Abbreviated Title: Minnelide for ASCP
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Title: A Phase II Trial of the Superenhancer Inhibitor Minnelide in Advanced Refractory Adenosquamous Carcinoma of the Pancreas (ASCP)

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Drug Names:	Minnelide
IND Number:	155631
Sponsor:	NCI CCR
Manufacturer:	Minneamrita Therapeutics
Supplier	Minneamrita Therapeutics

Commercial Agents: None

PRÉCIS

Background:

- Adenosquamous carcinoma of the pancreas (ASCP) is a highly aggressive variant of pancreatic ductal adenocarcinoma (PDA), the most common type of pancreas cancer.
- ASCP is estimated to account for 0.5-4% of the 55,000 people who are diagnosed with pancreatic cancer in the U.S. each year, making it a very rare tumor type.
- No prospective clinical trials specific to ASCP have ever been performed.
- Preclinical data in ASCP models indicate that an activated superenhancer network drives epigenetic changes which cause the prognostically unfavorable squamous differentiation.
- Genomic analysis of ASCP tumors identifies frequent amplification of *MYC.*
- Minnelide is a small molecule anti-superenhancer drug that inhibits *MYC.*
- The recommended dose of Minnelide has previously been established through clinical testing for other indications.

Primary Objective:

- To determine the single agent antitumor activity (disease control rate) of the anti-superenhancer agent Minnelide in participants with advanced, previously treated ASCP

Eligibility:

- Age ≥ 18 years
- Histologically confirmed ASCP or high suspicion for ASCP based on histologic analysis for squamous markers
- Participants with metastatic or locally advanced unresectable disease and progression on at least 1 prior treatment regimen

Design:

- This is a phase II single cohort clinical trial with one arm.
- The number of evaluable participants needed for the primary endpoint is 25; maximum accrual set at 55 participants (accounting for screen failures and inevaluable participants).
- Initial participants will receive Minnelide at 2 mg/day PO on Days 1-21 of a 28 day cycle. Later participants will receive a higher dose of 2.5 mg/day PO on the same schedule.
- Treatment will be continued for up to 12 cycles (1 year) in the absence of disease progression or unacceptable toxicity.
- Treatment response will be assessed by imaging every 2 cycles (8 weeks).
- Optional tumor biopsies will be requested mid-cycle 1 and at time of progression.
- A disease control rate of ≥ 40% in this highly refractory population would constitute a positive study. Up to 12 participants will treated be initially. If 3 of the 12 participants have a response, then up to 13 additional participants will be entered to determine the true response rate.

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Council for Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

- To determine the single agent antitumor activity (disease control rate) of the anti-superenhancer agent Minnelide in participants with advanced, previously treated ASCP

1.1.2 Secondary Objectives

- To determine the overall safety of Minnelide in participants with previously treated advanced ASCP
- To determine progression free survival (PFS) and overall survival (OS)

1.1.3 Exploratory Objectives

- Identify factors that influence participant response to treatment through detailed molecular analysis of participant tumor, serum and blood cell samples acquired at baseline, on-treatment and at time of progression
- Evaluate ctDNA as a marker of response in ASCP

1.2 BACKGROUND AND RATIONALE

1.2.1 Adenosquamous carcinoma of the pancreas (ASCP)

With a 5-year survival rate of <9%, pancreatic cancer is one of the most lethal types of cancer [1]. Every year about 55,000 people in the U.S. are diagnosed with pancreatic cancer, >90% of which have pancreatic ductal adenocarcinoma (PDA) [1]. It is now appreciated that there are at least 2 subtypes of PDA based on mRNA expression profiling: one that is more responsive to standard of care chemotherapy and has improved prognosis, and a second more aggressive type (variously termed squamous [2], quasimesenchymal [3] or basal-like [4]) that is highly refractory to standard chemotherapy and has a poorer overall survival [3-5]. Recently, it was appreciated that clades of

the poor prognosis basal-like subtype can co-exist in a heterogeneous mix with better prognosis "classical" subtype areas within tumors of the same patient [6]. Although there are no normal cells within the pancreas that have a squamous differentiation, basal-like subtype tumors may have focal areas of morphologically squamous differentiation marked by expression of squamous markers p63, CK5 and CK6. Areas of squamous differentiation are absent from better prognosis classical subtype tumors. Hibayashi et al recently reported that the squamous differentiation areas appear to genetically evolve from regular glandular differentiation areas through mutation of a chromatin modifier gene (such as *ARID1A, KMT2C*) concurrent with amplification of *MYC*. Anti-tumor treatment with standard chemotherapy agents is anticipated to select for outgrowth of these chemo-resistant areas with squamous differentiation.

Adenosquamous carcinoma of the pancreas (ASCP) is a rare variant of PDA that represents the extreme case of squamous differentiation. ASCP is histologically defined as a tumor containing both glandular and squamous differentiations with the squamous component constituting at least 30% of the tumor [7]. The arbitrary cutoff of 30% has been disputed in the literature [8, 9] with opponents noting that the evaluation is subjective and highly dependent on sampling [10] especially when fine-needle aspiration is used [11]. The reported prevalence of ASCP varies significantly in the literature ranging from 0.38% to 10% of all exocrine pancreatic cancers with most researchers reporting between 0.5% to 4% [12]. Pathologic diagnosis of ASCP is most straightforward in resected patients where the large quantity of tissue available allows for more conclusive determination of the percentage of squamous component in the tumor. Unfortunately, the majority of patients with ASCP do not present with resectable disease and diagnosis must be made from fine needle aspiration or core needle biopsy. Diagnosis using these methods is possible [13], however, definitive diagnosis of ASCP is more difficult as patients with some squamous component noted in the sample may be diagnosed with PDAC due to the pathologist's inability to conclusively quantitate a ≥30% squamous component.

The prognosis for patients with ASCP is worse than for those with conventional PDA. In one 38-patient single institution study, the median survival of patients with resected ASCP was 10.9 months compared to 17.9 months for patients with PDA [6]. In another study comparing the survival of patients with all stages of ASCP to those with matched stages of PDA, the overall median survival for ASCP was about 5 months shorter than that for PDA (6.51 vs. 11.0 months) [11]. As in PDA, patients with ASCP are most often diagnosed too late for surgical resection and there are no effective treatments for advanced disease. Due to its relative rarity, there have been no published prospective clinical trials specifically treating patients with ASCP and no guidelines exist for treating patients with advanced ASCP. Case studies in the literature typically cite treatment regimens similar to those for PDA. As demonstrated by the poorer prognosis of ASCP patients compared to PDA, those treatments are not optimized for ASCP.

Because of the rarity of the disease, very few genomic profiling studies have been carried out on ASCP tumors. Until recently, our understanding of the molecular and genomic characteristics of ASCP was very limited. Fang et al. reported the whole genome and whole exosome sequencing of 17 ASCP tumors and found that prevalence of KRAS (100%) and p53 (88%) mutations exceeds that of PDA [14]. A previous report by Borazanci et al also identified *KRAS* mutation in 100% of ASCP tumor samples (16/16) [12]. Also similar to PDA, copy number variant (CNV) analysis revealed gains at 8q (*MYC*) and 12p (*KRAS*) and losses corresponding to the loci of *CDKN2A, TP53* and *SMAD4*. Interestingly, compared to PDA, ASPC had much more extensive and frequent chromosome 3p loss, which includes the *WNT5A* gene (5/11 PDA versus 16/17 ASCP), a

chromosomal feature noted by the authors to also occur with high frequency in pure squamous cell carcinomas such as those of the head and neck, and lung [14]. More recently, Barrett et al described the genomic landscape of an additional 12 ASCP cases using tumor nuclei purified by a novel flow-sorting technique [15]. Frequent mutations in *KRAS* (92%, 11 of 12), *TP53* (83%) and *CDKN2A* (50%) were again noted, as well as frequent *MYC* amplification. As noted by Hibayashi et al, mutations targeting epigenomic regulators of chromatin organization were also common. These data support the theory that ASCP shares a common progenitor with PDA, but represents the most aggressive form of the disease, one that may evolve in PDA patients after multiple lines of standard treatment.

1.2.2 Minnelide, an anti-super enhancer drug that inhibits Myc

1.2.2.1 Mechanism of action

Minnelide is a prodrug (14-0-phosponooymethyl triptolide disodium salt) of triptolide. It rapidly releases the active compound triptolide when exposed to phosphatase in the bloodstream. History of triptolide use and study is extensive since it is a diterpenoid triepoxide found in the Chinese plant *Tripterygium wilfordii*. For an extensive review of the agent, please see [16]. Multiple mechanisms of action for triptolide/Minnelide have been reported. Minnelide is known to inhibit HSP70 expression and to cause HSP-70-dependent apoptosis in pre-clinical models of PDA, a tumor type that typically overexpresses HSP70 [17]. However, triptolide may induce apoptosis in pancreatic cancer cells by different pathways depending on the cell line [18] . A summary table reporting anti-tumor efficacy of Minnelide in various pre-clinical models is shown below in **Table 1**.

Table 1: Summary of Results in Primary Pharmacodynamic Studies with Minnelide™

Type of Study	Test System	Method of Administration	Results	Reference Number
Antitumor effect	Human pancreatic tumor cell lines	In vitro	Minnelide™ decreased cell viability of all pancreatic tumor cell types in the presence, but not absence, of alkaline phosphatase.	MN12-001
Antitumor effect	Human A2780 ovarian tumor cell line	In vitro	"Activated" Minnelide™ inhibited ovarian tumor cell proliferation in a concentration-dependent manner.	MN12-008
Efficacy – Orthotopic pancreatic tumor model	Athymic nude mice (Ncr *nu/nu*) implanted with MIA PaCa-2 tumor cells into the pancreatic tail	PO or IP	Once daily Minnelide™ treatment administered by the PO or IP routes were similarly effective at reducing tumor volumes and weights.	MN16-001

Type of Study	Test System	Method of Administration	Results	Reference Number
Efficacy – Orthotopic pancreatic tumor model	Athymic nude mice (Ncr *nu/nu*) implanted with MIA PaCa-2 tumor cells into the pancreatic tail	IP; QD or BID	Minnelide™ treatment markedly improved animal survival and significantly decreased pancreatic MIA PaCa-2 tumor volumes and weights.	MN12-002
Efficacy – Orthotopic pancreatic tumor model; comparison to gemcitabine treatment	Athymic nude mice (Ncr *nu/nu*) implanted with MIA PaCa-2 tumor cells into the pancreatic tail	IP; QD	Minnelide™ was more effective than gemcitabine at decreasing pancreatic MIA PaCa-2 tumor volumes and weights.	MN12-003
Efficacy – Orthotopic pancreatic tumor model	Athymic nude mice (Ncr *nu/nu*) implanted with S2-013 tumor cells into the pancreatic tail	IP; QD	Minnelide™ treatment significantly decreased pancreatic S2-013 tumor volumes and weights, and markedly reduced the spread of metastases.	MN12-004
Efficacy – Orthotopic pancreatic tumor model	Athymic nude mice (Ncr *nu/nu*) implanted with AsPC-1 tumor cells into the pancreatic tail	IP; QD	Minnelide™ markedly improved animal survival and significantly decreased pancreatic AsPC-1 tumor volumes and weights even when daily treatment was initiated after palpable tumors were observed.	MN12-005
Efficacy – Human pancreatic tumor xenograft model	SCID mice implanted subcutaneously with human pancreatic adenocarcinoma tumors	IP; QD	Minnelide™ markedly improved animal survival and significantly decreased tumor volumes and weights when daily treatment was initiated earlier (ATV~300 mm3) or later (ATV~1000 mm3) after tumor implantation.	MN12-006
Efficacy – Spontaneous pancreatic tumor-forming mouse model	KRasG12D; Trp53R172H; Pdx-1 Cre mice	IP; QD	Minnelide™ treatment resulted in decreased tumor volumes and weights in animals genetically engineered to spontaneously develop pancreatic tumors.	MN12-007
Efficacy – Subcutaneous ovarian tumor model	Athymic nude mice (Ncr *nu/nu*) implanted subcutaneously with A2780 tumor cells	IP; QD or 2 or 3 times weekly	Minnelide™ markedly improved animal survival and decreased A2780 tumor volumes when administered daily, but not when administered twice or thrice weekly.	MN12-008

Type of Study	Test System	Method of Administration	Results	Reference Number
Efficacy – Subcutaneous pancreatic tumor model; dosing schedule comparison	Athymic nude mice (Ncr *nu/nu*) implanted subcutaneously with MIA PaCa-2 tumor cells	IP; QD or every third day	Minnelide™ significantly decreased MIA PaCa-2 tumor volumes when administered daily, but not when administered every third day.	MN13-001
Efficacy – Minnelide™ in combination with gemcitabine plus abraxane (Gem/Abx)	Athymic nude mice (Ncr *nu/nu*) implanted with S2-VP10 tumor cells into the pancreatic tail	PO	Minnelide™ in combination with Gem/Abx was more effective than Minnelide™ or Gem/Abx treatment alone.	MN16-002
Efficacy – Minnelide™ in combination with gemcitabine	Athymic nude mice (Ncr *nu/nu*) implanted subcutaneously with MIA-Paca2 or AsPC-1 tumor cells	IP	Combination treatment of Minnelide™ and gemcitabine was more effective than when the agents were administered alone.	MN16-005
Efficacy – Minnelide™ in combination with paclitaxel	Athymic nude mice (Ncr *nu/nu*) implanted with S2-VP10 tumor cells into the pancreatic tail or subcutaneously into the flank	IP	Minnelide™ in combination with paclitaxel was more effective than the agents alone.	MN16-003
Efficacy – Minnelide™ in combination with TRAIL	Athymic nude mice (Ncr *nu/nu*) implanted subcutaneously with S2-VP10 tumor cells	IP	Combination treatment of Minnelide™ and TRAIL was highly effective at doses of agents that were not effective when administered individually.	MN16-004
Efficacy – Minnelide™ in combination with oxaliplatin	Athymic nude mice (Ncr *nu/nu*) implanted with MIA PaCa-2 tumor cells into the pancreatic tail	IP	Combination treatment of Minnelide and oxaliplatin was highly effective at doses of agents that were not effective when administered individually.	MN16-006

1.2.2.2 Minnelide clinical experience

1.2.2.2.1 Intravenous formulation

A Phase 1, Multi-Center, Open-Label, Dose-Escalation, Safety, Pharmacokinetic, and Pharmacodynamic Study of Minnelide™ given intravenously daily for 21 days followed by 7 days off was performed in patients with Advanced GI Tumors beginning in September of 2013 and completed in July of 2016 (NCT01927965) (reference provided in the investigator brochure). A

second dosing schedule of Minnelide™ given intravenously was explored during this Phase 1 study where patients received drug for five consecutive days with a two day rest period for 3 weeks of each 4 week cycle.

