drug caused the adverse event. "Reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. "Suspected adverse reaction" implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

C. Life-Threatening Adverse Event / Life Threatening Suspected Adverse Reaction

As defined in 21 CFR 312.32:

Definition: any adverse drug event or suspected adverse reaction is considered "life-threatening" if, in the view of the investigator, its occurrence places the patient at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

D. Serious Adverse Event (SAE) or Serious Suspected Adverse Reaction

As defined in 21 CFR 312.32:

Definition: an adverse event or suspected adverse reaction is considered "serious" if, in the view of the investigator, it results in any of the following outcomes:

- Death
- A life-threatening adverse event

- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Any other important medical event that does not fit the criteria above but, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

E. Protocol Exceptions

Definition: A planned change in the conduct of the research for one participant.

F. Deviation

Definition: Any alteration or modification to the IRB-approved research without prospective IRB approval. The term "research" encompasses all IRB-approved materials and documents including the detailed protocol, IRB application, consent form, recruitment materials, questionnaires/data collection forms, and any other information relating to the research study.

A minor or administrative deviation is one that does not have the potential to negatively impact the rights, safety, or welfare of participants or others or the scientific validity of the study.

A major deviation is one that does have the potential to negatively impact the rights, safety, or welfare of participants or others or the scientific validity of the study.

APPENDIX C: Reporting Timelines

Expedited Reporting Timelines				
Event	**HRPO**	**QASMC**	**FDA**	**Polaris**
Serious AND unexpected suspected adverse reaction			Report no later than 15 calendar days after it is determined that the information qualifies for reporting	Report to Polaris within 24 hours of Sponsor-Investigator notification.
Unexpected fatal or life-threatening suspected adverse reaction			Report no later than 7 calendar days after initial receipt of the information	
Unanticipated problem involving risk to participants or others	Report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day.	Report via email after IRB acknowledgment		
Pregnancy				Notify Polaris as they occur.
Overdose				Notify Polaris as they occur.
Major deviation	Report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day.			
A series of minor deviations that are being reported as a continuing noncompliance	Report within 10 working days.			
Protocol exception	Approval must be obtained prior to implementing the change			
Clinically important increase in the rate of a serious suspected adverse reaction of that list in the protocol or IB			Report no later than 15 calendar days after it is determined that the information qualifies for reporting	

Expedited Reporting Timelines				
Event	**HRPO**	**QASMC**	**FDA**	**Polaris**
Complaints	If the complaint reveals an unanticipated problem involving risks to participants or others OR noncompliance, report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1 working day. Otherwise, report at the time of continuing review.			
Breach of confidentiality	Within 10 working days.			
Incarceration	If withdrawing the participant poses a safety issue, report within 10 working days. If withdrawing the participant does not represent a safety issue and the patient will be withdrawn, report at continuing review.			

Routine Reporting Timelines				
Event	**HRPO**	**QASMC**	**FDA**	**Polaris**
Adverse event or SAE that does not require expedited reporting	If they do not meet the definition of an unanticipated problem involving risks to participants or others, report summary information at the time of continuing review	Adverse events will be reported in the toxicity table in the DSM report which is typically due every 6 months.	The most current toxicity table from the DSM report is provided to the FDA with the IND's annual report.	Notify monthly via AE notification or cross-reporting reports/documents.
Minor deviation	Report summary information at the time of continuing review.			
Complaints	If the complaint reveals an unanticipated problem involving risks to participants or others OR noncompliance, report within 10 working days. If the event results in the death of a participant enrolled at WU/BJH/SLCH, report within 1			

Routine Reporting Timelines				
Event	**HRPO**	**QASMC**	**FDA**	**Polaris**
	working day. Otherwise, report at the time of continuing review.			
Incarceration	If withdrawing the participant poses a safety issue, report within 10 working days. If withdrawing the participant does not represent a safety issue and the patient will be withdrawn, report at continuing review.			

Expedited Reporting Timelines for Secondary Sites				
Event	**WU (Coordinating Center)**	**Local IRB**	**FDA**	**Polaris**
Serious AND unexpected suspected adverse reaction	Report no later than 11 calendar days after it is determined that the information qualifies for reporting.	Report all applicable events to local IRB according to local institutional guidelines.	The research team at Washington University is responsible for reporting all applicable events to the FDA as needed.	The research team at Washington University is responsible for reporting all applicable events to Polaris as needed.
Unexpected fatal or life-threatening suspected adverse reaction	Report no later than 4 calendar days after initial receipt of the information.			
Unanticipated problem involving risk to participants or others	Report no later than 4 calendar days after initial receipt of the information.			
Adverse event or SAE that does not require expedited reporting	As per routine data entry expectations			
Protocol exception	Approval must be obtained prior to implementing the change.			

APPENDIX D: Washington University Unanticipated Problem Reporting Cover Sheet

SAE COVER SHEET- Secondary Site Assessment

Washington University HRPO#:	Sponsor-Investigator:
Subject Initials:	Subject ID:
Treating MD:	Treating Site:
EVENT TERM:	Admission Date:
EVENT GRADE:	Date of site's first notification:

Treating MD Event Assessment:

Is this event **possibly, probably, or definitely** related study treatment?

☐ yes ☐ no

If yes, please list which drug (if more than one)_____

Explain _____

_____

_____ _____ _____

Physician's Name **Physician's Signature** **Date**