CLINICAL STUDY PROTOCOL

Protocol Number:	PTC596-ONC-008-LMS
Protocol Title:	A Phase 2/3 Study to Evaluate the Efficacy and Safety of Unesbulin in Unresectable or Metastatic, Relapsed or Refractory Leiomyosarcoma
Name of Study Intervention:	Unesbulin
Study Phase:	Phase 2/3
Approval Date:	V1.0 20 December 2021 V2.0 31 January 2022 V3.0 28 April 2023 V4.0 28 November 2023
IND Number:	122916
EudraCT Number:	2022-000073-12
ClinicalTrials.gov:	NCT05269355
Sponsor:	PTC Therapeutics, Inc. 100 Corporate Court South Plainfield, New Jersey 07080 USA

PROTOCOL IDENTIFIERS AND STUDY PERSONNEL

Project Code	PTC596-ONC
Therapeutic Area	Oncology
PTC Therapeutics Substance Identifier	PTC596
International Nonproprietary Name	Unesbulin
Protocol Number	PTC596-ONC-008-LMS
Protocol Version	4.0
Protocol Version Date	28 November 2023
Protocol Phase	2/3
Protocol Title	A Phase 2/3 Study to Evaluate the Efficacy and Safety of Unesbulin in Unresectable or Metastatic, Relapsed or Refractory Leiomyosarcoma

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SIGNATURE PAGE

Sponsor's Approval

The protocol has been approved by PTC Therapeutics.

Responsible Medical Officers:

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Date

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The eSignature page is located on the last page.

INVESTIGATOR'S AGREEMENT

I have received and read the Investigator's Brochure for unesbulin (PTC596). I have read the PTC596-ONC-008-LMS protocol and agree to conduct the study as outlined. I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

_____
Printed Name of Investigator

_____
Signature of Investigator

_____
Date

SYNOPSIS

Name of Sponsor/Company: PTC Therapeutics, Inc.		
Name of Investigational Product: Unesbulin (PTC596)		
Name of Active Ingredient: 5-fluoro-2-(6-fluoro-2-methyl-1H-benzo[d]imidazole-1-yl)-N_4-(4-(trifluoromethyl)phenyl)pyrimidine-4,6-diamine		
Protocol Number: PTC596-ONC-008-LMS	**Phase:** 2/3	**Country:** Global
Title of Study: A Phase 2/3 Study to Evaluate the Efficacy and Safety of Unesbulin in Unresectable or Metastatic, Relapsed or Refractory Leiomyosarcoma		
Studied Period (years): The study period will be approximately 4 years.		

Objectives:

Primary:

- Progression-free survival (PFS) of unesbulin plus dacarbazine (DTIC) versus placebo plus DTIC

Secondary:

Efficacy:

- Overall survival (OS) of subjects treated with unesbulin plus DTIC versus placebo plus DTIC
- Antitumor activity of unesbulin plus DTIC versus placebo plus DTIC

Safety:

- Safety and tolerability of unesbulin plus DTIC versus placebo plus DTIC

Exploratory:

Efficacy:

- Evaluate the antitumor activity of unesbulin plus DTIC versus placebo plus DTIC in subjects with at least 4 prior lines of treatment

Pharmacokinetics (PK):

- Evaluate the PK of unesbulin in the presence of DTIC in subjects with leiomyosarcoma (LMS)
- Evaluate the PK of DTIC/5-amino-imidazole-4-carboxamide (AIC) alone and in the presence of unesbulin in subjects with LMS

Patient-reported outcomes (PROs):

- Compare the effect of unesbulin plus DTIC versus placebo plus DTIC on PROs, including health-related quality-of-life (HRQoL) assessments

Biomarkers:

- Measure genotype of subject tumors, assessed by blood sampling, at baseline and posttreatment

Endpoints:

Primary:

- PFS per Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 assessed by an independent central imaging laboratory

Secondary:

Efficacy:

The key secondary endpoint is OS.

Other secondary efficacy endpoints:

- Objective response rate (ORR; proportion of subjects with best overall response [BOR] of either complete response [CR] or partial response [PR])

- Disease control rate (DCR) or clinical benefit rate (CBR), defined as the proportion of subjects with BOR of CR, PR, or at least 3 months of stable disease (SD)
- Duration of response (DoR)

Safety:

- Vital signs, physical examination, electrocardiograms (ECG), laboratory abnormalities, Eastern Cooperative Oncology Group (ECOG) performance status (PS) scores, and adverse events (AEs)

Exploratory:

Efficacy:

- PFS, OS, ORR, DCR, and DoR in subjects with at least 4 prior lines of treatment

PK:

- PK parameters of unesbulin in subjects who receive unesbulin plus DTIC: C_{max}, T_{max}, and area under the concentration versus time curve from time zero to the last sampled time or the last non-zero concentration (AUC_{0-t})
- PK parameters of DTIC and its inactive metabolite AIC in subjects who receive unesbulin plus DTIC and placebo plus DTIC: C_{max}, T_{max}, and AUC_{0-t}

PROs:

- 30-item score European Organisation for the Research and Treatment of Cancer Quality-of-Life Questionnaire (EORTC QLQ-C30)
- EuroQol 5-level EQ-5D version (EQ-5D-5L)

Biomarkers:

- Genetic evaluation of the tumors by analyzing circulating cell-free tumor DNA (ccfDNA)

Study Design and Methodology:

This is an international, multicenter, randomized, double-blind, placebo-controlled, Phase 2/3 study to compare the safety and efficacy of unesbulin plus DTIC versus placebo plus DTIC in subjects with unresectable or metastatic, relapsed or refractory LMS who have received at least 1 prior line of systemic therapy.

Eligible subjects will be randomized 2:1 (unesbulin plus DTIC: placebo plus DTIC) to one of the following treatment groups:

- **Unesbulin plus DTIC**: Unesbulin 300 mg will be administered PO twice weekly (BIW) in each 3-week treatment cycle. DTIC 1000 mg/m^2 will be administered IV once every 21 days (Q21D).
- **Placebo plus DTIC**: Matching placebo will be administered PO BIW in each 3-week treatment cycle. DTIC 1000 mg/m^2 will be administered IV Q21D.

For approximately 300 subjects with 1 to 3 prior lines of treatment, randomization will be stratified as follows:

- Number of prior systemic therapies (1 or >1)
- ECOG PS score (0 or 1)
- Histological tumor type (uterine versus nonuterine LMS)

Forty-five subjects with at least 4 prior lines of treatment will be randomized and stratified as follows:

- ECOG PS score (0 or 1)
- Histological tumor type (uterine versus nonuterine LMS)

One treatment cycle will constitute 21 days. DTIC will be administered on Day 1 of each 3-week treatment cycle. Unesbulin/placebo tablets will be administered on Days 2 and 5 of Week 1 and Days 1 and 4 of Weeks 2 and 3 of each 3-week treatment cycle (Note: the preferable duration between 2 doses of unesbulin/placebo is approximately 72 hours).
Approximately 12 randomized subjects will be assessed for PK of unesbulin, DTIC, and AIC (an inactive metabolite of DTIC). For Cycles 1 and 3, rich PK sampling will occur on Day 1 for DTIC and AIC, and on Day 2 for unesbulin. The target is for approximately 7 PK evaluable subjects in the unesbulin group to complete Cycle 3. For Cycles 2 and 4, sparse PK sampling will occur on Day 1 for DTIC and AIC.
All subjects will receive treatment until evidence of disease progression, unacceptable toxicity, or other withdrawal criteria are met. No crossover will be permitted.
An independent Data Monitoring Committee (DMC) has been established and will review safety data as per the DMC charter. In addition, one interim efficacy analysis will be performed (by an external vendor) and reviewed by the DMC. Based on the results of this interim analysis, the DMC will make recommendations to either continue or stop the study. The DMC Charter (a separate document) and the DMC Statistical Analysis Plan (SAP) will provide detailed guidance for the conduct of this interim analysis.
Dose modifications/stopping criteria:
If treatment-related toxicities are observed, a stepped reduction in the dose of DTIC is permitted beginning with the starting dose of 1000 to 850 mg/m^2, followed by 700, 600, and 500 mg/m^2. The following guidelines should be observed:

- If a subject requires a dose reduction of DTIC to less than 500 mg/m^2, then DTIC should be discontinued.
- Subjects should receive all necessary supportive care including blood products, platelet transfusions, antiemetics, and antibiotics while being treated on this study.
- The use of granulocyte colony-stimulating factor (eg, pegfilgrastim) and erythropoietin or thrombopoietin-stimulating agents is permitted per institutional practice.