There were a total of 45 patients enrolled in the Phase 1 study, however 3 patients were enrolled but not dosed with study drug. The primary reason for discontinuation of treatment was disease progression. There were 6 deaths in the study – all occurred after the study drug had ended; though were within the 30-day follow-up period. These deaths were found to be related to progression of the patient's disease (pancreatic/gastric cancer), and not the study drug. There was also one patient who died from respiratory failure, unrelated to use of the study drug.

There were 3 patient who discontinued the study drug due to treatment emergent adverse events (TEAEs). All instances were considered Grade 3, and two of these TEAEs were considered to be probably related to use of the study drug. These two cases were also considered (DLTs). These events included: cerebellar toxicity, embolism, and reversible cerebellar dysfunction. A total of 28 Serious Adverse Events occurred in 17 patients and six were found to be related to the study drug.

Overall, the most commonly reported TEAEs (>20%) regardless of causality included: hypoalbuminaemia, anemia, hypoproteinemia, fatigue, neutropenia, leukopenia, thrombocytopenia, nausea, hypocalcemia, diarrhea, hyperphosphatemia, constipation, vomiting, hyponatremia, lymphopenia, abdominal pain, dehydration, hyperglycemia, peripheral edema, hypophosphatemia, and headache.

32 patients had at least 1 AE that was considered related to the study drug. Adverse events of neutropenia grade 3 and 4 of short durations have been observed at all dose levels and were determined to be drug related. The neutropenia resolved within a couple of days of not receiving treatment. Neutropenic fever and neutropenic infection were observed in three patients and determined to be drug related. Anemia and thrombocytopenia grade 3 and 4 were observed in 22% of the patients and determined to be drug related. Other side effects of any grade that were possibly related to the drug included nausea, vomiting, diarrhea, constipation, anorexia, stomatitis, cerebellar toxicity, embolism and dyspnea, which occurred in ≤ 3% of the patients.

Pharmacokinetic and tumor response data have been reported (https://cancerres.aacrjournals.org/content/75/15_Supplement/CT207): Pharmacokinetic analysis from 21 patients indicated rapid conversion of Minnelide to triptolide within 30 minutes. Peak triptolide concentrations occurred within 5 minutes of the end of infusion. Triptolide half-life was less than 30 minutes. Clearance was complete within 6 hours except for the patient with reversible cerebellar toxicity. Reduction of HSP70 levels was observed in all subjects except those treated in the lowest dose cohort, consistent with expected bioactivity. Radiologic response following 2 cycles was measured by RECIST criteria in 10 participants: 1 participant (gastric) had a partial response and 6 participants (5 pancreas, 1 rectal) had stable disease.

Subsequently, patients with stage IV PDA that had progressed on standard chemotherapy were enrolled to a Phase II study testing the recommended phase II dose (RP2D) of IV Minnelide (0.67 mg/m^2 IV on D1-21 of a 28 day cycle). This study has completed accrual of 19 participants (NCT03117920). The trial results are not yet reported.

1.2.2.2.2 Oral Formulation

An oral formulation of Minnelide was pursued to overcome the logistical difficulties of administering daily intravenous injections to patients for 3 out of every 4 weeks. A Phase I trial

dose escalation study of oral Minnelide given alone or in combination with nab-paclitaxel for patients with advanced solid tumors was started in October 2017 and is ongoing (NCT03129139) (reference provided in the investigator brochure). To date, there have been a total of 67 patients accrued, 43 in the monotherapy regimen, and 24 to the combination regimen. Monotherapy has been tested at 5 different dose levels: 1.00, 1.25, 1.50, 1.75 and 2.00 mg/day given days 1-21 of a 28 days cycle. The 2 mg/day dosing for this schedule was determined to be safe.

More recently, Minnelide was tested at a dose of 2.5mg given on days 1-21 of a 28 days cycle (NCT03129139). As of November 3, 2022, 6 patients had been treated at this dose. One DLT of grade 4 thrombocytopenia was observed but no other significant safety issues were identified. This dose was established as safe to give oral Minnelide on this schedule.

Composite safety data are available for 67 patients, 43 in the monotherapy regimen, and 24 in the combination regimen. The primary reason for discontinuation of treatment has been disease progression. There have been 6 deaths on the study, 5 of which were assessed as not related to Minnelide and which occurred after discontinuation of the study drug. One patient on the 1.25 mg monotherapy regimen died from G5 related sepsis. One patient on the combination regimen at 0.25mg of Minnelide died from G5 sepsis, which after investigation and review of pharmacokinetic data, was found to be unrelated to Minnelide.

There have been 6 patients that have discontinued the study drug due to a treatment emergent adverse event (TEAE). Four instances were considered Grade 5 (as described above), one was Grade 3, one was Grade 2. One of the TEAE's were considered to be probably related to use of the study drug, three possibly related (Two Grade 5 sepsis, one Grade 3 hypokalemia, one Grade 2 neutrophil count decrease), and the other two not related (Grade 5 disease progression). A total of 56 Serious Adverse Events have occurred in 33 patients and 11 were found to be related to the investigational product. The 11 related SAE's, two grade five sepsis, a grade four sepsis, a grade three nausea, a grade three polymicrobial bacteremia, a grade three intractable nausea, a grade three hypokalemia event, a grade three worsening leukocytosis, a grade three blood infection, and a grade two enteritis were on the monotherapy regimen. A grade five sepsis and a grade two esophagitis were on the combination therapy regimen. Overall, the most commonly reported TEAE (> 20%) regardless of grade or causality have included: Anemia, Abdominal Pain, Anorexia, Vomiting, Diarrhea, Nausea, Fatigue, Hypokalemia, and Hypoalbuminaemia. 22 patients had at least 1 AE that was considered definitely, probably, or possibly related to the study drug. Grade 3 – Grade 5 TEAE's reported in ≥ 3% of patients regardless of causality to data are as follows:

- Grade 3 Adverse Events: Abdominal Pain 10%, Anemia 15%, Alkaline Phosphatase Increased 4%, Dehydration 3%, Diarrhea 6%, Hypoalbuminemia 6%, Hypokalemia 10%, Hyponatremia 6%,, Nausea 3%, Neutrophil Counts Decreased 13%, Platelet Count Decreased 3%, Thrombocytopenia 3%, Urinary Tract Infection 3%, Vomiting 3%, Weight decreased 3%, White Blood Cell Count Decreased 3%.
- Grade 4 Adverse Events: Leukopenia 3%, Neutropenia 7, Sepsis 3%.
- Grade 5 Adverse Event: Disease Progression 9%

Of note, no cerebellar toxicity has been observed with the oral formulation.

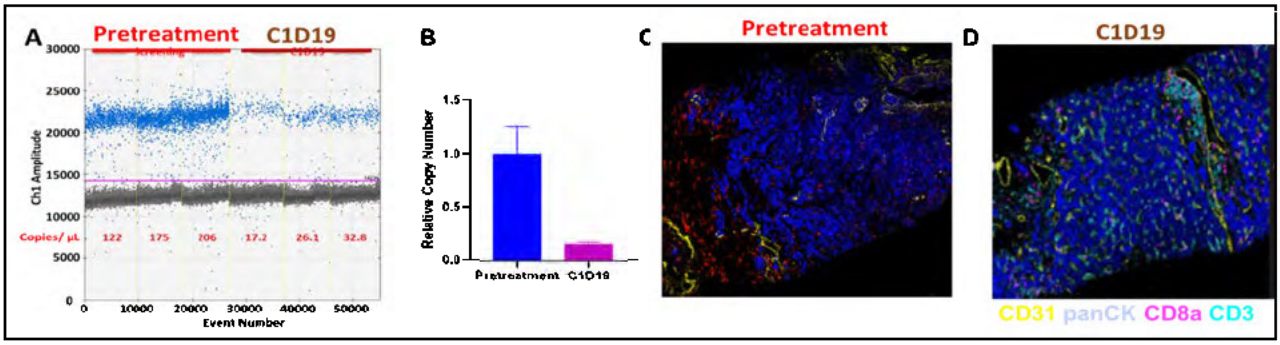
Pharmacokinetic data was obtained in 33 patients receiving oral Minnelide at seven different dose levels from 0.50 mg/day to 2.00 mg/day. Peak triptolide drug levels were observed within 60

minutes after administration of the oral dose in 26 of 31 patients. No serum triptolide was detected by 240 minutes post-administration in all 23 patients with data at this timepoint.

1.2.3 Evidence for Minnelide activity in ASCP

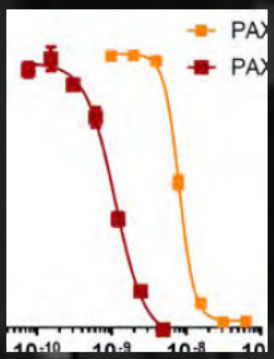
In concert with its activity against HSP70, Minnelide is also a small molecule inhibitor of the XPB subunit of the TFIIH transcription complex[19]. Similar to what has been seen upon inhibiting CDK7, another subunit of the TFIIH complex, inhibition of XPB subunit by Minnelide can suppress a superenhancer complex which regulates c-Myc [19]. Recently, it was found that IV Minnelide treatment reduces *MYC* expression in PDA patient circulating tumor cells (CTCs) (**Figure 1**A-B). Further, IV Minnelide treatment results in changes to the tumor immune milleu. Specifically, increased infiltration of B and T cells (**Figure 1**C-D) in PDA patient tumor tissue was observed following IV Minnelide treatment, suggesting that Minnelide may induce changes in the immune compartment in addition to suppressing c-Myc expression.

Figure 1: *Minnelide suppresses MYC expression and induces B and T cell infiltration in PDA patients. A) MYC RNA transcripts detected by digital PCR in circulating tumor cells (CTCs) isolated from 3 paired patient samples: pretreatment (screening) and post-treatment at Day 19 of Cycle 1 (C1D19). B) Quantification of MYC expression levels in the CTCs before and after Minnelide treatment. C and D) Multi-parameter CyTOF analysis of biopsies taken from patients before (C) and after (D) treatment with Minnelide shows increase B cell (CD31$^+$, yellow) and T cell (CD3$^+$, light blue) infiltration*



As *MYC* appears to be a key determinant in ASCP, inhibition of c-Myc with Minnelide might be advantageous for this patient population. Minnelide anti-tumor cell efficacy was tested against 2 ASCP PDX-derived cell lines. Minnelide was found to have anti-tumor efficacy with single digit nM IC_{50}'s (**Figure 2**A). Minnelide treatment resulted in down-regulation of Myc protein levels as predicted (**Figure 2**B). In the PAX265 PDX, Minnelide (at 0.42 mg/kg daily dosing) significantly inhibited tumor growth in mice after 2 weeks of treatment (**Figure 2**C). These pre-clinical studies demonstrate that ASCP is sensitive to treatment with Minnelide.

Figure 2 . *Antitumor activity of Minnelide in ASCP models. A) Dose response curves of Minnelide in two ASCP cell lines (PAX217 and PAX265. Cells were treated with Minnelide for 3 days. B) Down-regulation of MYC protein level in PAX265 cells detected by Western blotting. C)Growth inhibition of PAX265 PDX xenograft tumors by Minnelide . Mice were treated with Minnelide at 0.42 mg/kg, QD, i.p. for 2 weeks. N=5. *P <0.05.*



Appendix A

a	≥	µ
pl	≥	µ
he obm	≥	g/dL or ≥ 5.6 mmol/L*

creatinine OR measured or calculated [b]creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 × ULN OR ≥ 45 mL/min for participant with creatinine levels >1.5 × institutional ULN
total bilirubin	≤1.5 ×ULN OR direct bilirubin ≤ULN for participants with total bilirubin levels >1.5 × ULN
AST (SGOT) and ALT (SGPT)	≤2.5 × ULN (≤5 × ULN for participants with liver metastases)
International normalized ratio (INR) OR – prothrombin time (PT) – activated partial thromboplastin time (aPTT)	≤1.5 × ULN unless participant is receiving anticoagulant therapy as long as PT or PTT is within therapeutic range of intended use of anticoagulants

ALT (SGPT) = alanine aminotransferase (serum glutamic pyruvic transaminase); AST (SGOT) = aspartate aminotransferase (serum glutamic oxaloacetic transaminase); GFR = glomerular filtration rate; ULN=upper limit of normal.

[a] Criteria must be met without erythropoietin dependency and without packed red blood cell (pRBC) transfusion within last 2 weeks.

[b] Creatinine clearance (CrCl) should be calculated per institutional standard.

Note: This table includes eligibility-defining laboratory value requirements for treatment; laboratory value requirements should be adapted according to local regulations and guidelines for the administration of specific chemotherapies.

2.1.1.9 The effects of Minnelide on the developing human fetus are unknown. For this reason, women of child-bearing potential (WOCBP) and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation, and for 6 months (women) and 3 months (men) after the last dose of trial treatment. Male participants must also refrain from donating sperm during this period. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

2.1.2 Exclusion Criteria

2.1.2.1 Has uncontrolled vomiting or medical condition which inhibits oral ingestion or digestion because the study treatment is administered orally.

2.1.2.2 Pregnant and/or women who are breast feeding are excluded from this study because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with Minnelide.

2.1.2.3 Is currently participating and receiving trial therapy, or has participated in a trial of an investigational agent/therapy or used an investigational device within 3 weeks of the first planned treatment on this study.

2.1.2.4 Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to Cycle 1/Day 1

2.1.2.5 Requires use of ondansetron or another prohibited medication (see Section **4**). Note that other 5-HT_3 inhibitors are NOT prohibited.

2.1.2.6 Has received major surgery within the last 4 weeks, minor endoscopic procedure such as biliary stenting within the last 2 weeks, or percutaneous procedure such as hepatic biopsy or celiac plexus block within 24 hours of planned treatment start date. Note: participant must have recovered adequately from the toxicity and/or complications from the intervention prior to starting therapy.

2.1.2.7 Has a known additional malignancy that is progressing or requires active treatment. Exceptions include basal cell carcinoma of the skin or squamous cell carcinoma of the skin that has undergone potentially curative therapy or in situ cervical cancer.

2.1.2.8 Has known active central nervous system (CNS) metastases and/or carcinomatous meningitis. **Participants with previously treated brain metastases may participate** if: a) follow-up brain imaging after central nervous system (CNS)-directed therapy shows no evidence of progression at ≥ 4 weeks since treatment, AND b) participant has stability of baseline neurologic symptoms without receiving immunosuppressive-doses of systemic corticosteroid (physiologic replacement doses are permitted) x7 days or increases in other supportive medications that treat neurologic symptoms such as anti-epileptics x14 days. Participants with carcinomatous meningitis are excluded regardless of clinical stability.

2.1.2.9 Has an active infection requiring systemic therapy.

2.1.2.10 Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the participant's participation for the full duration of the trial, or is not in the best interest of the participant to participate, in the opinion of the treating investigator.

2.1.2.11 Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

2.1.2.12 Has known uncontrolled or poorly controlled human immunodeficiency virus (HIV) infection. HIV is considered uncontrolled or poorly controlled if an HIV-infected individual is not taking highly active anti-retroviral therapy or has a detectable viral load within the previous 6 months.

2.1.2.13 Has active HBV or HCV or is currently under treatment for HBV or HCV. Active HBV or HCV does not include previously cleared HBV or HCV or successfully cured HBV or HCV through treatment

2.1.2.14 Has received a live vaccine within 30 days of planned start of trial therapy.

Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g. Flu-Mist®) are live attenuated vaccines, and are not allowed.

2.1.2.15 History of allergic reactions attributed to compounds of similar chemical or biologic composition to Minnelide

2.2 RECRUITMENT STRATEGIES

This protocol may be abstracted into a plain language announcement posted on NIH websites and on NIH social media platforms.

2.3 SCREENING EVALUATION

2.3.1 Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the participant has signed a consent include the following:

- Email, written, in person or telephone communications with prospective participants
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images
- Review of existing photographs or videos
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes

2.3.2 Screening activities performed after a consent for screening has been signed

The following activities will be performed only after the participant has signed the consent for screening on this study. Assessments performed at outside facilities or on another NIH protocol within the timeframes below may also be used to determine eligibility once a participant has signed the 000254 consent for screening.

Screening of potential participants must be performed within 21 days prior to first dose of trial medication (unless otherwise noted below). All necessary laboratory values and assessment reports must be available and reviewed prior to signing study consent for treatment.

The following will be completed as part of the screening visit:

2.3.2.1 Confirmation of diagnosis: Obtain archival tumor specimen (primary or metastatic site) for all participants for review by NCI Laboratory of Pathology; may be performed at any time prior to eligibility determination (no time limit). If archival tumor specimen is unavailable for confirmation of diagnosis, then fresh tumor biopsy will be performed. If fresh biopsy is done for diagnosis, sufficient tissue for baseline research biopsy may be obtained at the same time; refer to Section **5.1.3**.

2.3.2.2 Medical history: review of concurrent baseline conditions (using NCI CTCAE version 5.0), prior cancer therapy (including documentation of prior surgery, adjuvant or neoadjuvant chemotherapy and radiotherapy).

2.3.2.3 Physical examination, including vital signs, height (cm) and weight (kg): review of organ systems, weight, and vital signs (i.e., temperature, pulse, respirations, blood pressure). Height is at screening only.

2.3.2.4 Performance status (ECOG): an assessment of activities of daily living; see **Appendix A: Performance Status Criteria**

2.3.2.5 Clinical laboratories

- Complete blood count (CBC) with differential: includes Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils, WBC, RBC, Hemoglobin, Hematocrit, MCV, RDW, Platelet
- Serum chemistries: Sodium (NA), Potassium (K), Chloride (CL) Total CO2 (Bicarbonate), Creatinine, Glucose, Urea nitrogen, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, Albumin, Calcium, Magnesium, and Phosphorus; including creatinine clearance (if needed; see Section **2.1.1.8**)
- Coagulation panel: PT/INR and aPTT
- Urinalysis including; protein, specific gravity, glucose, and blood
- Hepatitis testing: HBsAg and HCV RNA (qualitative) if not performed in the last 3 months
- Pregnancy test: Serum β-HCG pregnancy test for women of child-bearing potential

2.3.2.6 Electrocardiogram (ECG)

2.3.2.7 Imaging scans: Computed tomography (CT) / magnetic resonance imaging (MRI) scan as clinically indicated to document disease status (including chest, abdomen, pelvis, and other regions as clinically indicated). In addition, a brain scan may be required to exclude brain metastases if clinically indicated by participant history or exam.

2.3.2.8 Concomitant medications: review to include notation of all medications taken within 30 days prior to treatment initiation

2.4 Participant Registration and Status Update Procedures

Registration and status updates (e.g., when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found at: https://nih.sharepoint.com/sites/NCI-CCR-OCD-Communications/SitePages/OEC-Administrative---Clinical-Research-(ADCR).aspx?Mode=Edit.

2.4.1 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of reversible factors (e.g., active infection requiring systemic treatment, low blood counts or pregnancy) may be rescreened.

2.4.2 Treatment Assignment Procedures

Cohorts

Number	Name	Description
1	Advanced ASCP	Participants with previously treated, advanced ASCP

Arms

Number	Name	Description
1	Minnelide	Treat with Minnelide PO daily, Days 1-21 of each 28 day cycle

Arm Assignment

Participants in Cohort 1 will be directly assigned to Arm 1.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is a Phase II single arm, single cohort study that will enroll up to 25 evaluable participants with advanced ASCP that has been previously treated. In order to account for screen failures and inevaluable patients (i.e., participants who are not evaluable for the primary endpoint of response), the accrual ceiling will be set at 55 participants.

Accrual may continue up to 15 treated participants while the primary objective is being determined but no new participants may be accrued once it is known that this criteria has not been met (see Sections **10.3** and **10.5.6**).

Initial participants will self-administer Minnelide at 2 mg PO daily on Days 1-21 of each 28 days cycle. Those joining the study after modification dated 3/10/23 will receive Minnelide at 2.5 mg PO daily on Days 1-21 of each 28 days cycle. Participants initially enrolled at the 2 mg dose may be increased to the 2.5 mg dose at the discretion of the study team if they have been tolerating the lower dose (i.e., intrapatient dose escalation is permitted). Treatment will continue for up to 12 cycles (1 year) in the absence of disease progression or unacceptable toxicity. After initiation of treatment, restaging scans will be conducted approximately every 2 cycles or 8 weeks. Optional biopsies will be requested mid-cycle 1 and at time of progression.

3.2 DRUG ADMINISTRATION

3.2.1 Minnelide

- Study participants will be provided Minnelide capsules (0.5 mg) (see **Appendix B: Participant Medication Diary**). Quantity of each container will be sufficient for 1 cycle; resupplies will be provided as necessary at the beginning of each cycle. Any remaining capsules and containers will be returned to the investigational pharmacy for accountability.
- Containers will be white opaque, round HDPE bottle (120 cc, 38mm screw neck (Heavy Weight), induction sealed with a 38mm child-resistant white polypropylene cap with liner.
- Capsules will be Size 2 White/White opaque capsules are filled with 150 mg blended powder that contains 0.50 mg Minnelide ™ free acid.
- Medication will be stored at controlled cold temperature (2–8°C, excursions permitted 0–15°C) in the site investigational pharmacy.
- Medication will be provided to participants by the site investigational pharmacy on receipt of a prescription from the investigator. The medication will be provided in a bag with ice packs for the transfer home and participants will be instructed to store the medication in a refrigerator at home.

- Doses will be self-administered at home daily for 21 days and then 7 days without dose for each treatment cycle. The maximum number of cycles will be 12.
- The daily dose will be 2 mg (four capsules) or 2.5 mg (five capsules). Participants will be instructed to take the dose after fasting for at least 30 minutes prior to Minnelide administration, with a recommended 90-minute fast post dose. If needed, 60-minute fast post dose is acceptable. Capsules may be taken with 240 mL of water.
- If the medication dose is more than 6 hours late then it should be skipped.
- Any missed doses will be reported during the study visits. Do not try to make up missed doses or continue taking past the 21 allotted days for that cycle.
- **NOTE:** Upon return of each supply, the study staff will record in the source documents (e.g., medical record) the number of capsules returned at each visit.

3.2.2 Supportive Medications for risk of nausea and emesis

- Participants with baseline nausea and/ or emesis will be continued on their home medication regimen. If this contains ondansetron (a prohibited medication), then ondansetron will be transitioned to an acceptable alternative such as granisetron.
- Participants who do not have a prescribed anti-emetic regimen at baseline or those whose current anti-emetic regimen is deemed insufficient will be prescribed sufficient supply of oral granisetron (1 mg, PRN) to use until first return visit (at 14 days) in case increased nausea/ emesis occurs while taking Minnelide. An alternative anti-emetic may be prescribed in case of participant allergy, intolerance or preference. Refills will be prescribed as needed at subsequent visits.

3.3 Dose Modifications

Two dose-reductions per participant (see **Table 2** below) will be permitted in response to either hematologic or non-hematologic toxicities for criteria as defined in the sub-sections below.

Table 2: Minnelide Dose Reduction

Minnelide Dose Reductions	Minnelide Dose (Low)	Minnelide Dose (High)
Full-dose	2 mg/ day	2.5 mg/ day
1st occurrence of AE requiring dose reduction	1.5 mg/ day (75%)	2.0 mg/day (80%)
2nd occurrence of AE requiring dose reduction	1.0 mg /day (50%)	1.5 mg/day (60%)
3rd occurrence of AE requiring dose reduction	No further Minnelide given	No further Minnelide given

A new cycle of Minnelide should not be started if the participant's laboratory values, clinical status or medical conditions no longer meet initial study eligibility criteria as per the assessments to be completed prior to each cycle (see Study Calendar, Section **3.7**) with Minnelide held until these values reach eligibility criteria unless otherwise described below (i.e., Section **3.3.1** to **3.3.4**).

If treatment is delayed for > 2 weeks due to any drug-related toxicity, the participant must be 1) dose-reduced (as defined above) or 2) discontinued from treatment with Minnelide capsules and

followed for safety. Cycles of Minnelide are never skipped. If participants are delayed receiving the next cycle of Minnelide for > 2 weeks due to complications of disease, co-morbid conditions, scheduling problems or any reason other than drug-related toxicity, Minnelide should be continued at previously administered dose when these issues have resolved to meet requirements of study eligibility criteria. In such cases where a new cycle of Minnelide is delayed, the duration of the current cycle lengthens to >28 days. A new cycle does not start until a participant takes the first dose of Minnelide for that next cycle.

As defined below, individual doses of Minnelide may be held in response to lab abnormalities or or clinical issues. If Minnelide treatment must be paused in this way, these missed doses are not administered later. Minnelide treatment for the cycle will still finish on Day 21.