No dose reduction of unesbulin/placebo is permitted. Guidelines for unesbulin/placebo dosing when treatment-related toxicities continue beyond the stepwise dose reduction of DTIC is detailed in this protocol.

Sample Size Justification:
The sample size was computed based on the comparison of PFS between subjects randomized to unesbulin plus DTIC and subjects randomized to placebo plus DTIC.
The median PFS was assumed to be approximately 1.5 to 2.5 months for the placebo plus DTIC group and more than 4 months for the unesbulin plus DTIC group. With an overall one-sided alpha level of 0.025, a total of 245 events are required to achieve at least 90% power at a hazard ratio of 0.5. Assuming 19 months of enrollment time, a minimum follow-up period of 6 months for the primary endpoint, and approximately 11.5% uniform dropout rate, a total of 300 subjects are planned to be randomized.
One interim analysis will be performed when approximately 36% of the PFS events occur (approximately 88 events). The study may stop for both efficacy and futility. The study may stop for efficacy if the observed hazard ratio is less than 0.447, and the study may stop for futility if the observed hazard ratio is more than 0.67.
The alpha allocation will be controlled separately at the interim and the final analysis timepoints by using the Lan-DeMets spending function that approximates the

O'Brien-Fleming approach. The family-wise significance level is 0.025, with 0.000184 allocated at the interim analysis and 0.024816 allocated at the final analysis.
For the key secondary endpoint OS, sample size is based on detecting a hazard ratio of 0.625 (median OS of 10 months versus 16 months, for placebo plus DTIC vs unesbulin plus DTIC, respectively) and a minimum 15-month follow-up. With 184 events (among 265 subjects) a 1-sided 0.025 level log-rank test will have approximately 84% power. A separate group sequential design and monitoring will be performed for OS, with one interim analysis performed when at least 60% of the OS events occur and a final OS analysis when 184 events occur. The alpha allocation will be controlled by using the Lan-DeMets spending function that approximates the O'Brien-Fleming approach.
Data from the additional 45 subjects enrolled with at least 4 prior lines of therapy will not be included in the primary analysis; rather, the efficacy analysis of data from these subjects will be considered exploratory and included as a sensitivity analysis only.

Number of Subjects (Planned):
Randomized: Approximately 345 (300 subjects with 1 to 3 prior lines of systemic therapy for the primary analysis and 45 subjects with at least 4 prior lines of systemic therapy for the exploratory analysis)

Inclusion Criteria:

1. Subject is willing and able to provide informed consent
2. Willingness and ability to comply with scheduled visits, drug administration plan, laboratory tests, other study procedures, and study restrictions
3. Disease status including:
    a. Histological or cytological confirmation of LMS arising at any anatomic site except bone sarcoma
    b. Unresectable or metastatic, relapsed or refractory disease
    c. Measurable disease per RECIST 1.1 criteria
    d. Disease progression on previous treatment before screening or intolerability to other oncology treatments

Demographics:

4. Age ≥18 years
5. Male or female

Performance status:

6. ECOG PS score of 0 or 1

Hematopoietic:

7. Absolute neutrophil count ≥1500/mm^3 without the use of growth factors in the past 7 days
8. Platelet count ≥100000/mm^3 without platelet transfusion in the past 14 days
9. Hemoglobin ≥9 g/dL (packed red blood cell transfusion is not allowed within 7 days)

Hepatic:

10. Bilirubin ≤ upper limit of normal (ULN) except for those patients with Gilbert's syndrome
11. Aspartate aminotransferase and alanine aminotransferase <3 times the ULN
12. Subjects with liver metastases may be enrolled

Pulmonary:

13. Subjects with well-controlled asthma (eg, use of rescue medications <2 times per week over the last 12 months) or chronic obstructive pulmonary disease (eg, no exacerbations over the prior 3 months) may be enrolled.

Renal:

14. Creatinine <1.5 times normal OR creatinine clearance ≥ 60 mL/min

Prior therapeutics:

15. Toxicity from prior therapies recovered to Grade ≤1 or subject's baseline, except for alopecia. In addition, endocrinopathies associated with prior immunotherapy-based treatments that are well controlled on replacement medication are not exclusionary.

Chemotherapy and targeted therapy:

16. At least 1 prior systemic cytotoxic or targeted therapy regimen for LMS, which may include but is not limited to single-agent doxorubicin or other anthracycline, doxorubicin plus ifosfamide, trabectedin, pazopanib, or gemcitabine with or without docetaxel

Surgery:

17. At least 4 weeks since prior surgery and recovered in the opinion of investigator

Other:

18. Capable of swallowing oral medication
19. Women of childbearing potential (WOCBP; as defined by the Clinical Trials Facilitation and Coordination Group [CTFG]) must have a negative serum pregnancy test at screening and agree to abstinence or the use at least one of the following highly effective forms of contraception (with a failure rate of <1% per year when used consistently and correctly) (Clinical Trials Facilitation and Coordination Group 2020). Contraception or abstinence must be continued for the duration of the study and for at least 6 months after the last dose of study drug:
    - Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
        - Oral
        - Intravaginal
        - Transdermal
    - Progestogen-only hormonal contraception associated with inhibition of ovulation:
        - Oral
        - Injectable
        - Implantable
    - Intrauterine device
    - Intrauterine hormone-releasing system
    - Bilateral tubal occlusion
    - Vasectomized partner with confirmed azoospermia

    All females will be considered of childbearing potential unless they are postmenopausal (at least 12 months consecutive amenorrhea in the appropriate age group without other known or suspected cause) or have been sterilized surgically (eg, bilateral salpingectomy, hysterectomy, bilateral oophorectomy).
20. Lactating females are not eligible unless they have agreed not to breastfeed their infants during treatment and for a period of 1 month following completion of treatment.
21. Males who are sexually active with WOCBP who have not had a vasectomy must agree to use a barrier method of birth control from the start of study drug administration through at least 6 months after the last dose of study drug. Males should not donate sperm from the start of study treatment through at least 6 months after the last dose of study drug).

Exclusion Criteria:

1. Received temozolomide or DTIC at any time
2. Any other systemic anticancer therapy including investigational agents ≤3 weeks before initiation of study treatment. Additionally, subjects may not have received radiation ≤3 weeks before initiation of study treatment.
3. Known intolerance to DTIC or one or more of the excipients in unesbulin.
4. Co-existing active infection or any co-existing medical condition likely to interfere with study procedures, including:
    a. Significant cardiovascular disease (New York Heart Association Class III or IV cardiac disease), myocardial infarction within the past 6 months, unstable angina, congestive heart failure requiring therapy, unstable arrhythmia or a need for antiarrhythmic therapy, or evidence of ischemia on ECG, marked baseline prolongation of QT/QTc (corrected QT) interval, eg, repeated demonstration of a QTc interval >500 msec (Long QT Syndrome [congenital])
5. Human immunodeficiency virus, hepatitis B virus, or hepatitis C virus positivity
6. History of solid organ transplantation

Therapeutics:

7. Known or suspected allergy or immediate or delayed hypersensitivity to unesbulin or DTIC, their excipients, or any agent given in this study

Gastrointestinal:

8. Bowel obstruction, malabsorption, or other contraindication to oral medication
9. Gastrointestinal disease or other conditions that could affect absorption. Active peptic ulcer disease, active gastritis, or previous history of gastric perforation within the last 2 years
10. Inflammatory bowel disease (including ulcerative colitis and Crohn's disease), diverticulitis, cholecystitis, symptomatic cholangitis, or appendicitis

Wounds/surgery:

11. Serious non-healing wound, ulcer, or bone fractures
12. Major surgery, open biopsy, or significant traumatic injury that has not recovered, in the opinion of the investigator, within 28 days of baseline
13. Mucosal or internal bleeding

Concomitant medications:

14. Concomitant strong CYP1A2 inhibitors (such as fluoroquinolones [broad spectrum quinolone antibiotics, including enoxacin and ciprofloxacin] and selective serotonin reuptake inhibitor [SSRI] agents fluvoxamine and fluoxetine) should be avoided on the same day that DTIC or unesbulin/placebo is administered. CYP1A2 inhibitors may inhibit the conversion of DTIC to its active metabolite and may increase the exposure of unesbulin.
15. Concomitant use of moderate CYP1A2 inducers (such as phenytoin, rifampin, ritonavir, teriflunomide, and barbiturates). Chronic use of marijuana should be avoided, but irregular recreational use may be permitted at the discretion of the treating investigator. CYP1A2 inducers may increase the conversion of DTIC to its active metabolites.
16. Coadministration of acid-reducing agents should be avoided approximately 4 hours before and after unesbulin/placebo administration.

17. Immunization with a live vaccine within 30 days before starting study drug due to the risk of serious and life-threatening infections.

Other:

18. Prior malignancies, other than LMS, that required treatment or have shown evidence of recurrence (except for non-melanoma skin cancer, adequately treated cervical carcinoma in situ, prostate cancer in situ or any other low risk malignancy that is approved by the medical monitor) during the 5 years before initiation. Cancer treated with curative intent more than 5 years previously and without evidence of recurrence is not an exclusion.
19. Known coagulopathy or bleeding diathesis. Subjects on anticoagulation should be monitored closely and International Normalized Ratio and/or activated partial thromboplastin time (APTT)/prothrombin time (PT) should be within the required range where applicable.
20. Prior or ongoing clinically significant illness, medical or psychiatric condition, medical history, physical findings, ECG findings, or laboratory abnormality that, in the investigator's opinion, could affect the safety of the subject, or alter the absorption, distribution, metabolism, or excretion of the study drugs, or could impair the assessment of study results.
21. History of brain metastases or leptomeningeal disease at any time in subject's history, including treated central nervous system (CNS) disease

Investigational product, dosage, and mode of administration:

Unesbulin tablets for oral administration will be provided in strengths of 50 and 200 mg. Subjects will be treated with unesbulin 300 mg PO BIW in combination with DTIC administered IV Q21D. One treatment cycle will constitute 21 days. Unesbulin is to be administered on Days 2 and 5 of Week 1 and on Days 1 and 4 of Weeks 2 and 3.

Matching placebo tablets will be provided and should be administered on the same schedule as unesbulin.

Based on the results from a food effect assessment in the ongoing Phase 1b Study PTC596-ONC-007-LMS, it is recommended that unesbulin/placebo be taken with food.

Duration of treatment:

Treatment will continue for each subject until evidence of disease progression, unacceptable toxicity, or any of the following reasons: withdrawal of consent by subject, pregnancy, significant noncompliance, withdrawal by investigator, or study discontinuation by PTC or regulatory authority.

Reference therapy, dosage, and mode of administration:
DTIC for injection will be provided (by the site or by the sponsor) and will be reconstituted with Sterile Water for Injection, USP. The resulting solution contains 10 mg/mL of DTIC having a pH of 3.0 to 4.0. The reconstitution step may be modified based on institutional policy and or the availability of a specific strength of the DTIC vial to obtain a resulting DTIC solution of 10 mg/mL. The calculated dose of the resulting solution is drawn into a syringe. The reconstituted solution is further diluted with 5% Dextrose Injection, USP or 0.9% Sodium Chloride injection and administered as an IV infusion over 1 hour ±30 minutes. Within 30 minutes before DTIC administration, the following premedication regimen is recommended: fosaprepitant 150 mg IV once, dexamethasone 20 mg PO once, and ondansetron 16 mg PO once. The premedication regimen as per institutional practice is allowed.
Placebo tablets will be supplied and administered in a blinded manner to subjects BIW in combination with DTIC per the same dosing schedule as for unesbulin.

Criteria for Evaluation:
Efficacy: Response assessments will be performed by radiological imaging using RECIST 1.1 criteria every 6 weeks (±7 days) until disease progression. After Cycle 8, scans may be performed every 9 weeks. If any subject responds with a CR or PR at any assessment, then the subsequent confirmatory scan should be performed 4 weeks (+5 days) after the previous scan where the objective response was first evidenced. Assessment of response and the primary endpoint, PFS, will be performed by an independent central reader who will be blinded to treatment assignment.
Subjects should be followed with the same imaging procedure throughout the study. Computed tomography scans or magnetic resonance imaging may be used as clinically indicated. If the subject withdraws for reasons other than tumor progression, an end-of-treatment (EOT) scan should be performed if the last scan was ≥3 weeks earlier.
Survival follow-up will continue every 3 months until the later of 184 events or 2 years after the last subject is randomized.
PRO: HRQoL will be assessed using the EORTC QLQ-C30 and the EQ-5D-5L.
PK: For the subset of subjects who provide consent, blood samples per PK sample interval will be collected for the determination of DTIC, AIC, and unesbulin. PK sample collection, processing, storage, and shipment will be performed according to instructions outlined in the laboratory manual separate from this protocol. The collected PK samples may also be used for further exploratory analysis of unesbulin and DTIC metabolism.
Safety: Subjects will be monitored closely for AEs and laboratory abnormalities during the study. Safety assessments will include vital signs, physical examination, electrocardiograms, laboratory values, ECOG PS scores, and AEs.
Biomarkers: Blood samples (10 mL) will be collected at screening (predose) and prior to dosing on Day 1 of Cycle 3 and at the time of disease progression or EOT for all subjects and analyzed for exploratory circulating soluble biomarkers, and genetic evaluation of tumors will be performed.

Statistical Methods:

The primary objective of this study is to compare PFS of unesbulin plus DTIC versus placebo plus DTIC per RECIST 1.1.

Efficacy: All hypothesis testing will be one-sided based on a significance level of 0.025.

For subjects with 1 to 3 prior lines of treatment, the distribution of PFS will be compared in the 2 randomized groups via log-rank test stratified by prior lines of therapy (1 versus >1), ECOG PS score (0 versus 1), and LMS origin (uterine versus nonuterine). The hazard ratio and the corresponding 95% CI will be estimated in a stratified Cox proportional hazards model using randomized group as the single covariate. The PFS curves for each randomized group will be estimated using the Kaplan-Meier (KM) product-limit method. Two-sided, 95% CIs for median PFS will be computed by Brookmeyer and Crowley method (using log-log transformation). PFS rates at 3, 6, 12, 18, 24, and 30 months will also be estimated using KM estimates on the PFS curve for each randomized group. Associated two-sided 95% CIs will be calculated using the Greenwood formula.

OS will be compared between the 2 treatment groups. Two analyses are planned for OS: the first (interim) at the time of the final analysis for PFS (provided PFS is significant), at which point the information fraction for OS will be at least 60% and a second (final) analysis when 184 events have accumulated. The same statistical models applied to the analysis of PFS will be applied to the analysis of OS.

BOR will be summarized by response category for each treatment group. ORR will be computed in each treatment group along with the exact 95% CI using Clopper-Pearson method. An estimate of the difference in ORRs and corresponding 95% CI will be calculated using Cochran-Mantel-Haenszel methodology and adjusted by the same stratification factors as in primary analysis of PFS.

For subjects with at least 4 prior lines of treatment, a sensitivity analysis will be performed to explore the efficacy for this population.

PK: PK of unesbulin, DTIC, and AIC will be analyzed using standard compartmental or noncompartmental analysis methods, as appropriate for the data. The impact of unesbulin on the PK of DTIC and AIC will be investigated.

Safety: The safety analysis will be performed for all treated subjects. Descriptive statistics of safety will be presented using National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events Version 5.0 (CTCAE V5.0) by treatment group. All treatment-emergent AEs will be tabulated by system organ class and Medical Dictionary for Regulatory Affairs (MedDRA) preferred term. Laboratory abnormalities, including hematology and chemistry, will be summarized using worst grade per NCI CTCAE V5.0 criteria.