3.3.1 Neutropenia and Febrile Neutropenia

Minnelide should be halted and growth factor should be given to all participants experiencing grade ≥ 3 neutropenia until ANC exceeds 1500/ mm^3. After recovery of ANC, dose reduction (see Section **3.3**) to next lowest level of Minnelide is required to continue therapy, except if grade ≥ 3 neutropenia occurs within first 1-2 weeks of cycle 1. Transient grade ≥3 neutropenia has been observed during the first 1-2 weeks of cycle 1 in some participants on prior study of oral Minnelide. In this situation, Minnelide should be held until ANC recovers to Grade 1 or better, but can subsequently be resumed at the previous dose. Any subsequent neutropenia events will require dose reduction as specified in Section **3.3**.

Febrile neutropenia should always be treated as a medical emergency; Grade 5 sepsis has been observed in patients receiving Minnelide treatment. Minnelide treatment should be halted immediately and growth factor administered to all participants experiencing febrile neutropenia until ANC exceeds 1500/ mm^3. Pan-cultures should be taken as per standard of care and appropriate empiric antibiotic treatment should be promptly initiated. In addition, Minnelide should be held and no new cycle started until ANC exceeds 1500/ mm^3 AND prescribed antibiotic course has been completed. Upon resumption of Minnelide, dose reduction is required for each episode of febrile neutropenia as per Section **3.3**.

3.3.2 Thrombocytopenia

Minnelide should be held for grade 4 platelet count decrease or for grade ≥ 3 platelet count decrease that is accompanied by new clinically significant bleeding. The current cycle of Minnelide may be resumed at a decreased dose when platelet count exceeds 50,000/ mm^3. Dose reductions should be made as specified in Section **3.3**. To start next cycle, platelets must recover to that specified by eligibility criteria and Minnelide should be administered at the decreased dose. Any subsequent grade 4 thrombocytopenia events or grade 3 events associated with clinically significant bleeding will require dose reduction as specified in **Table 2**.

3.3.3 Anemia

Minnelide should be held for grade ≥3 anemia. The current cycle may be resumed when Hgb exceeds 8.0 g/dL. Transfusion may be given to increase Hgb. Minnelide should be dose-reduced if participant requires transfusion 2 or more times due to drug-related anemia or if Hgb has not recovered to at least 8.0 g/dL by the scheduled start of the next cycle. Dose reductions should be made as specified in Section **3.3**. Any subsequent occurrences of anemia requiring transfusion or failure of Hgb to recover to at least 8.0 g/dL by the scheduled start the next cycle will require further dose reduction as specified in Section **3.3**.

3.3.4 Non-hematologic toxicity

Minnelide should be held for any clinically significant grade ≥ 3 non-hematologic toxicity. The present cycle of Minnelide may be resumed when toxicity resolves to at least grade 1 (or to baseline in participants with liver metastases and elevated transaminases). If the clinically significant toxicity is attributable to study drug, then Minnelide should be dose-reduced when resumed. If the adverse event is judged to be unrelated or unlikely to be related to Minnelide, then Minnelide may be resumed at the previous dose.

Asymptomatic grade ≥ 3 electrolyte abnormalities than can be rapidly corrected with supplementation are not considered clinically significant.

Nausea, vomiting or diarrhea that resolve within 48 hrs of initiating appropriate supportive medications are not considered clinically significant. **NOTE:** See contraindication for concomitant use of ondansetron in Section **4**.

3.4 On Treatment Evaluations

The following describes all tests and procedures to be conducted during treatment. Refer to Study Calendar (Section **3.7**) for timing and applicable windows.

For each time period, consider the following order of assessments:

- Baseline/Cycle 1: The results of all assessments, with the exception of tumor markers, must be available and reviewed prior to initiation of Minnelide administration to ensure results still meet standards defined in eligibility criteria. The cycle should be delayed if these criteria are not met (refer to Dose Modifications, Section **3.3**).
- Subsequent Cycles: The results of all assessments, with the exception of tumor markers, must be available and reviewed prior to initiation of Minnelide administration in a new cycle to confirm eligibility for ongoing treatment administration.
- Mid-cycle – Cycle 1/Subsequent Cycles: There is no specific order of events for these days. The study team must review results of laboratory studies within 2 calendar days of the blood draw.
- Unscheduled Visits: In the event of an unscheduled/unplanned visit (e.g., additional clinical assessment(s) due to toxicity), the investigator should use best clinical judgement as to the necessary assessments. In the event that the decision is made to continue treatment, all tests/assessments as required by the next visit on the Study Calendar (Section **3.7**) should still be conducted (or repeated) within the applicable windows. If a decision is made to discontinue treatment, the participant should move to the End of Treatment visit (Section **3.5**), with tests/assessments completed (or repeated) within the applicable windows.

If a physical exam and vital signs are required by the study calendar, then an in-person clinic visit is required. If only symptom assessment is required, a telehealth visit may be conducted so that participants need not travel to the NIH Clinical Center for evaluation unless preferred by the participant or felt to be clinically indicated by the provider. Given the standard nature of the planned clinical laboratories, interlaboratory variability is not a concern and participants may have clinical labs drawn locally, with results sent to the NIH study team (e.g., via fax); in these cases, an appropriate member of the study team will follow-up remotely to discuss any symptoms and to make recommendations/adjustments, as necessary.

The following is a description of all procedures:

- Symptom assessment: verbal review of participant's current symptoms
- Physical exam, including vital signs and weight: review of organ systems, weight (kg), and vital signs (i.e., temperature, pulse, respirations, blood pressure). After initiation of study drug, symptom-directed physical examinations will be performed as clinically indicated in the investigator's judgment.
- Performance status (ECOG): an assessment of activities of daily living; see **Appendix A: Performance Status Criteria**
- Clinical laboratories:
    - CBC with differential: includes Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils, WBC, RBC, Hemoglobin, Hematocrit, MCV, RDW, Platelet
    - Serum Chemistries: Sodium (NA), Potassium (K), Chloride (CL) Total CO2 (Bicarbonate), Creatinine, Glucose, Urea nitrogen, Alkaline Phosphatase, ALT/GPT, AST/GOT, Total Bilirubin, Direct Bilirubin, Albumin, Calcium, Magnesium, Phosphorus
    - Urinalysis: protein, specific gravity, glucose, and blood
    - Pregnancy test: Serum β-HCG for women of child-bearing potential
    - Serum tumor markers: CA19-9 and CEA (or CA-125 if not a secretor of CA19-9 and CEA)
- Imaging scans: CT scans or MRI as required for evaluation (including chest, abdomen, pelvis, and other regions as clinically indicated); may be adjusted to assess additional known sites of disease, as needed. Performed up to 3 days prior to start of every other cycle (typically odd numbered cycles). If radiologic response to treatment is observed (see Section **6.3**), confirmatory scans may be performed at 4 week interval (e.g., start of next cycle), and in this case subsequent scans should continue prior to every other cycle thereafter (i.e., prior to even numbered cycles).
- Adverse events and Concomitant medication review: Adverse events (assessed using the NCI CTCAE v5.0) and concomitant medication will be continuously monitored throughout the study until 30 days from last dose of Minnelide or start of new anticancer treatment, whichever comes first. Adverse events that occur beyond 30 days after the last administration will be recorded as noted in Section **6.1**.
- Minnelide administration, including dispensing and adherence review: Study drug will be dispensed at each cycle and instructed to be self-administered by participants per schedule-see Section **3.2**. Participants will be given a dosing diary (see **Appendix B: Participant Medication Diary**); to be reviewed at each noted visit for drug adherence/accountability.
- Contraception review: Review of contraception use for men and women of childbearing potential. See Section **2.1.1.9**.
- Correlative research samples: Refer to Section **5**.
- NIH Advance Directives Form: All participants will be offered the opportunity to complete an NIH advance directives form. This should be done preferably at baseline but can be done at any time during the study as long as the capacity to do so is retained. The completion of the form is strongly recommended, but is not required.

3.5 END OF TREATMENT ASSESSMENTS

The participant will continue on study drug until there is evidence of tumor progression, intolerable toxicities, completion of 12 cycles, or withdraws from treatment (refer to Section **3.9.1**). The "End of Treatment" assessments (Section **3.7**) will be performed 7-35 days after completing the last dose of study drug or before the initiation of a new anti-cancer treatment, whichever comes first. If the participant cannot return to the study center for this visit, the participant may complete assessments locally, including seeing a local physician for a physical exam and local clinical laboratories, and the study team will follow-up with the participant remotely. Imaging scans need only be performed at end of treatment, if not performed within the last 4 weeks.

3.6 FOLLOW-UP PERIOD

3.6.1 30-day Follow-up Visit

The mandatory Safety Follow- up visit should be conducted approximately 30 (+ 5 days) after the last dose of study drug and before initiation of a new anti-cancer treatment, whichever comes first (see assessments, Section **3.7**). In addition, the study team will confirm contact information for participant and a designated family member, and remind participant of follow-up contact that will be conducted for survival status.

3.6.2 Survival Follow-up

Overall survival will be collected until 1 year post-last dose of Minnelide. Participants will be contacted remotely (e.g., phone or email) approximately every 90 days. If participants are still being seen at the NIH, this information may be obtained from the medical records.

3.7 STUDY CALENDAR

Procedure	Screening	Cycle 1					Subsequent Cycles		End of treatment visit	Follow-up	
		Baseline/ Day 1		Day 8	Day 15	Day 22	Day 1	Day 15		30-day Follow-up	Survival Follow-up
Window(s):	Section 2.3.2	≤7 days	≤3 days	±2 days	±2 days	±2 days	≤3 days	±2 days	Section 3.5	Section 3.6.1	Section 3.6.2
Informed Consent	X										
Confirmation of diagnosis	X										
Medical History	X										
Symptom Assessment	X		X	X	X	X	X	X			
Physical Exam	X	X			X		X		X		
Vital Signs	X	X			X		X		X		
Height	X										
Weight	X	X					X				
Performance Status (ECOG)	X	X			X		X		X		
Clinical laboratories											
CBC with differential	X		X	X	X	X	X	X	X		
Serum chemistries	X		X	X	X	X	X	X	X		
PT/INR and aPTT	X										
Urinalysis	X		X				X		X		
HBsAg and HCV RNA	X										
Pregnancy Test (serum)	X		X				X		X	X	
Serum Tumor Markers			X				X		X		
ECG	X								X		
Imaging scans (CT/MRI)	X						X (every other cycle)		X		
Adverse Events		X		X	X	X	X	X	X	X	
Concomitant Medications	X	X			X		X		X	X	
Review Participant Dosing Diary and Drug Accountability			X		X		X				
Dispense Minnelide			X				X				

Procedure	Screening	Cycle 1				Subsequent Cycles		End of treatment visit	Follow-up	
		Baseline/ Day 1	Day 8	Day 15	Day 22	Day 1	Day 15		30-day Follow-up	Survival Follow-up
Minnelide administration		Continuous PO daily dosing for 21 days, followed by 7 days off in week 4 of each cycle.								
Review Contraception		X		X		X		X	X	
Review Contact Information								X	X	
Correlative research	See Section 5.2 for windows for Correlative Research samples.									
Blood Sample(s)		X		X		X (C2D1 only)		X (or PD)		
Tumor Biopsy (optional)		X		X				X (or PD)		
Archival Tissue	X	X								
NIH Advance Directives Form		X								
Survival		X X								

NOTE: Other tests/assessments should be completed as clinically indicated. For a description of all on study assessments, see Section 3.4.

3.8 COST AND COMPENSATION

3.8.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures are performed outside the NIH Clinical Center, participants may have to pay for these costs if they are not covered by an insurance company. Medicines that are not part of the study treatment will not be provided or paid for by the NIH Clinical Center.

3.8.2 Compensation

Participants will not be compensated on this study.

3.8.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant/guardian as appropriate. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

No reimbursement is provided to participants enrolled at sites other than NIH Clinical Center.

3.9 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to removal from study, effort must be made to have all participants complete a safety visit (See **Study Calendar**).

3.9.1 Criteria for removal from protocol therapy

A participant must be discontinued from the protocol therapy for any of the following reasons:

- Completed 12 cycles of trial treatment
- Confirmed disease progression
- Participants request to be withdrawn from active therapy
- Unacceptable adverse experiences as described in Section **3.3**
- Inter-current illness that prevents further administration of treatment
- Investigator Discretion
- The participant has a confirmed positive serum pregnancy test
- Noncompliance with trial treatment or procedure requirements

3.9.2 Off-Study Criteria

A participant who meets one of the following criteria will be considered Off Study:

- Participant requests to be withdrawn from study
- Death
- Screen failure

3.9.3 Lost to Follow-up

A participant will be considered lost to follow-up if he or she fails to return for three scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within three business days and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, an IRB-approved certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

4 CONCOMITANT MEDICATIONS/MEASURES

Necessary supportive measures for optimal medical care may be given throughout the study, including IV antibiotics to treat infections, blood components, and antiemetics. Additional care will be administered as indicated by the treating physician and the participant's medical need.