SCHEDULE OF ASSESSMENTS

Table 1: Schedule of Study Assessments and Procedures

Assessment	**Screening**	**Cycle 1**							**Cycle 2**							**Cycle 3 +**							**EOT[a]**	**FUP[a]**	**sFUP[a]**
Study Week		**1**			**2**		**3**		**1**			**2**		**3**		**1**			**2**		**3**				
Cycle Day	**-28 to -1**	1	2	5	8	11	15	18	1	2	5	8	11	15	18	1	2	5	8	11	15	18			
Time Window[b]	**-**	2	1	1	2	1	2	1	2	1	1	2	1	2	1	2	1	1	2	1	2	1	1	3	3
Informed consent	X																								
Eligibility	X																								
Medical history	X																								
Con. meds[c]	X	Throughout study																						X	
Pathology review[d]	X																								
Physical exam[e]	X	X							X							X							X		
Height	X																								
Weight[f]	X	X							X							X							X		
Vital signs	X	X							X							X							X		
ECOG PS	X	X							X							X							X		
Adverse events	X	Throughout study																						X	
Laboratory tests[g]	X	X			X		X		X			X		X		X			X		X		X		
HIV, hepatitis serologies	X																								
Urine/serum pregnancy test[h]	X	X[h]							X[h]							X[h]									
Urinalysis	X																						X		
ECG (single) for subjects not in the PK assessment[i]	X	X							X							X							X		
ECG (single) for subjects in the PK assessment[j]	X	X	X						X							X	X								
Tumor imaging[K]	X															X							X		
Survival		Throughout study																							X
PROs[l]	X	X							X							X							X		
Biomarker sampling[m]	X															X							X		
Blood for PK[n]		X	X						X							X	X								
Dispense unesbulin/placebo		X							X							X									
Study drug accountability[o]					X		X		X			X		X		X			X		X		X		
DTIC (IV)[p]		X							X							X									

Assessment	**Screening**	**Cycle 1**							**Cycle 2**							**Cycle 3 +**							**EOT[a]**	**FUP[a]**	**sFUP[a]**
Study Week		**1**			**2**		**3**		**1**			**2**		**3**		**1**			**2**		**3**				
Cycle Day	**-28 to -1**	1	2	5	8	11	15	18	1	2	5	8	11	15	18	1	2	5	8	11	15	18			
Time Window[b]	**-**	2	1	1	2	1	2	1	2	1	1	2	1	2	1	2	1	1	2	1	2	1	1	3	3
Unesbulin or placebo (PO) in clinic or home[q]			X	X	X	X	X	X		X	X	X	X	X	X		X	X	X	X	X	X			

Abbreviations: AE, adverse events; AIC, 5-amino-imidazole-4-carboxamide; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CBC, complete blood count; CMP, complete metabolic panel; Con., concomitant; DTIC, dacarbazine; ECG, electrocardiogram; ECOG, Eastern Cooperative Oncology Group; EOI, end of infusion; EORTC QLQ-C30, 30-item score European Organisation for the Research and Treatment of Cancer Quality-of-Life Questionnaire; EOT, end-of-treatment; EQ-5D-5L, EuroQol 5-level EQ-5D version; FUP, follow-up; OS, overall survival; PK, pharmacokinetics; PROs, patient-reported outcomes; PS, performance status; RBC, red blood cell; SAE, serious adverse event; sFUP, survival follow-up; SOI, start of infusion; WBC, white blood count

[a] The EOT visit should be performed within 30 days after the end of treatment or until recovery from or stabilization of the AE, whichever occurs latest. The EOT imaging should be performed if the last scan was ≥3 weeks earlier. For subjects requiring immediate initiation of a new treatment option, the EOT visit may be completed earlier. A safety FUP assessment should be performed by phone at 30 days (±3 days) after the EOT to assess AEs and concomitant medications. sFUP assessments should be performed by phone every 3 months (±7 days) after the EOT until the later of 184 events or 2 years after the last subject has been randomized.

[b] Specified time window is ± days. Unesbulin/placebo should not be dosed on consecutive days, and preferably at least 3 days (72 hours) between each unesbulin/placebo dose. The window for completion of the activities associated with Cycle 1 Day 1 and Cycle 2 Day 1 may be divided between a 2-day span.

[c] Concomitant medications involve a complete medication reconciliation, including medications used for tolerability (eg, antiemetics and antidiarrheals).

[d] Pathology review to confirm the histologic diagnosis of leiomyosarcoma is required. An outside pathology report documenting leiomyosarcoma is acceptable.

[e] Full physical examination should be done at screening and EOT visits. Symptom-directed physical examination can be done at all other timepoints.

[f] Assess weight on the day of DTIC administration; modify dose of DTIC if >10% change from baseline (or the last time the dose was changed).

[g] Local laboratory testing is acceptable. Screening laboratory assessments and urinalysis must be collected within 3 weeks of Cycle 1 Day 1. Cycle 1 Day 1 laboratory assessments may be collected up to 24 hours before the first dose, but this window may be extended up to 72 hours for sites if it is impacted by laboratory working hours. For subsequent cycles, laboratory assessments may be collected up to 48 hours before dosing. On Day 1 Week 1 of each cycle, all laboratory assessments should be performed. On Day 1 of Weeks 2 and 3 of Cycles 1, 2, and 3, a CBC and CMP should be assessed for each subject. After Cycle 3, clinical laboratory assessments are required only once every cycle instead of on a weekly basis. If DTIC is held due to toxicity (see Section 3.3), then laboratory assessments should be completed while dosing with unesbulin/placebo continues. If DTIC is interrupted or held, then laboratory assessments should be done within 24 hours of resuming dosing with DTIC and continue on a weekly basis for the first 3 weeks after DTIC reintroduction. For subsequent cycles, the clinical laboratory assessments may be performed at a minimum of once per cycle or more frequently per the investigator's discretion to ensure that the subject meets the protocol-defined safety criteria in Section 3.3.

Laboratory tests include: (1) CBC (hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration), WBC count, WBC differential (may be automated or manual as per institutional standards), RBC count, lymphocytes, monocytes, neutrophils, band neutrophils (if reported), eosinophils, basophils, platelets; and (2) CMP: albumin, alkaline phosphatase, total bilirubin, bicarbonate, blood urea nitrogen, calcium, chloride, creatinine, glucose, potassium, total protein, AST, ALT, and sodium.

[h] **For women of childbearing potential, a serum pregnancy test should be performed at screening (with a window of 7 days) and a urine pregnancy test should be performed within 24 hours of Day 1 of Cycle 1. A urine or serum pregnancy test should be performed within 24 hours prior to dosing on Day 1 of Cycle 2 and continuing every 2 cycles beginning at Cycle 4.**

[i] **For subjects not participating in the PK assessment**, a single ECG should be collected on Day 1 of the first 3 treatment cycles only, approximately 2 hours after DTIC infusion. If DTIC is interrupted, then the single ECG should be collected approximately 2 hours after DTIC infusion and recorded on an unscheduled case report form page.

[j] **For subjects participating in the PK assessment**, a single ECG should be taken as follows:

- Predose and 1.5, 4, and 8 hours postdose on Day 1 of Cycles 1 and 3 concurrent with intensive PK sampling for DTIC/AIC.

- Predose and 2, 4, 6, and 8 hours postdose on Day 2 of Cycles 1 and 3 concurrent with intensive PK sampling for unesbulin.
- Approximately 2 hours after DTIC infusion on Cycle 2.

[k] Pretreatment, baseline imaging should be performed ≤14 days before Cycle 1 Day 1. On-treatment imaging should be performed every 6 weeks (±7 days). After Cycle 8, scans may be performed every 9 weeks. If any subject responds with a CR or PR at any assessment, then the subsequent confirmatory scan should be performed 4 weeks (+5 days) after the previous scan where the objective response was first evidenced. If the subject withdraws from the study for reasons other than progression of disease, EOT imaging should be performed if the last scan was ≥3 weeks earlier. Subjects should be followed with the same imaging procedure throughout the study. Computed tomography scans or magnetic resonance imaging may be used as clinically indicated.

[l] PROs include EORTC QLQ-C30 and EQ-5D-5L.

[m] Blood samples (10 mL) to assess circulating biomarkers will be collected at screening (predose) and prior to dosing on Day 1 of Cycle 3 and at the time of disease progression or EOT. Samples will be processed, stored, and shipped according to instructions in the laboratory manual.

[n] For subjects participating in the PK assessments:

- **PK for DTIC/AIC**: On Day 1 of Cycles 1 and 3, blood samples will be collected at predose, 0.5, 1.0 (within 10 minutes before EOI), 1.25 (approximately 15 minutes post EOI), 1.5, 2, 4, 8, 10, and 24 hours postdose (SOI). On Day 1 of Cycles 2 and 4, sparse blood samples will be collected at predose, 1.25, and 4 hours postdose (SOI). If DTIC is interrupted or held in Cycle 3, then the same intensive PK sampling schedule for DTIC should be followed on the day that DTIC resumes in the subsequent cycle. For PK assessments, DTIC must be administered the day before unesbulin/placebo dosing. DTIC should not be administered on the same day as unesbulin/placebo.
- **PK for unesbulin**: On Day 2 of Cycles 1 and 3, blood samples will be collected at predose, 1, 2, 3, 4, 6, 8, 10, and 24 hours postdose. PK sample collection, processing, storage, and shipment will be performed according to instructions outlined in the laboratory manual separate from this protocol. If DTIC is interrupted or held in Cycle 3, then the same intensive PK sampling schedule for unesbulin/placebo should be followed on the day after DTIC resumes in the subsequent cycle. For PK assessments, DTIC must be administered the day before unesbulin/placebo dosing. DTIC should not be administered on the same day as unesbulin/placebo.
- The collected PK samples may also be used for further exploratory analysis of unesbulin and DTIC metabolism.