No concomitant cytotoxic therapy, whether conventional or investigational, will be allowed during this study. All concomitant medications and supportive therapy must be recorded on the appropriate CRF.

Palliative radiotherapy may be permitted at the discretion of the PI while the participant is undergoing treatment.

All concomitant medications received within 30 days before the first dose of trial treatment and at the 30 day (+ 5 days) follow-up visit should be recorded.

In vitro studies suggest that Minnelide has a low potential for CYP450 related drug-drug interactions.

Minnelide is a substrate for but not an inhibitor of P-glycoprotein. Co-administration with inhibitors of glycoprotein is permitted with caution.

Ondansetron may not be given with Minnelide. It was noted in the Phase 1 study that participants receiving ondansetron in conjunction with Minnelide had higher serum Minnelide concentrations. Follow-up of this observation in murine models confirmed that co-administration of ondansetron with Minnelide increased Minnelide exposures. The mechanism for this interaction is unknown. **Other 5-HT_3 receptor antagonists did not produce this interaction and are acceptable**.

5 CORRELATIVE STUDIES FOR RESEARCH

Note: Platforms and procedures for analysis may be adjusted based upon current technology and/or collaborations in place at the time of actual analyses.

5.1 BIOSPECIMEN COLLECTION

Please note that details for collection, processing, storing and shipping will be described in the Trial Lab Manual.

5.1.1 Blood Sample

Blood samples will be collected for all enrolled participants at the time points described in the Specimen Collection Table (Section **5.2**).

5.1.1.1 Cytokine and immune cell profiling

Peripheral immune cell profiling and cytokine analysis will be performed to determine the effect of Minnelide on the systemic immune environment in ASCP participants. Peripheral immune cells will be analyzed by multiparameter flow cytometry to identify potential changes in 10 classic subsets (CD4 and CD8 T cells, B cells, NK cells, NK-T cells, conventional and plasmacytoid dendritic cells, T regulatory cells, MDSCs, and monocytes) and 121 refined subsets relating to their maturation and function.

5.1.1.2 CTCs and circulating tumor DNA (ctDNA)

Analysis of CTCs and ctDNA will be performed to identify potential early biomarkers of response to Minnelide therapy. CTCs will be enumerated and characterized. If CTCs are sufficient, they will be enriched and analyzed for gene expression and mutation. Mutational status of genes in ctDNA including, but not necessarily limited to, KRAS will be evaluated as well as copy number variation of MYC and potentially other genes of interest will be assessed.

5.1.2 Archival Tumor Tissue

All participants will be asked to provide an archival tumor tissue specimen to identify overall mutational landscape of tumor or results of prior tumor mutational analysis by an FDA approved platform (e.g., Foundation One).

If information from genomic mutational analysis previously performed on a participant's tumor sample using another CLIA-certified platform is not available, tissue will be analyzed by NCI Laboratory of Pathology (LP) COMPASS program. Results will be returned to participants. Whole exome sequencing (WES) or whole genome sequencing (WGS) may be performed on selected samples.

A minimum of 10-unstained slides will be requested for TruSight analysis by NCI LP.

5.1.3 Fresh Tumor Tissue/ recent archival tumor tissue

Participants may elect to provide tissue for additional research studies designed to better understand the treatment effect of Minnelide on their tumor. Participants will be provided with the following options to provide tumor tissue for the analyses described below:

1. Participants who have had recent core or excisional biopsy of a tumor lesion (within 90 days prior to Cycle 1/Day 1 and since completion of last anti-cancer therapy) may consent to use this tissue for baseline analysis for the purposes of this study even if it was collected for another reason.
2. Participants lacking a recent core or excisional biopsy of a tumor lesion may consent to undergo new biopsy of a tumor lesion for research purposes unless tumor is considered inaccessible or biopsy is otherwise considered not in the participant's best interest.
3. Participants on study will be asked to consent to biopsy after the completion of 2 weeks of treatment (~Cycle 1 Day 15), and at the time of disease progression unless tumor is considered inaccessible or biopsy is otherwise considered not in the participants best

interest.

Consent for biopsy will be obtained by the interventionalist at the time of the procedure. If the participant refuses the optional biopsy, the refusal will be documented in the medical record.

Each tumor biopsy will consist of approximately 5 passes, and will be processed, stored and shipped as per the Trial Lab Manual. In case of insufficient tissue to complete all assays described below, priority will be given to completing the analyses listed first in the **Specimen Collection Table**.

5.1.3.1 IHC Studies

IHC analysis will be performed by collaborators at TGen to determine the expression of MYC and other super-enhancer regulated genes such as HSP70 and COL1A2 in samples from Minnelide treated participants.

5.1.3.2 Assay for Transposase Accessible Chromatin using sequencing (ATAC-seq)

ATAC-seq analysis will be performed by collaborators at TGen in samples from Minnelide treated participants to determine effects of Minnelide treatment on chromatin accessibility.

5.1.3.3 Multiplex IHC for immune cells

Changes in the profile of immune cells infiltrating the tumor following Minnelide treatment will be assessed by the Gulley/ Sader lab(s). Tumor biopsies will also be analyzed by multiplexed, multispectral IHC and assessed for immune subsets, include including MDSCs, Tregs, $CD4^+$ or $CD8^+$ T cells, and M1/M2 tumor-associated macrophages (TAMs). If there are sufficient samples, functional markers such as PD1, PDL1, Tim3, CTLA4 and CD40 may also be included.

5.1.3.4 Digital Spatial Genomics

Digital spatial genomics will be performed at TGen on selected samples to determine the effects of Minnelide treatment on the localization of different cell populations within the tumor microenvironment.

5.1.3.5 Single cell RNA-seq

Single cell RNA-seq will be performed by collaborators at TGen on selected samples to determine changes in cell population composition and cell state within the ASCP tumor microenvironment upon Minnelide treatment.

5.1.3.6 Establishment of participant-derived xenograft (PDX) and organoid models

PDX/Organoid models will be established by collaborators at TGen if residual tissue remains after other analyses are completed. Those models will be used to study the biology of ASCP and test new therapeutic regimens for ASCP and may also be used to help understand mechanisms of resistance to Minnelide.

5.2 SPECIMEN COLLECTION TABLE

Specimen Type	Test/assay (listed in order of precedence)	Specimen Type (Amount)	Collection (timepoints)	Location of specimen analysis^ (CCR lab database, if applicable)
Archival or fresh tumor tissue obtained at any time prior to start of study. FFPE acceptable. If multiple acceptable specimens are available, analysis should be completed with the most recent. (Optional)	Tumor mutational analysis WES or WGS on selected	10-unstained slides If sufficient tissue available after other analyses below	Baseline	NCI LP BPC (TBD*) (Labmatrix)
Fresh tumor tissue (Optional)	IHC for PD biomarkers ATAC-seq Multiplex IHC for immune cell changes Digital spatial genomics and/ or single cell RNA-seq	Unstained slides; or, 5-18 gauge cores at each timepoint	Baseline Cycle 1 Day 15 (±7 days)	TGen Gulley/ Sater (Labmatrix)
Fresh tumor tissue (Optional)	PDX/ organoid model generation Tumor mutational analysis WES or WGS on selected	2-18g cores	At the time of disease progression	TGen NCI LP BPC (TBD*) (Labmatrix)

Specimen Type	Test/assay (listed in order of precedence)	Specimen Type (Amount)	Collection (timepoints)	Location of specimen analysis^ (CCR lab database, if applicable)
Serum	Cytokine markers	1- 4 mL red SST tubes at each timepoint	Baseline Cycle 1 Day 15 (±7 days) At the time of disease progression	BPC (Cao) (Labmatrix)
Blood	ctDNA	1- Streck 10-cc Cell-Free DNA BCT tube for each timepoint	Baseline Cycle 1 Day 15 (±7 days) Cycle 2 Day 1 (±3 days) At the time of disease progression	Developmental Therapeutics Branch Clinical Translation Unit (DTBCTU) (Labmatrix)
Blood	CTCs	2- Streck 10-cc Cell-Free DNA BCT tubes at each timepoint	Baseline Cycle 1 Day 15 (±7 days) Cycle 2 Day 1 (±3 days) At the time of disease progression	DTBCTU (Labmatrix)
PBMC	Immune cell subset profiling by flow cytometry	2 - 10 cc sodium heparin (green top) at each timepoint	Baseline Cycle 1 Day 15 (±7 days)	Schlom (Labmatrix)

*Laboratory for planned analyses has not been finalized. Samples to be retained by the Biospecimen Processing Core (BPC) until distribution. Tube size for bloods may be adjusted based on supply available or change in assay, etc., if maximum volume is not exceeded.

Please note that tubes and media may be substituted based on availability with the permission of the PI or laboratory investigator.

^ The location of specimen processing or analysis may be adjusted with the permission of the PI or laboratory investigator

5.3 SAMPLE STORAGE, TRACKING AND DISPOSITION

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

5.3.1 Biospecimen Processing Core (BPC)

5.3.1.1 Handling and Processing of Specimens

The samples will be placed immediately on wet ice and refrigerated. The date and exact time of each blood draw should be recorded on the sample tube.

For sample pickup, page [redacted].

For immediate help, call [redacted] (main BPC number) or, if no answer, [redacted] (main clinical pharmacology lab number).

For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov.

5.3.1.2 Biospecimen Processing Core

All samples sent to the BPC will be barcoded, with data entered and stored in the Labmatrix utilized by the BPC. This is a secure program, with access to Labmatrix limited to defined BPC personnel, who are issued individual user accounts. Installation of Labmatrix is limited to computers specified by BPC. These computers all have a password restricted login screen. All BPC personnel with access to participant information complete the NIH online Protection of Human Subjects course.

Labmatrix creates a unique barcode ID for every sample and sample box, which cannot be traced back to participants without Labmatrix access. The data recorded for each sample includes the participant ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Participant demographics associated with the clinical center participant number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80°C according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in Labmatrix. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described below. The

study will remain open so long as sample or data analysis continues. Samples from consenting participants will be stored until they are no longer of scientific value or if a participant withdraws consent for their continued use, at which time they will be destroyed. If, at any time, a participant withdraws consent, the participants data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved. The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of Section **7.2**.

Sample barcodes are linked to participant demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the Labmatrix. It is critical that the sample remains linked to participant information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

Note: All other CCR lab systems/databases at which samples will be stored/analyzed are as noted in the Specimen Collection Table (e.g., Labmatrix; Section **5.2**).

5.3.2 Specimen Labeling, Storage

All specimens (blood and tissue) collected during the course of this study will follow storage, handling and labeling procedures that ensure security, confidentiality, and sample integrity are maintained. All samples are tracked by distinct identification labels that include a unique participants identifier and data of specimen collection. Thus, samples will be de-identified of personal data, with access to personal data restricted to the study investigators.

All cryopreserved samples are tracked for freezer location and storage criteria in the Investigators designated laboratory at specified temperature with alarm systems in place, and as per the Trial Lab Manual.

5.3.3 Protocol Completion/Sample Destruction

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

The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of Section **7.2**

5.4 SAMPLES FOR GENETIC/GENOMIC ANALYSIS

5.4.1 Description of the scope of genetic/genomic analysis

Tumor tissue may undergo somatic Whole Exome Sequencing (WES), Whole Genome Sequencing (WGS) and single cell RNA sequencing to look for the molecular basis of the participant's cancer as described in Sections **5.1.2** and **5.1.3**.

5.4.2 Description of how privacy and confidentiality of medical information/biological

specimens will be maximized

Every effort will be made to keep participants' personal information, biospecimens and resultant data confidential.

Precautions will be taken to protect the privacy of participants. The Clinical Investigator or a member of the study staff will maintain a master enrollment log containing participant names and their assigned study-specific numbers and the consent forms. All of these documents will be stored in locked cabinet(s) at the Clinical Site. Only the study Clinical Investigators or the study coordinator(s) will have routine access to the master enrollment log and participant identifiable information. The informed consent form discloses the limits of confidentiality and the fact that participants may be requested to disclose such information by a third party. This is a risk of participation in the study and the participants will be informed of this risk. Given the uniqueness of one's genetic information, we cannot guarantee that participant's genetic information will never be able to be linked back to them.

Samples will be stored at TGen or NCI CC. Samples will not be stored with any participant identifiers. Research laboratory staff will not see any identifying participant information that could be linked to the sample. Research staff will receive only de-identified and coded information.

Data from the study may be published in scientific journals and/or public data sharing databases (such as dbGaP). All data published or shared will not include any participant identifiers.

Participants samples and data will be covered by a certificate of confidentiality, providing additional protections to study participants.

5.4.3 Management of Results

Participants will be contacted if a clinically actionable gene variant is discovered. Clinically actionable findings for the purpose of this study are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of incidental findings that is current at the time of primary analysis. Participants will be contacted at this time with a request to provide a sample to be sent to a CLIA certified laboratory. If the research findings are verified in the CLIA certified lab, the participant will be offered the opportunity to come to NIH (at our expense) to have genetic education and counseling to explain this result. If the participant does not want to come to NIH, a referral to a local genetic healthcare provider will be provided (at their expense).

This is the only time during the course of the study that incidental findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into a 21 CFR Part 11-compliant data capture system provided by the NCI CCR and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

Document AEs from the first study intervention, Study Day 1, through 30 (+5) days following the last dose of study medication, whichever occurs later. Beyond 30 days after the last intervention, only adverse events which are serious and related to the study intervention need to be recorded.