[o] Subject medication diary is given to subjects to record unesbulin/placebo dosing at home and will be provided at Day 1 of each cycle or at other visits, as needed, to ensure compliance. Subject should bring the diary back at all scheduled visits for review with the research staff to assess treatment compliance.

[p] Within 30 minutes before DTIC administration, the following premedication regimen is recommended: fosaprepitant 150 mg IV once, dexamethasone 20 mg PO once, and ondansetron 16 mg PO once. The premedication regimen as per institutional practice is allowed. Please refer to Section 3.3 for instructions on DTIC interruptions and dose modifications due to toxicity.

[q] For subjects participating in the PK assessment, unesbulin/placebo will be administered at the clinic on the day of intensive PK sampling (Day 2 of Cycle 1 and Cycle 3 except in cases of DTIC interruption) and at home on other scheduled unesbulin/placebo dosing days. For all other subjects (ie, those not in the PK assessment), unesbulin/placebo should be taken by the subject at home.

Note: Treatment cycles will repeat until the subject meets the criteria for withdrawal from the study. All windows refer to business days (exclude weekends and holidays). Activities designed to be completed in a single day, such as PK sampling from before dosing to hour 8 postdose, may not be split up between the 2 days of a window.

If DTIC is interrupted and toxicity resolves as per criteria in Section 3.3, then clinical laboratory (ie, CBC and CMP) and body weight assessments must be performed within 24 hours prior to DTIC reintroduction. A single ECG must also be performed approximately 2 hours after reintroduction of DTIC if the interruption occurs within the first 3 treatment cycles. If DTIC is interrupted or held in Cycle 3, then the same intensive PK sampling schedule should be followed upon reintroduction of DTIC as described in footnote “n”.

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or Specialist Term	Explanation
AE	Adverse event
AIC	5-amino-imidazole-4-carboxamide
ALT	Alanine aminotransferase
AME	Absorption, metabolism, and excretion
ANC	Absolute neutrophil count
APTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
AUC	Area under the concentration-time curve
AUC_{0-t}	Area under the concentration versus time curve from time zero to the last sampled time or the last non-zero concentration
BCRP	Breast cancer resistance protein
BIW	Twice weekly
BOR	Best overall response
CBC	Complete blood count
CBR	Clinical benefit rate
ccfDNA	Circulating cell-free tumor DNA
CL	Clearance
CMP	Complete metabolic panel
CNS	Central nervous system
CR	Complete response
CTCAE V5.0	Common Terminology Criteria for Adverse Events Version 5.0
CTFG	Clinical Trials Facilitation and Coordination Group
DCR	Disease control rate
DMC	Data Monitoring Committee
DoR	Duration of response
DTIC	Dacarbazine
ECG	Electrocardiogram
eCRF	Electronic case report form
ECOG	Eastern Cooperative Oncology Group
EOI	End of infusion
EORTC QLQ-C30	30-item score European Organisation for the Research and Treatment of Cancer Quality-of-Life Questionnaire
EOT	End-of-treatment
EQ-5D-5L	EuroQol 5-level EQ-5D version
FUP	Follow-up
GCP	Good Clinical Practice
HRQoL	Health-related quality-of-life
ICF	Informed consent form
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ITT	Intent-to-treat
KM	Kaplan-Meier
LLN	Lower limit of normal
LMS	Leiomyosarcoma
MedDRA	Medical Dictionary for Regulatory Affairs
mITT	Modified Intent-to-treat
NCI	National Cancer Institute
OATP1B1, OATP1B2	Organic anion transporter polypeptide transporters
ORR	Objective response rate
OS	Overall survival

Abbreviation or Specialist Term	Explanation
PFS	Progression-free survival
PLT	Platelets
P-gp	P-glycoprotein
PK	Pharmacokinetic/s
PR	Partial response
PRO	Patient-reported outcome
PS	Performance status
PT	Prothrombin time
Q21D	Once every 21 days
RECIST	Response Evaluation Criteria in Solid Tumors
RP2D	Recommended Phase 2 dose
RSI	Reference Safety Information
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Stable disease
SEER	Surveillance, Epidemiology, and End Results
sFUP	Survival follow-up
SOI	Start of infusion
SOP	Standard operating procedures
SSRI	Selective serotonin reuptake inhibitor
STS	Soft-tissue sarcoma
SUSAR	Suspected unexpected serious adverse reaction
TEAE	Treatment-emergent adverse events
ULN	Upper limit of normal
Vz	Volume of distribution
WBC	White blood cell
WOCBP	Women of childbearing potential

1. INTRODUCTION

1.1. Disease Background

Leiomyosarcoma (LMS) is a common type of soft-tissue sarcoma (STS), comprising 10% to 28% of all STS (Toro 2006, Ducimetière 2011, Ferrari 2011, Friedman 2018, Nagar 2018, Parikh 2018, Saltus 2018, Bessen 2019). In 2021, it is estimated that there will be 13460 new cases of soft-tissue cancer, and an estimated 5350 people will die of this disease (Howlader 2021). The 5- and 10-year trends in incidence of new STS have been slowly increasing (<1%) for both sexes. Death rates have been rising at an average of 0.1% each year from 2010 through 2018. The estimated 5-year survival of all STS is 65% based on National Cancer Institute (NCI) Surveillance, Epidemiology, and End Results data collected from 2000 to 2017 (Howlader 2021).

The underlying etiology of LMS is poorly understood. These cancers of mesenchymal origin can arise from any site in the body with the most common locations including the abdomen, retroperitoneum, large blood vessels, and uterus (Gladdy 2013). These tumors display nuclear atypia and mitotic activity and are characterized by complex cytogenetic and molecular changes (Gibault 2011, Miettinen 2014, Guo 2015). The Cancer Genome Atlas project reported low mutational burden in LMS compared with other tumors (Cancer Genome Atlas Research Network 2017).

LMS is one of the more aggressive sarcoma subtypes, with the highest risk of recurrence and decreased disease-specific survival (Pisters 1996, Svarvar 2007, Wang 2011, Gladdy 2013, Miettinen 2014, Worhunsky 2015, George 2018). The best chance for a cure in localized LMS is complete surgical resection, but complete resection at the time of diagnosis may be limited by anatomical constraints, and recurrence is common (George 2018).

There has been little progress in the treatment of unresectable or metastatic, relapsed or refractory LMS. Due to the low mutational burden and lack of driver mutations in LMS (Cancer Genome Atlas Research Network 2017), the disease has not been amenable to treatment with immunological or targeted therapies. First-line treatments include the combinations of cytotoxic agents doxorubicin (with or without ifosfamide) and gemcitabine with docetaxel (Hensley 2008, Ratan 2016, In 2017, George 2018).

Several chemotherapeutic agents and regimens have shown efficacy and are recommended in unresectable or metastatic, relapsed or refractory LMS as second-line treatment or later, including dacarbazine (DTIC), gemcitabine with docetaxel, trabectedin, and pazopanib (Ratan 2016, George 2018). The cytotoxic agent trabectedin and the angiogenic receptor tyrosine kinase inhibitor pazopanib were approved for treatment of LMS in the second- or later-line setting and provide a progression-free survival (PFS) benefit of 4.2 to 4.6 months with no demonstrated improvement in overall survival (OS) (Van Der Graaf 2012, Demetri 2016). Due to the low mutational burden and lack of driver mutations in LMS (Cancer Genome Atlas Research Network 2017), the disease has not been amenable to treatment with immunological or targeted therapies.

Thus, the very modest improvements in PFS and lack of improvement in OS by these approved products suggest there remains an urgent and unmet medical need for new therapeutic options in LMS. There is no uniform consensus for the standard of care recommended for second-line therapy or beyond in STS (NCCN 2021). Current guidelines suggest that choice of treatment should be based on histology and molecular subtype, as well as patient characteristics (In 2017, Gronchi 2021). Participation in a clinical trial is strongly recommended by the National Comprehensive Cancer Network (NCCN 2020).