SAE assessment after the 30 days will be documented by a licensed physician listed on the 1572 in the source documents.

All adverse events related to study intervention will be recorded in the study database, regardless of grade. Abnormal grade 1 or grade 2 laboratory values not considered related to study intervention will be recorded in the database as an AE **only** if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact. AE's related to changes in creatinine, AST, ALT and bilirubin are always considered clinically significant.
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the participant's outcome.

The PI (or PI designee) evaluation of each AE not captured in the clinical database determining that it meets the criteria above will be documented in the source documents. Note: the investigator performing the assessment must be a licensed clinician listed on the FDA form1572.

End of study procedures: Data will be stored according to HHS, FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

What data will be shared?

I will share human data generated in this research for future research as follows:

_X_ Coded, linked data in an NIH-funded or approved public repository.

___ Coded, linked data in another public repository.

_X_ Coded, linked data in BTRIS (automatic for activities in the Clinical Center)

_X_ Identified or coded, linked data with approved outside collaborators under appropriate agreements.

___ I will not share human data generated in this research for future research. If checked, explain:

How and where will the data be shared?

Data will be shared through

_X_ An NIH-funded or approved public repository. Insert name or names:clinicaltrials.gov.

___ Another public repository. Insert name or names: _____.

_X_ BTRIS (automatic for activities in the Clinical Center)

_X_ Approved outside collaborators under appropriate individual agreements.

_X_ Publication and/or public presentations.

When will the data be shared?

_X_ Before publication.

_X_ At the time of publication or shortly thereafter.

6.2.2 Genomic Data Sharing Plan

Unlinked genomic data will be deposited in public genomic databases such as dbGaP in compliance with the NIH Genomic Data Sharing Policy.

6.3 RESPONSE CRITERIA

For the purposes of this study, participants should be re-evaluated for response every 8 weeks. In addition to a baseline scan, confirmatory scans should also be obtained 4-8 weeks (not less than 4) 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 (22). 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.

6.3.1 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:

- By chest x-ray: ≥20 mm;
- By CT scan:
    - Scan slice thickness 5 mm or under: as ≥10 mm
    - Scan slice thickness >5 mm: double the slice thickness
- With calipers on clinical exam: ≥10 mm.

All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

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

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 participant, 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.

6.3.2 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 participant 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.(23-25) 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.(26)

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:

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

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

6.3.3 Response Criteria

6.3.3.1 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 of 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 of diameters while on study.

6.3.3.2 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 participant 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).

6.3.3.3 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 participant's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Participants 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	

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*

* 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: Participants 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 Participants 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

6.3.4 Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

6.4 Toxicity Criteria

The following adverse event management guidelines are intended to ensure the safety of each participant while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm).

7 NIH REPORTING REQUIREMENTS / DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING / IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements. Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reviewed by the OHSRP in the NIH eIRB system will also be reported to the NCI Clinical Director/designee; therefore, a separate submission for these reports is not necessary.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention should be reported via email unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 NIH REQUIRED DATA AND SAFETY MONITORING PLAN

7.4.1 Principal Investigator/Research Team

The clinical and scientific research teams will meet on a weekly basis when participants are being actively treated on the trial to discuss each participant and overall progress of the study.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in Section **7.2.1** will be submitted within the appropriate timelines.

The principal investigator will review adverse event and response data on each participant to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

8 SPONSOR PROTOCOL/SAFETY REPORTING

8.1 DEFINITIONS

8.1.1 Adverse Event

Any untoward medical occurrence in a participant or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2))

8.1.2 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse event (see Section **8.1.3**)
- Inpatient hospitalization or prolongation of existing hospitalization
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.
    - A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient or subject convenience) is not considered a serious adverse event.
    - Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the participant or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.3 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the participant or subject 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. (21CFR312.32)

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 5.0.

8.1.5 Relationship to Study Product

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.2 Assessment of Safety Events

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to Section **6.1**. All serious adverse events recorded from the time of first investigational product administration must be reported to the sponsor with the exception of any listed in Section **8.4**.

8.3 Reporting of Serious Adverse Events

Any AE that meets protocol-defined serious criteria or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety. Any exceptions to the expedited reporting requirements are found in Section **8.4**.

All SAE reporting must include the elements described in Section **8.2**.

SAE reports will be submitted via an electronic SAE reporting system (e.g. HiLIT). In the event of system downtime or issues , SAE reports will be submitted using the CCR SAE Report form to the sponsor at: OSROSafety@mail.nih.gov. CCR SAE report form and instructions can be found at: https://nih.sharepoint.com/:u:/r/sites/NCI-CCR-OCD-Communications/SitePages/Forms-and-Instructions.aspx?csf=1&web=1&e=uWBXtI

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

8.4 Waiver of Expedited Reporting to Sponsor (CCR)

As death/hospitalization due to disease progression are part of the study objectives (OS, DCR), and captured as endpoints in this study, they will not be reported in expedited manner to the sponsor. However, if there is evidence suggesting a causal relationship between the study drug and the event, report the event in in an expedited manner according to Section **8.3**.

8.5 Safety Reporting Criteria to the Pharmaceutical Collaborators

Reporting will be per the collaborative agreement.

8.6 Reporting Pregnancy

All required pregnancy reports/follow-up to OSRO will be submitted to: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. Forms and instructions can be found here: https://nih.sharepoint.com/:u:/r/sites/NCI-CCR-OCD-Communications/SitePages/Forms-and-Instructions.aspx?csf=1&web=1&e=uWBXtI.

8.6.1 Maternal exposure

If a participant becomes pregnant during the course of the study, the study treatment should be discontinued immediately, and the pregnancy reported to the Sponsor no later than 24 hours of when the Investigator becomes aware of it. The Investigator should notify the Sponsor no later than 24 hours of when the outcome of the Pregnancy becomes known.

Pregnancy itself is not regarded as an SAE. However, congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (Section **8.1.2**) should be reported as SAEs.

The outcome of all pregnancies should be followed up and documented.

8.6.2 Paternal exposure

Male participants should refrain from fathering a child or donating sperm during the study and for 3 months after the last dose of Minnelide .

Pregnancy of the participant's partner is not considered to be an AE. The outcome of all pregnancies occurring from 3 months after the last dose of Minnelide should, if possible, be followed up and documented. Pregnant partners may be offered the opportunity to participate in an institutional pregnancy registry protocol (e.g., the NIH IRP pregnancy registry study) to provide data about the outcome of the pregnancy for safety reporting purposes.

8.7 Regulatory Reporting for Studies Conducted Under CCR-Sponsored IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

All serious events will be reported to the FDA at least annually in a summary format.

8.8 SPONSOR PROTOCOL DEVIATION REPORTING

A Protocol Deviation is defined as any non-compliance with the clinical trial Protocol, Manual of Operational Procedures (MOP) and other Sponsor approved study related documents, GCP, or protocol-specific procedural requirements on the part of the participant, the Investigator, or the study site staff inclusive of site personnel performing procedures or providing services in support of the clinical trial.

It is the responsibility of the study Staff to document any protocol deviation identified by the Staff or the site Monitor in the CCR Protocol Deviation Tracking System (PDTS) online application. The entries into the PDTS online application should be timely, complete, and maintained per CCR PDTS user requirements.

In addition, any deviation to the protocol should be documented in the participant's source records and reported to the reviewing IRB per their guidelines. OSRO required protocol deviation reporting is consistent with E6(R2) GCP: Integrated Addendum to ICH E6(R1): 4.5 Compliance with Protocol; 5.18.3 (a), and 5.20 Noncompliance; and ICH E3 16.2.2 Protocol deviations.

9 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure:

- that the rights of the participants are protected;
- that the study is implemented per the approved protocol, Good Clinical Practice and standard operating procedures; and,
- the quality and integrity of study data and data collection methods are maintained.

Monitoring for this study will be performed by NCI CCR Office of Sponsor and Regulatory Oversight (OSRO) Sponsor and Regulatory Oversight Support (SROS) Services contractor. Clinical site monitoring activities will be based on OSRO standards, FDA Guidance E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1) March 2018, and applicable regulatory requirements.

Details of clinical site monitoring will be documented in a Clinical Monitoring Plan (CMP) developed by OSRO. CMPs will be protocol-specific, risk-based and tailored to address human subject protections and integrity of the study data. OSRO will determine the intensity and frequency of monitoring based on several factors, including study type, phase, risk, complexity, expected enrollment rate, and any unique attributes of the study and the site. The Sponsor will conduct a periodic review of the CMP to confirm the plan's continued appropriateness. A change to the protocol, significant or pervasive non-compliance with GCP, or the protocol may trigger CMP updates.

OSRO SROS Monitoring visits and related activities will be conducted throughout the life cycle of each protocol. The first activity is before the study starts to conduct a Site Assessment Visit (SAV) (as warranted), followed by a Site Initiation Visit (SIV), Interim Monitoring Visit(s) (IMVs), and a study Close-Out Visit (COV).

Some monitoring activities may be performed remotely, while others will occur at the study site(s). Monitoring visit reports will describe visit activities, observations, and associated action items or follow-up required for resolution of any issues, discrepancies, or deviations. Monitoring reports

will be distributed to the study PI, NCI CCR QA, CCR Protocol Support Office, coordinating center (if applicable), and the Sponsor regulatory file.

The site Monitor will inform the study team of any deviations observed during monitoring visits. If unresolved, the Monitor will request that the site Staff enter the deviations in the CCR Protocol Deviation Tracking System (PDTS) for deviation reporting to the Sponsor and as applicable per institutional and IRB guidance.

10 STATISTICAL CONSIDERATIONS

10.1 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
To determine the single agent antitumor activity (disease control rate) of the anti-superenhancer agent Minnelide in participants with advanced, previously treated ASCP	Assess disease control rate in the study population, where disease control = best response of CR, PR or at least 16 weeks of stable disease	Establishing disease control in 8 or more of 25 participants with advanced previously treated ASCP would be considered clinically significant.
Secondary		
Determine Minnelide safety in participants with advanced, previously treated ASCP	Identify Adverse Events of grade 2 and above (per CTCAE criteria, version 5.0) related to Minnelide that occur from start of treatment to 30 days after last treatment. Assess rate of Serious Adverse Events related to Minnelide that occur from start of treatment to 30 days after last treatment.	Descriptive endpoint to assess toxicity profile of the study drug in this population.
Assess alternative measures of anti-tumor activity	Determine median time until disease progression, switch to a new systemic treatment or death in the treated population. Data cut-off will occur 1 year after last Minnelide treatment. Determine median time until death in the treated population. Data cut-off will occur 1 year after last Minnelide treatment. Determine objective response rate of Minnelide.	Descriptive endpoints that may help to determine sample size needed for future Phase 3 study

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Exploratory		
Identify factors that influence participant response to treatment through detailed molecular analysis of participant tumor, serum and blood cell samples acquired at baseline, on-treatment and at time of progression	For each patient with available samples, we will describe the following properties and consider associations with patient response to therapy:	These endpoints have been selected to help understand why the drug may or may not work, to allow better design of future studies in this patient population.
	Tumor genetic mutations at baseline and at time of progression	Do specific genetic profiles give a patient higher or lower likelihood or response? Does treatment change mutational profile?
	Treatment-related change (between pre-treatment baseline and ~C1D15) in:	
	1) protein expression of Myc in tumor cells, 2) protein expression HSP70 in tumor cells and 3) change in accessibility of tumor cell chromatin	Has the drug reached the tumor? Is predicted bioactivity seen?
	4) Populations and locations of tumor infiltrating immune cells, 5) circulating cytokines (as above and at time of progression), and 6) circulating immune cell subsets (as above, start of C2 and at time of progression)	Does Minnelide change the host and/or tumor immune environment?
	7) Change in tumor-associated cell milleu with Minnelide treatment	Does Minnelide alter the tumor microenvironment?
	Changes in CTCs at baseline, ~C1D15, start of C2 and at time of progression	Does Minnelide clear CTCs?

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Evaluate ctDNA as a marker of response in ASCP	Percent of patients with detectable KRAS mutant ctDNA; and, concordance in change of detectable KRAS mutant ctDNA and disease control status as assessed at baseline, ~C1D15, start of C2, and at time of progression.	KRAS mutation is nearly ubiquitous in this population and mutant ctDNA may serve as a reliable early blood-based biomarker of response. This aim is hypothesis-generating

10.2 Statistical Hypothesis

10.2.1 Primary Efficacy endpoints:

Disease control rate defined as (CR+PR+ stable x 16 weeks) using RECIST criteria.

10.2.2 Secondary Efficacy endpoints:

- Safety of Minnelide
- PFS and OS

10.3 Sample Size Determination

The primary objective of this trial is to determine if the CR, PR and SD >16 weeks (clinical benefit) rate will be adequate using Minnelide without stratification for dose. The trial will be conducted using a Simon optimal two-stage phase II trial design [20][19] to rule out an unacceptably low clinical benefit rate of 20% (p0=0.20) in favor of an improved clinical benefit rate of 40% (p1=0.40). With alpha=0.10 (probability of accepting a poor treatment=0.10) and beta=0.20 (probability of rejecting a good treatment=0.20), the first stage will enroll a total of 12 evaluable participants. If 0-2 of these 12 participants have clinical benefit, then no further participants will be accrued. If 3 or more of the first 12 participants have clinical benefit, then accrual would continue until a total of 25 evaluable participants have been enrolled. As this is a rare participant population, trial accrual may continue for up to 15 participants while this determination is being made, but no new participants may be accrued to the trial once it is known that this criteria has not been met. If single agent activity is insufficient to meet criteria for study continuation, consideration will be given to exploring combination therapy including Minnelide in this population. If there are 3-7 participants with clinical benefit out of 25 participants, this would be an uninterestingly low clinical benefit rate. If there are 8 or more of 25 (32%) who experienced clinical benefit, this would be sufficiently interesting to warrant further study in later trials. Under the null hypothesis (20% clinical benefit rate), the probability of early termination is 55.8%.

It is expected that approximately one participant may initiate treatment on this trial every 1-2 months; thus, accrual to the trial may be completed in 3 years. In order to allow for screen failures and a small number of inevaluable participants, the accrual ceiling will be set at 55 participants.

10.4 Populations for Analyses

Intention to treat: any participants who enroll onto the trial and provide consent and who receive at least one dose of the assigned agent will be included in the efficacy and safety evaluations.

10.4.1 Evaluable for toxicity

All participants will be evaluable for toxicity from the time of their first treatment with Minnelide.

10.4.2 Evaluable for objective response:

Only those participants who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for objective response. These participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

10.4.3 Evaluable Non-Target Disease Response:

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

10.5 STATISTICAL ANALYSES

10.5.1 General Approach

The fraction of all evaluable participants who experience clinical benefit (CR+PR +SD>16 weeks) will be reported along with a confidence interval.

10.5.2 Analysis of the Primary Endpoint

The fraction of evaluable participants who experience clinical benefit will be reported along with a 95% two-sided confidence interval.

10.5.3 Analysis of the Secondary Endpoints

PFS and OS will be determined using the Kaplan-Meier method and will be reported along with a 95% confidence interval for the median.

Objective response rate (ORR) will be calculated as the percentage of patients with CR or PR as defined by RECIST 1.1 criteria.

Analysis of safety is as described in the Section **10.5.4**.

10.5.4 Safety Analyses

The fraction of participants who experience a toxicity, by grade and type of toxicity, will be tabulated.

10.5.5 Baseline Descriptive Statistics

Baseline demographic characteristics will be reported.

10.5.6 Planned Interim Analyses

As indicated in the two-stage design, the number of responses after 12 evaluable participants have been treated will be noted and will be used to determine if enrollment to the second stage of accrual may continue.

10.5.7 Sub-Group Analyses

None.

10.5.8 Tabulation of individual Participant Data

None.

10.5.9 Exploratory Analyses

The following are the planned exploratory analyses:

- Utilize participant blood and biopsy tissue acquired on this protocol to identify factors that may be important in determining participant response to treatment (techniques involved immune phenotyping analysis, whole genome sequencing, ATAC-seq, Digital Spatial Profiling (Nanostring GeoM) and effect of the study drug on tumor a circulating cell populations.
- Evaluate ctDNA as a marker of response in ASCP

The exploratory objectives are intended to collect data for use in planning future scientific investigations or clinical research. These analyses are expected to be performed first using descriptive techniques, reporting descriptive statistics including confidence intervals when appropriate. Any statistical tests performed for evaluation of exploratory objectives will be done without formal adjustment for multiple comparisons, but in the context of the number of tests performed.

11 COLLABORATIVE AGREEMENTS

11.1 AGREEMENT TYPE

Clinical trial agreement (CTA # 01208-21) with Minneamrita is executed.

12 HUMAN SUBJECTS PROTECTIONS

12.1 RATIONALE FOR SUBJECT SELECTION

All participants meeting the criteria listed in Section **2.1** are eligible for enrollment regardless of race, ethnicity, sex, nationality, or English-language ability.

Participants with ASCP are specifically being recruited to this study because the preclinical evidence supports efficacy of the trial intervention in this participant population.

12.2 PARTICIPATION OF CHILDREN

ASCP is typically a disease of adults. Because ASCP does not typically occur in children and no dosing or adverse event data are currently available on the use of Minnelide in participants <18 years of age, children are excluded from this study.

12.3 RISK/BENEFIT ASSESSMENT

12.3.1 Known Potential Risks

12.3.1.1 Minnelide

Minnelide given in oral form is still under investigation. At this stage, the major treatment-related toxicities appear to be related to myelosuppression, including neutropenia, febrile neutropenia and neutropenic sepsis. At least one death due to sepsis attributed to Minnelide has been reported on studies of IV and oral Minnelide.

12.3.1.2 Blood Collection

Side effects of blood draws include pain and bruising, lightheadedness, and rarely, fainting. Up to 60.5 mL of research blood may be collected at any visit but no more than 178 ml in an 8 week period.

12.3.1.3 Urine Collection

No physical risks are associated with urine collection.

12.3.1.4 Electrocardiogram

Some skin irritation can occur where the ECG/EKG electrodes are placed. The test is completely painless, and generally takes less than a minute to perform.

12.3.1.5 Imaging

In addition to the radiation risks discussed below, CT scans may include the risks of an allergic reaction to the contrast. Participants might experience hives, itching, headache, difficulty breathing, increased heartrate and swelling. Furthermore, the IV catheter used to administer the contrast may cause bleeding, infection or inflammation of the skin and vein with pain and swelling. Participants undergoing gadolinium enhanced MRIs may also be at risk for kidney damage. MRIs include the additional risk of damage to hearing.

12.3.1.6 Biopsy Collection

The risks of the optional research biopsies include pain, bleeding and infection at the biopsy site. In addition, as the biopsies may be collected under CT guidance, participants in this study may be exposed to radiation as discussed below.

12.3.1.7 Risks from Radiation Exposure

On this study, patients will receive up to 3 CT-guided biopsies and up to 5 CT scans. The total radiation dose for research purposes will be approximately 8.9 rem. The risk of getting cancer from the radiation exposure in this study is 0.9% and of getting a fatal cancer is 0.4%.

12.3.2 Known Potential Benefits

Pre-clinical studies of Minnelide in ASCP models support our hypothesis that participant tumors will be sensitive to this drug. Better control of ASCP provided by Minnelide may improve tumor-related symptoms such as pain and fatigue and may slow disease progression. Treatment with these drugs have the potential to provide direct benefit to participants.

12.3.3 Assessment of Potential Risks and Benefits

Advanced ASCP that has progressed after previous treatment has a grim prognosis with median survival of no more than 3 months. There are no standard treatments for this disease. Available research suggests that Minnelide may be effective against ASCP. These potential benefits are balanced against the risk of drug toxicity. While Minnelide can cause serious toxicities, these toxicities have been successfully managed with supportive measures and dose reduction in other clinical studies. In addition, the frequent clinical and laboratory assessments that have been built into this protocol will further minimize risk to participants.

12.4 Consent Process and Documentation

The informed consent document will be provided as a physical or electronic document to the participant for review prior to consenting. A designated study investigator* will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with policy, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of the participant. Whether in person or remote, the privacy of the participants will be maintained. Consenting investigators (and participant, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to participant) or as described below, with a manual (non-electronic) signature on the electronic document. When required, witness signature will be obtained similarly as described for the investigator and participant.

Manual (non-electronic) signature on electronic document:

When a manual signature on an electronic document is used for the documentation of consent at the NIH Clinical Center, this study will use the following to obtain the required signatures:

- Adobe platform (which is not 21 CFR Part 11 compliant); or,
- iMedConsent platform (which is 21 CFR Part 11 compliant)

During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations (if remote consent); the same screen may be used when in the same location but is not required.

Both the investigator and the participant will sign the document using a finger, stylus or mouse.

Note: Refer to the CCR SOP PM-2, Obtaining and Documenting the Informed Consent Process for additional information (e.g., verification of participant identity when obtaining consent remotely) found at: https://nih.sharepoint.com/sites/NCI-CCR-OCD-Communications/SitePages/OEC-Administrative---Clinical-Research-(ADCR).aspx?Mode=Edit.

13 REGULATORY AND OPERATIONAL CONSIDERATIONS

13.1 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, the Investigational New Drug (IND) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and

will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and as applicable, Food and Drug Administration (FDA).

13.2 Quality Assurance and Quality Control

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Council for Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

13.3 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NIH has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

13.4 Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict

confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the/each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the NCI CCR. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical site(s) and by NCI CCR research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NIH.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

14 PHARMACEUTICAL INFORMATION

14.1 MINNELIDE – IND # 155631

14.1.1 Source/Acquisition and Accountability

14.1.1.1 Supplier

Minneamrita Therapeutics, LLC, 17939 Cachet Isle Drive, Tampa, FL 33647

Aizant Drug Research Solutions Pvt. Ltd. ("Aizant") manufactures and packages Minnelide ™ Capsules under cGMP for clinical trial material (CTM).

14.1.1.2 Receipt of Minnelide ™ Supplies.

Minnelide ™ will be received by the pharmacy department. The pharmacy department should review and confirm receipt of the IMP is as per the paperwork received with the drug. Pharmacy must ensure that ordering and delivery records for the IMP are retained in the Pharmacy Site File. Drug accountability will be recorded on a study-specific IMP accountability log. Shipments of IMP received at pharmacy will be logged on the accountability log, which will be kept in the

Pharmacy Site File.

14.1.1.3 Minnelide ™ Accountability

The Investigator or a delegated individual (e.g. pharmacist), must ensure that the study drug is dispensed in accordance with hospital standard operating procedures and applicable regulatory requirements. Full drug accountability records must be maintained for Minnelide ™.

The Minnelide ™ provided for this study is for use only as directed in the study protocol. IMP related documentation will be provided to the site in a pharmacy pack. It is the Investigator's responsibility to establish a system for handling the IMP to endure that:

- Deliveries of IMPs by Minneamrita Therapeutics LLP are correctly received by a responsible person (e.g. pharmacist or suitable pharmacy designee) and are handled and stored correctly and safely.
- Minnelide ™ is dispensed only to study participants, and in accordance with the protocol
- A dispensing record (which will include the identification of the participant to whom the IMP was dispensed, the date of dispensing, the quantity of IMP dispensed, and the date and quantity of any unused IMP returned to pharmacy) is accurately maintained. Any discrepancies must be accounted for on the appropriate form.

In the case that any study drug is damaged, the NCI CCR coordinating team must be contacted for reconciliation and replacement.

14.1.1.4 Dispensing of Minnelide ™

Minnelide ™ will be dispensed by the pharmacy in accordance with a trial-specific prescription. A prescription template will be provided in the pharmacy file, although site will be permitted to use their own clinical trial template prescription if suitable. Prior approval of the final prescription template by Minneamrita Therapeutics is required. Minnelide ™ will be dispensed on the day of administration i.e. days 1-21 of each cycle.

14.1.1.5 Return or Destruction of IMP

At study termination or at the request of Minneamrita Therapeutics any quantities of IMP that have expired or remain unused must be destroyed according to the site's local standard operating procedures and only following permission by OSRO. OSRO will seek approval from Minneamrita Therapeutics, as required, per the study agreement. Minneamrita Therapeutics will not carry out IMP destruction. Certificates of destruction must be provided by the site and copies must be retained in the Pharmacy Site File.

14.1.2 Toxicity

Results from nonclinical pharmacological, pharmacokinetic, and toxicological studies evaluating Minnelide ™ given intravenously or orally support the rationale for evaluation of Minnelide ™ in cancer participants. Plasma concentrations of Minnelide ™ and triptolide associated with key responses and exposure margins calculated against these key responses which reflect toxicological findings considered relevant or possibly relevant to human risk.

In definitive 28-day repeat dose toxicity studies, a no-observed adverse-effect level (NOAEL) for Minnelide ™ in female dogs was identified to be 0.05 mg/kg/day and considered the highest non-

severely toxic dose (HNSTD); the NOAEL for Minnelide ™ in rats or male dogs could not be determined. Target organ toxicities indicated reproductive organ accumulation.

In humans, 42 participants with advanced GI tumors were given Minnelide ™ intravenously in a phase 1 open-label dose-escalation safety, pharmacokinetic and pharmacodynamic study. Doses were given daily for 21 days followed by 7 days off or each day for 5 days with two days off for three weeks followed by a 7 day rest period. The primary reason for the discontinuation of treatment was disease progression. There were 6 deaths in the study, but these occurred after the study drug had ended and were within the 30-day follow-up period. The deaths that occurred during the study were found to be not related to the study drug, and were found to be related to progression of the participant's disease (pancreatic/gastric cancer). There was one participant who died from respiratory failure, and this was also found to be not related to use of the study drug. There were 3 participant who discontinued the study drug due to a treatment emergent adverse events (TEAEs). All instances were considered Grade 3, and two of these TEAEs were considered to be probably related to use of the study drug. These two cases were also considered (DLTs). These events included: cerebellar toxicity, embolism, and cerebellar dysfunction. A total of 28 Serious Adverse Events occurred in 17 participants and six were found to be related to the study drug.