1.2. Study Rationale

Unesbulin is an investigational, orally bioavailable, novel small molecule being developed by PTC Therapeutics (PTC) for the treatment of LMS. Unesbulin binds to a unique site within the colchicine-binding site of tubulin, which is considered an important target for destabilization of tubulin polymerization and microtubule formation (Lu 2012, Jernigan 2021). By inhibiting microtubule formation, unesbulin arrests tumor cells, including cancer stem cells, in G2/M phase and induces tumor cell apoptosis. Microtubule-targeting agents have been successfully used for years as tumoricidal agents. However, their use has been limited by broad toxicities and poor pharmacologic properties until recent approvals of eribulin and nab-paclitaxel, both of which are administered intravenously and are associated with risk of peripheral neurotoxicity, but neither of which are approved for LMS (Kawai 2017).

Unesbulin is administered orally and demonstrates attractive pharmacological properties and a favorable pharmacokinetic (PK) profile, including a long circulating half-life, effective biodistribution, and lack of P-glycoprotein (P-gp) substrate activity. P-gp is a promiscuous drug efflux pump, and elevated P-gp expression in cancer cells has been linked to reduced chemotherapeutic responses and poor clinical outcome in various cancer types including both blood cancers and solid tumors (Zhang 2018). Many commonly used anticancer drug classes such as taxanes (paclitaxel), vinca alkaloids (vinblastine), and anthracyclines (doxorubicin) are P-gp substrates and suffer from this limitation. Since unesbulin also crosses the blood-brain barrier, it is an attractive candidate for many oncological indications, including neuro-oncology. Furthermore, while most tubulin binding agents have been associated clinically with peripheral neuropathy (Kawai 2017), there has been no evidence of peripheral neuropathy with unesbulin.

The antitumor efficacy of unesbulin was evaluated in vivo in studies utilizing xenograft models of LMS. Unesbulin as a single agent and in combination with other standard-of-care anticancer agents, including DTIC, has demonstrated efficacy in rodent models of LMS. Notably, in a study where the efficacy of unesbulin was evaluated as monotherapy and in combination with DTIC using an SK-LMS-1 mouse model of LMS, the combination of unesbulin and DTIC was markedly more effective than either agent alone. The combination of unesbulin (through Day 98) and DTIC (21 mg/kg intraperitoneal once daily for 5 days) was highly effective in suppressing tumor growth. At Day 165 (when the study ended), tumors were not visible in 5 of the original 8 mice that received the combination treatment, whereas mice treated with unesbulin or DTIC alone showed progressive tumor growth.

Based on strong nonclinical data for the unesbulin and DTIC combination, along with an expected favorable toxicity profile for the combination, PTC initiated an ongoing, Phase 1b study of unesbulin in combination with DTIC for the treatment of unresectable or metastatic, relapsed or refractory LMS (Study PTC596-ONC-007-LMS). The primary objectives of the study are to determine the maximum tolerated dose, recommended Phase 2 dose (RP2D), and safety of unesbulin in combination with DTIC. Subjects are treated with unesbulin orally twice weekly (BIW) in combination with DTIC administered IV once every 21 days (Q21D).

Preliminary results for the 18 subjects exposed to the combination therapy of unesbulin at various dose levels with DTIC in Study PTC596-ONC-007-LMS demonstrated that the 300 mg BIW dose exhibited favorable efficacy and safety profiles with manageable toxicity compared with the 400 and 200 mg dose levels. Most cases of Grade 3-4 neutropenia and thrombocytopenia resolved within days without the need for treatment discontinuation or administration of growth factors or immunostimulants.

Together with the available nonclinical and PK data from the unesbulin clinical studies, PTC considers the dose of 300 mg unesbulin BIW in combination with DTIC to be the appropriate RP2D.

In addition to Study PTC596-ONC-007-LMS, an initial Phase 1 study (PTC596-ONC-001-AST) in subjects with advanced solid tumors was completed, and 2 investigator-initiated Phase 1b studies, PTC596-ONC-004-OVA (subjects with newly diagnosed ovarian cancer) and PTC596-ONC-006-DPG (pediatric subjects with diffuse interstitial pontine glioma or high-grade glioma), are ongoing.

For similar reasons in the design of Study PTC596-ONC-007-LMS, PTC considers DTIC to be the most appropriate comparator for the intended population in Study PTC596-ONC-008-LMS. DTIC induces cytotoxicity by acting as an alkylating agent. It is a widely available generic drug that is often used as monotherapy or in combination with other therapies in advanced STS. DTIC as a monotherapy can achieve a PFS of 1.5 to 2.6 months (Hensley 2017, Blay 2019), and it is an acceptable choice to consider in the refractory setting for LMS because it is relatively well tolerated and given on a convenient schedule. Additionally, there is existing precedent for DTIC use as a single-agent comparator in the development of new treatments for LMS. During the clinical development of trabectedin for LMS, single-agent DTIC was used as the comparator arm during randomized Phase 2 and 3 studies (Demetri 2016, Patel 2019).

Additional information, including a description of unesbulin's chemistry, pharmacology, safety, and preliminary antitumor activity in models of LMS is provided in the current version of the Investigator's Brochure (IB).

1.3. Risk/Benefit Assessment

The risk/benefit assessment of the addition of unesbulin to DTIC is driven by the unmet medical need associated with unresectable or metastatic, relapsed or refractory LMS.

A Phase 1b study of unesbulin in combination with DTIC was initiated based on the compelling preclinical data for the unesbulin and DTIC combination, an expected favorable toxicity profile for the combination, noting the need for novel therapies in this disease, and specifically for an improvement over DTIC's activity as monotherapy. The preliminary clinical data from Study PTC596-ONC-007-LMS are consistent with the promising nonclinical data and support the continuous development of unesbulin in combination with DTIC. DTIC was well tolerated in combination with unesbulin. The most common treatment-related Grade 3 or 4 treatment-emergent adverse events (TEAEs) were associated with myelosuppression. These events occurred less frequently among subjects treated with unesbulin 300 mg as compared with unesbulin 400 mg, which further supports the selection of the unesbulin 300 mg dose. Most cases of Grade 3-4 neutropenia and thrombocytopenia resolved within days without the need for treatment discontinuation or administration of growth factors or immunostimulants. The observed adverse events (AEs) are expected to be manageable with regular clinical and laboratory monitoring, as with other cytotoxic therapies.

The purpose of this Phase 2/3 study is to compare the efficacy and safety of unesbulin 300 mg plus DTIC versus placebo plus DTIC in subjects with unresectable or metastatic, relapsed or refractory LMS who have received at least 1 prior line of systemic therapy. To mitigate potential study drug-related toxicities, dose adjustment guidelines and stopping criteria are included. The study will be conducted in compliance with the protocol, Good Clinical Practice (GCP), and applicable regulatory requirements.

Additional information and guidelines to mitigate potential risks of unesbulin, such as drug-drug interactions, are included in the IB.

In consideration of the limited treatment options and unmet medical need for patients with advanced LMS, PTC believes unesbulin in combination with DTIC provides an acceptable benefit-risk profile for continued development in patients with unresectable or metastatic, relapsed or refractory LMS.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1. Primary Objective

The primary objective is to assess PFS of unesbulin plus DTIC versus placebo plus DTIC.

2.2. Secondary Objectives

- Evaluate OS of subjects treated with unesbulin plus DTIC versus placebo plus DTIC
- Evaluate the antitumor activity of unesbulin plus DTIC versus placebo plus DTIC
- Evaluate safety and tolerability of unesbulin plus DTIC versus placebo plus DTIC

2.3. Exploratory Objectives

- Evaluate the antitumor activity of unesbulin plus DTIC versus placebo plus DTIC in subjects with at least 4 prior lines of treatment
- Evaluate the PK of unesbulin in the presence of DTIC in subjects with LMS
- Evaluate the PK of DTIC/5-amino-imidazole-4-carboxamide (AIC) alone and in the presence of unesbulin in subjects with LMS
- Compare the effect of unesbulin plus DTIC versus placebo plus DTIC on patient-reported outcomes (PROs), including health-related quality-of-life (HRQoL) assessments
- Measure genotype of subject tumors, assessed by blood sampling, at baseline and posttreatment

2.4. Primary Endpoint

- PFS per Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 assessed by an independent central imaging laboratory

2.5. Secondary Endpoints

The key secondary endpoint is OS.

Other secondary efficacy endpoints:

- Objective response rate (ORR; proportion of subjects with best overall response [BOR] of either complete response [CR] or partial response [PR])
- Disease control rate (DCR) or clinical benefit rate (CBR), defined as the proportion of subjects with BOR of CR, PR, or at least 3 months of stable disease (SD)
- Duration of response (DoR)

Safety:

- Vital signs, physical examination, electrocardiograms (ECG), laboratory abnormalities, Eastern Cooperative Oncology Group (ECOG) performance status (PS) scores, and AEs

2.6. Exploratory Endpoints

Efficacy:

- PFS, OS, ORR, DCR, and DoR in subjects with at least 4 prior lines of treatment

PK:

- PK parameters of unesbulin in subjects who receive unesbulin plus DTIC: C_{max}, T_{max}, and area under the concentration versus time curve from time zero to the last sampled time or the last non-zero concentration (AUC_{0-t})
- PK parameters of DTIC and its inactive metabolite AIC in subjects who receive unesbulin plus DTIC and placebo plus DTIC: C_{max}, T_{max}, and AUC_{0-t}

PROs:

- 30-item score European Organisation for the Research and Treatment of Cancer Quality-of-Life Questionnaire (EORTC QLQ-C30)
- EuroQol 5-level EQ-5D version (EQ-5D-5L)

Biomarkers:

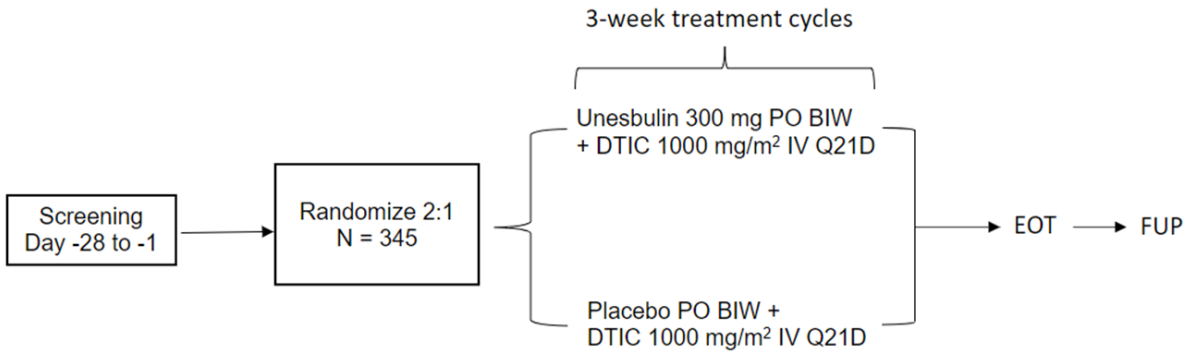
- Genetic evaluation of the tumors by analyzing circulating cell-free tumor DNA (ccfDNA)

3. INVESTIGATIONAL PLAN

3.1. Overall Study Design

This is a randomized, double-blind, placebo-controlled, Phase 2/3 study to compare the efficacy and safety of unesbulin plus DTIC versus placebo plus DTIC in subjects with unresectable or metastatic, relapsed or refractory LMS who have received at least 1 prior line of systemic therapy.

Figure 1: Study Schematic



Abbreviations: AE, adverse event; BIW, twice weekly; DTIC, dacarbazine; EOT, end-of-treatment; FUP, follow-up; OS, overall survival; Q21D, once every 21 days
Note: All subjects will receive treatment until evidence of disease progression, unacceptable toxicity, or other withdrawal criteria are met. An EOT assessment will be performed within 30 days after the end of treatment or until recovery or stabilization of the AE, whichever occurs latest. For subjects requiring immediate initiation of a new treatment option, an EOT visit may be completed earlier. A safety FUP assessment should be performed by phone at 30 days (±3 days) after the EOT. Survival FUP assessments will occur by phone every 3 months (±7 days) after the EOT visit until the later of 184 events or 2 years after the last subject is randomized.

Eligible subjects will be randomized 2:1 (unesbulin plus DTIC: placebo plus DTIC) to one of the following treatment groups:

- Unesbulin plus DTIC: unesbulin 300 mg administered PO BIW in each 3-week treatment cycle. DTIC 1000 mg/m^2 will be administered IV Q21D.
- Placebo plus DTIC: matching placebo tablets administered PO BIW in each 3-week treatment cycle. DTIC 1000 mg/m^2 will be administered IV Q21D.

For subjects with 1 to 3 prior lines of treatment, randomization will be stratified as follows:

- Number of prior systemic therapies (1 or >1)
- ECOG PS score (0 or 1)
- Histological tumor type (uterine versus nonuterine LMS)

For subjects with at least 4 prior lines of treatment, randomization will be stratified as follows:

- ECOG PS score (0 or 1)
- Histological tumor type (uterine versus nonuterine LMS)

One treatment cycle will constitute 21 days. DTIC will be administered on Day 1 of each 3-week treatment cycle. Unesbulin or placebo tablets will be administered on Days 2 and 5 of Week 1 and Days 1 and 4 of Weeks 2 and 3 of each 3-week treatment cycle (Note: the preferable duration between 2 doses of unesbulin/placebo is approximately 72 hours). Based on the results from a food effect assessment in the ongoing Phase 1b Study PTC596-ONC-007-LMS, it is recommended that unesbulin/placebo be administered with food.

All subjects will receive treatment until evidence of disease progression, unacceptable toxicity, or other withdrawal criteria are met. No crossover will be permitted.

Subjects will undergo weekly assessments throughout the study. The schedule of all study efficacy, safety, PK, and laboratory assessments is shown in Table 1, including a window for completion of the activities associated with each visit. Of note, activities designed to be completed in a single day, for example, PK sampling from before dosing to 8 hours postdose, may not be split up between the 2 days of a window.

An end-of-treatment (EOT) assessment should be performed within 30 days after the end of treatment or until recovery from or stabilization of the AE, whichever occurs latest. For subjects requiring immediate initiation of a new treatment option, the EOT visit may be completed earlier. The EOT assessments are outlined in Table 1. A safety follow-up assessment should be performed by telephone call approximately 30 days (±3 days) after the EOT to assess AEs and concomitant medications. Survival follow-up (sFUP) assessments should be performed by phone every 3 months (±7 days) after the EOT until the later of 184 events or 2 years after the last subject is randomized.

PK Assessment

Approximately 12 randomized subjects will participate in a PK substudy at selected sites. These subjects will be assessed for PK of unesbulin, DTIC, and AIC. The target is for approximately 7 PK evaluable subjects in the unesbulin group to complete Cycle 3.

For the DTIC/AIC PK assessment, blood samples will be collected following an intensive schedule at predose and up to 24 hours postdose on Day 1 of Cycles 1 and 3. Sparse blood samples will be collected at predose and 1.25 and 4 hours postdose (start of infusion) on Day 1 of Cycles 2 and 4.

For unesbulin PK assessment, blood samples will be collected following an intensive schedule at predose and up to 24 hours postdose on Day 2 of Cycles 1 and 3.

PK sample collection, processing, storage, and shipment will be performed according to instructions outlined in the laboratory manual separate from this protocol. The collected PK samples may also be used for further exploratory analysis of unesbulin and DTIC metabolism.

Data Monitoring

An independent Data Monitoring Committee (DMC) will be established and will review the interim safety data as per the DMC charter. In addition, one interim efficacy analysis will be performed (by an external vendor) and reviewed by the DMC. Based on the results of this interim analysis, the DMC will make recommendations to either continue or to stop the study. The DMC Charter (a separate document) and the DMC Statistical Analysis Plan (SAP) will provide detailed guidance for the conduct of the interim analysis.

3.2. Number of Subjects

Assuming 19 months of enrollment time and a minimum of 6 months of follow-up for the primary endpoint, approximately 345 subjects are planned to be randomized: 300 subjects with 1 to 3 prior lines of systemic therapy and an additional 45 subjects with at least 4 prior lines of systemic therapy. Enrollment into both cohorts will occur in parallel, but enrollment will be capped at 45 in the cohort of subjects with 4 or more prior lines of systemic therapy.