Overall, the most commonly reported TEAE (> 20%) regardless of causality included: hypoalbuminaemia, anemia, hypoproteinemia, fatigue, neutropenia, leukopenia, thrombocytopenia, nausea, hypocalcemia, diarrhea, hyperphosphatemia, constipation, vomiting, hyponatremia, lymphopenia, abdominal pain, dehydration, hyperglycemia, peripheral edema, hypophosphatemia, and headache.

32 participants had at least 1 AE that was considered related to the study drug. Adverse events of neutropenia grade 3 and 4 of short durations have been observed at all dose levels and were determined to be drug related. The neutropenia resolved within a couple of days of not receiving treatment. Neutropenic fever and neutropenic infection were observed in three participants and determined to be drug related. Anemia and thrombocytopenia grade 3 and 4 were observed in 22% of the participants and determined to be drug related. Other side effects of any grade that were possibly related to the drug included nausea, vomiting, diarrhea, constipation, anorexia, stomatitis, cerebellar toxicity, embolism and dyspnea, which occurred in ≤ 3% of the participants.

In an ongoing phase 1 multi-center, open-label, dose-escalation, safety, pharmacokinetic and pharmacodynamic study of Minnelide ™ capsules were given alone or in combination with Protein-Bound Paclitaxel to participants with Advanced Solid Tumors. This study is still ongoing.

To date, there have been a total of 67 participants enrolled and dosed in the Phase 1 study, 43 in the monotherapy regimen, and 24 in the combination regimen. The primary reason for discontinuation of treatment has been disease progression. There have been 6 deaths on the study, 5 of which were assessed as not related to Minnelide and were after discontinuation of the study drug. One participant on the 1.25mg monotherapy regimen passed away from G5 related sepsis. One participant on the combination regimen at 0.25mg of Minnelide ™ plus protein bound paclitaxel passed away from G5 sepsis, which after investigation and review of pharmacokinetic data, was found to be unrelated to Minnelide ™. There have been 6 participants that have discontinued the study drug due to a treatment emergent adverse event (TEAE). Four instances were considered Grade 5, one was Grade 3, one was Grade 2. Only one of these TEAE's were considered to be probably related to use of the study drug three possibly related (Two Grade 5

sepsis, one Grade 3 hypokalemia, one Grade 2 neutrophil count decrease), and the other two not related (Grade 5 disease progression). A total of 56 Serious Adverse Events have occurred in 33 patients and 11 were found to be related to the investigational product. The 11 related SAE's, two grade five sepsis, a grade four sepsis, a grade three nausea, a grade three polymicrobial bacteremia, a grade three intractable nausea, a grade three hypokalemia event, a grade three worsening leukocytosis, a grade three blood infection, and a grade two enteritis were on the monotherapy regimen. A grade five sepsis and a grade two esophagitis were on the combination therapy regimen..

Overall, the most commonly reported TEAE (> 20%) regardless of grade or causality have included: anemia, abdominal pain, anorexia, vomiting, diarrhea, nausea, fatigue, hypokalemia, and hypoalbuminemia. 43 participants had at least 1 AE that was considered definitely, probably, or possibly related to the study drug.

Grade 3 Adverse Events: Abdominal Pain 10%, Anemia 15%, Alkaline Phosphatase Increased 4%, Dehydration 3%, Diarrhea 6%, Hypoalbuminemia 6%, Hypokalemia 10%, Hyponatremia 6%, Nausea 3%, Neutrophil Counts Decreased 13%, Thrombocytopenia 3%, Urinary Tract Infection 3%, Vomiting 3%, Weight decreased 3%, White Blood Cell Count Decreased 3%.

Grade 4 Adverse Events: Leukopenia 3%, Neutropenia 7%, Sepsis 3%.

Grade 5 Adverse Event: Sepsis Disease Progression 9%.

14.1.3 Formulation and Preparation

The oral dosage form of product, Minnelide ™ Capsules, is Minnelide ™ drug substance blended with excipients, filled in hard gelatin capsules.

Compendial grade excipients include Avicel PH101 (filler: microcrystalline cellulose, MCC), Colloidal silicon dioxide (glidant: Aerosil 200), magnesium oxide DC (diluent: granular) and magnesium stearate (lubricant). Minnelide ™ API powder (<1 wt %) is blended with the excipients (>99 wt % total). The bulk powder blend is filled in capsules:

- Size 2 White/White opaque capsules are filled with 150 mg blended powder that contains 0.50 mg Minnelide ™ free acid.

Aizant Drug Research Solutions Pvt. Ltd. ("Aizant") manufactures and packages Minnelide ™ Capsules under cGMP for clinical trial material (CTM).

14.1.4 Stability and Storage

The finished product is stored at Controlled Cold Temperature (2–8° C, excursions permitted 0–15° C). Participants will be provided cooler bags to transport medication home and instructed to store the medication in their refrigerator at home.

14.1.5 Administration Procedures

The oral dosage form of product, Minnelide ™ Capsules, is Minnelide ™ drug substance blended with excipients, filled in hard gelatin capsules.

Minnelide ™ Capsules for this study are white, opaque, hard gelatin size 2 capsules containing 0.5mg free acid Minnelide ™ for oral administration.

Two to five Minnelide ™ Capsules will be given orally once daily for 21day followed by a 7-day rest period. This cycle will be repeated every 28 days as long as there is no evidence of progressive disease and the treatment is associated with acceptable toxicity.

14.1.6 Incompatibilities

14.1.6.1 Pregnancy and Lactation

Teratogenic and nonteratogenic effects of Minnelide ™ have not yet been investigated. Women who are pregnant or may become pregnant should not be administered Minnelide ™. In the event that Minnelide ™ is inadvertently administered to pregnant participants, the clinician should discuss all available alternatives. Women of child-bearing potential and men must agree to use adequate contraception prior to study entry, for the duration of study participation and for 6 months (women) or 3 months (men) following last treatment. No data exist on the excretion of Minnelide ™ in milk or on the effects of Minnelide ™ on nursing infants.

14.1.6.2 Special Populations

No nonclinical or clinical data exist on the use of Minnelide ™ in special populations. No information exists regarding the effects of Minnelide ™ on pediatric or geriatric population.

14.1.6.3 Overdose

There is currently no data available regarding overdosage of Minnelide ™. In the event of accidental overdose, participants should not be administered any further Minnelide ™ and should be monitored until resolution of any possibly-related toxicities.

14.1.6.4 CYP 450

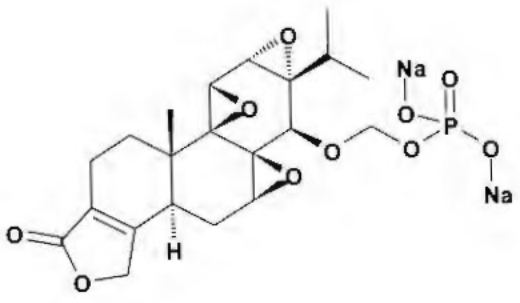
In vitro results indicate that Triptolide did not inhibit or induce CYP 450. No excretion studies have yet been conducted with Minnelide ™.

14.1.7 Additional Information

14.1.7.1 Chemical Formula

[(3b*S*,4a*S*,5a*R*,6*S*,6a*S*,7a*S*,7b*S*,8a*S*,8b*S*)-6a-isopropyl-8b-methyl-1-oxo-1,3,3b,4,4a,6,6a,7a,7b,8b,9,10-dodecahydrotrioxireno[2',3':6,7:2',3':4,4a:2',3':8,8a]naphtho[2,1-*e*]isobenzofuran-6-yl]oxymethyl dihydrogen phosphate

Structure



14.1.7.2 Molecular Weight

514.38

$C_{21}H_{25}O_{10}P \cdot 2Na$ (disodium salt)
$C_{21}H_{27}PO_{10}$ (free acid)

14.1.7.3 Mechanism of Action

Minnelide ™ is a prodrug that was designed to rapidly release the antitumor molecule, triptolide, through the action of phosphatases in the bloodstream. Triptolide is a diterpenoid triepoxide found in the Chinese plant *Tripterygium wilfordii,* which has widely been used as a natural medicine in China for hundreds of years, particularly in the treatment of autoimmune and inflammatory diseases. Triptolide has been shown to inhibit proliferation and induce apoptosis in tumor cell lines in vitro and in animal models of cancer. However, triptolide has undesirable physicochemical properties that limit its usage as a therapeutic product and Minnelide ™ was designed to overcome these issues. Based on current evidence Minnelide is believed to act as an antisuperenhancer through its interaction with the transcription factor TFIIH with evidence that this interaction down regulates Myc.

15 LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	Adverse Event/Adverse Experience
ALT	alanine aminotransferase
ALP	alkaline phosphatase
ANC	absolute neutrophil count
aPTT	activated partial thromboplastin time
ASPC	Advanced Refractory Adenosquamous Carcinoma of the Pancreas
AST	aspartate aminotransferase
ATAC	Assay for Transposase Accessible Chromatin
β-HCG	beta-Human Chorionic Gonadotropin
BID	bis in die (twice a day)
BPC	Biospecimen Processing Core
BSA	Body surface area
BUN	Blood urea nitrogen
CBC	Complete blood count
CC	Clinical Center
CCR	Center for Cancer Research
CFR	Code of Federal Regulations
CMP	Clinical Monitoring Plan
CNS	Central nervous system
CONSORT	Consolidated Standards of Reporting Trials
COV	Close-Out Visit
CR	Complete response
CrCl	Creatinine clearance
CT	Computed Tomography
CTC	Circulating tumor cells
CTCAE	Common Terminology Criteria for Adverse Events
ctDNA	Circulating tumor DNA
CTM	clinical trial material
DLT	Dose-limiting toxicity
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group – performance status
EKG	Electrocardiogram
FDA	Food and Drug Administration
FDG	Fluorodeoxyglucose
FFPE	Formalin fixed paraffin embedded
GCP	Good Clinical Practice
GFR	Glomerular filtration rate
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
HBsAg	Hepatitis B antigen

Abbreviation	Definition
HBV	Hepatitis B virus
HCV	Hepatitis C virus
H&P	History and Physical
Hgb	Hemoglobin
HHS	Human and Health Services
HIV	Human immunodeficiency virus
HNSTD	Highest non-severely toxic dose
ICH	International Council for Harmonisation
IMP	Investigational Medicinal Product
IMV	Interim Monitoring Visit
IND	Investigational New Drug Application
INR	International normalized ratio
IP	Intraperitoneal
IRB	Institutional Review Board
LP	Laboratory of Pathology
MRI	Magnetic Resonance Imaging
NCI	National Cancer Institute
NIH	National Institutes of Health
NOAEL	no-observed adverse-effect level
NSF	Nephrogenic systemic fibrosis
OHSRP	Office for Human Subjects Research Protection
OS	Overall survival
OSRO	Office of Sponsor and Regulatory Oversight
PD	Progressive Disease
PDA	Pancreatic ductal adenocarcinoma
PDX	Participant-derived xenograft
PET	Positron Emission Tomography
PFS	Progression free survival
PI	Principal Investigator
PO	Per os (by mouth)
PR	Partial Response
PT	Prothrombin time
QA	Quality Assurance
QC	Quality Control
QD	Quaque die (one a day)
RNA	Ribonucleic Acid
RP2D	Recommended Phase II dose
SAE	Serious Adverse Event/Serious Adverse Experience
SAV	Site Assessment Visit
SD	Stable Disease
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase

Abbreviation	Definition
SIV	Site Initiation Visit (SIV)
SOP	Standard Operating Procedure
TAM	Tumor-associated macrophages
TEAE	Treatment emergent adverse events
ULN	Upper limit of normal
WES	Whole exome sequencing
WGS	Whole genome sequencing
WOCBP	Women of childbearing potential

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17 APPENDICES

17.1 APPENDIX A: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able	80	Normal activity with effort; some signs or symptoms of disease.

	to carry out work of a light or sedentary nature (e.g., light housework, office work).	70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

17.2 APPENDIX B: PARTICIPANT MEDICATION DIARY

Protocol/Study Number_____ Cycle #_____

Participant Name_____ Participant Study ID _____

INSTRUCTIONS TO THE PARTICIPANT:

1. Complete one form for each Cycle.
2. Keep the study medication in the original container, in the refrigerator at home.
3. You will take ____capsules of 0.5 mg Minnelide each day. Fast at least 2 hours before and 1 hour after taking Minnelide. Swallow with water. Do not crush or chew.
4. Record the date and what time you took them.
5. If you have any comments or notice any side effects, please record them in the Comments column.
6. Bring any empty containers and containers with unused study drug and all pages of your medication diary to your next study appointment or clinic visit.
7. If a dose is missed: log the date, leave time blank and add a comment that the dose was missed/not taken and reason (for example: couldn't swallow, forgot, vomited). Call the Study Team for instructions on what to do.

Date Dose Taken	Day	Minnelide	
		Time Dose Taken	Comments (side effects or missed doses)
	1		
	2		
	3		
	4		
	5		
	6		

	7		
	8		
	9		
	10		
	11		
	12		
	13		
	14		
	15		
	16		
	17		
	18		
	19		
	20		
	21		

Participant's Signature: _____ Date: _____

Staff Signature: _____Date: _____