3.3. Dose Adjustment and Stopping Criteria

Tumor response assessments should continue uninterrupted every 6 to 9 weeks regardless of DTIC dosing/interruptions (see Schedule of Assessments).

Safety laboratory assessments (ie, complete blood count [CBC] and complete metabolic panel [CMP]) will continue as per the Schedule of Assessments regardless of DTIC dosing/interruptions.

The dose of unesbulin/placebo should not be reduced or held. However, any subject who experiences an intolerable toxicity should be withdrawn from the study drug if continuing would place them at risk based on ongoing benefit-risk assessment by the Investigator as described in Section 6.1.2.

If treatment-related toxicities are observed, a stepped reduction in the dose of DTIC is permitted throughout the study, from the starting DTIC dose of 1000 to 850 mg/m^2, followed by 700, 600, and 500 mg/m^2. The following guidelines should be observed:

- If a subject requires a dose reduction of DTIC to less than 500 mg/m^2, then DTIC should be discontinued.
- Subjects should receive all necessary supportive care including blood products, platelet transfusions, antiemetics, and antibiotics while being treated on this study.
- The use of granulocyte colony-stimulating factor (eg, pegfilgrastim) and erythropoietin or thrombopoietin-stimulating agents is permitted per institutional practice and is encouraged prophylactically.

If/when DTIC is on hold, unesbulin/placebo administration should continue uninterrupted BIW. DTIC should be reintroduced as soon as possible upon resolution of the hematological or nonhematological toxicity to a clinically acceptable level. Study staff should adhere to the following steps when DTIC is held due to toxicity during any treatment cycle:

- All procedures should be completed as per the Schedule of Assessments while unesbulin/placebo dosing continues.
- DTIC should be reintroduced at least 48 hours after unesbulin dosing. After DTIC is reintroduced, dosing should continue every 21 days regardless of when it is introduced in a 3-week treatment cycle with unesbulin/placebo. DTIC should not be administered on the same day as unesbulin/placebo dosing.
- At the time when DTIC is reintroduced, clinical laboratory (ie, CBC and CMP) and body weight assessments must be performed within 24 hours prior to DTIC reintroduction. A single ECG must also be performed approximately 2 hours after reintroduction of DTIC if the interruption occurs within the first 3 treatment cycles. If DTIC is interrupted or held

in Cycle 3, then the same intensive PK sampling schedule for DTIC and unesbulin/placebo should be followed in the subsequent cycle as described in footnote "n" of the Schedule of Assessments.

For instructions for managing hematological and nonhematological toxicities, refer to Table 2 and Table 3, respectively.

Table 2: Interventions for Common Hematological Toxicities by Grade

Toxicity Grade	DTIC Intervention
Neutropenia	
Grade 1 (ANC <1500/mm^3 to <LLN)	Maintain same dose.
Grade 2 (ANC 1000/mm^3 to <1500/mm^3)	Maintain same dose.
Grade 3 (ANC 500 to <1000/mm^3)	Hold DTIC until resolved to ≤Grade 2, then: If resolved ≤7 days, resume at original dose level. If resolved >7 days or second or subsequent occurrence, resume DTIC at one dose level lower. Consider addition of colony-stimulating factor (eg, pegfilgrastim) if not already employed.
Grade 4 (ANC <500/mm^3 or febrile neutropenia)	Hold DTIC until resolved to ≤Grade 2, then restart at one dose level lower. Consider addition of colony-stimulating factor (eg, pegfilgrastim) if not already employed.
Thrombocytopenia	
Grade 1 (PLT 75000 to <LLN)	Maintain same dose.
Grade 2 (PLT 50000 to <75000)	Hold DTIC until resolved to ≤Grade 1, then: If resolved ≤7 days, resume at original dose level. If resolved >7 days or second or subsequent occurrence, resume DTIC at one dose level lower.
Grade 3 (PLT 25000 to <50000)	Hold DTIC until resolved to ≤Grade 1, then restart at one dose level lower.
Grade 4 (PLT <25000) or thrombocytopenia with clinically significant bleeding	

Abbreviations: ANC, absolute neutrophil count; DTIC, dacarbazine; LLN, lower limit of normal; PLT, platelets

Table 3: Interventions for Nonhematological Toxicities by Grade

Toxicity Grade	DTIC Intervention
Grade 1 or 2	Maintain same dose.
Grade 3 or 4 Manageable with supportive care	Hold treatment until Grade ≤2, then restart at one dose level lower.
Grade 3 or 4 **Not manageable with supportive care**	Hold treatment until Grade ≤2, then restart at one dose level lower.

Abbreviations: DTIC, dacarbazine

Note: Supportive care includes blood products, platelet transfusions, antiemetics, and antibiotics while being treated on this study. The use of granulocyte colony-stimulating factor (eg, pegfilgrastim) is permitted as per institutional practice and is encouraged prophylactically.

3.4. End of Study Definition

The end of the study is defined as the date of the last visit of the last subject in the study.

3.5. Criteria for Study Termination

Treatment will continue for each subject until evidence of unacceptable toxicity, disease progression, or another reason as detailed in Section 6.

4. SELECTION AND WITHDRAWAL OF SUBJECTS

The following eligibility criteria are designed to select subjects for whom study participation is considered appropriate. All relevant medical and non-medical conditions should be taken into consideration when deciding whether this protocol is suitable for a particular subject. Eligibility criteria may not be waived. Any questions regarding eligibility should be discussed with the PTC Medical Monitor before enrollment.

4.1. Subject Inclusion Criteria

Subjects must meet all criteria to be eligible for enrollment into the study.

1. Subject is willing and able to provide informed consent
2. Willingness and ability to comply with scheduled visits, drug administration plan, laboratory tests, other study procedures, and study restrictions
3. Disease status including all of the following:
    a. Histological or cytological confirmation of LMS arising at any anatomic site except bone sarcoma
    b. Unresectable or metastatic, relapsed or refractory disease
    c. Measurable disease per RECIST 1.1 criteria
    d. Disease progression on previous treatment before screening or intolerability to other oncology treatments.

Demographics:

4. Age ≥18 years
5. Male or female

Performance status:

6. ECOG PS score of 0 or 1

Hematopoietic:

7. Absolute neutrophil count ≥1500/mm^3 without the use of growth factors in the past 7 days
8. Platelet count ≥100000/mm^3 without platelet transfusion in the past 14 days
9. Hemoglobin ≥9 g/dL (packed red blood cell transfusion is not allowed within 7 days)

Hepatic:

10. Bilirubin ≤ upper limit of normal (ULN) except for those patients with Gilbert's syndrome
11. Aspartate aminotransferase and alanine aminotransferase <3 times ULN
12. Subjects with liver metastases may be enrolled

Pulmonary:

13. Subjects with well-controlled asthma (eg, use of rescue medications <2 times per week over the last 12 months) or chronic obstructive pulmonary disease (eg, no exacerbations over the prior 3 months) may be enrolled.

Renal:

14. Creatinine <1.5 times normal OR creatinine clearance ≥60 mL/min

Prior therapeutics:

15. Toxicity from prior therapies recovered to Grade ≤1 or subject's baseline, except for alopecia. In addition, endocrinopathies associated with prior immunotherapy-based treatments which are well controlled on replacement medication are not exclusionary.

Chemotherapy and targeted therapy:

16. At least 1 prior systemic cytotoxic or targeted therapy regimen for LMS, which may include but is not limited to single-agent doxorubicin or other anthracycline, doxorubicin plus ifosfamide, trabectedin, pazopanib, or gemcitabine with or without docetaxel

Surgery:

17. At least 4 weeks since prior surgery and recovered in opinion of investigator

Other:

18. Capable of swallowing oral medication
19. Women of childbearing potential (WOCBP; as defined by the Clinical Trials Facilitation and Coordination Group [CTFG]) must have a negative serum pregnancy test at screening and agree to abstinence or the use of at least one of the following highly effective forms of contraception (with a failure rate of <1% per year when used consistently and correctly) (Clinical Trials Facilitation and Coordination Group 2020). Contraception or abstinence must be continued for the duration of the study and for at least 6 months after the last dose of study drug:
    - Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
        - Oral
        - Intravaginal
        - Transdermal
    - Progestogen-only hormonal contraception associated with inhibition of ovulation:
        - Oral
        - Injectable
        - Implantable
    - Intrauterine device
    - Intrauterine hormone-releasing system

- Bilateral tubal occlusion
- Vasectomized partner with confirmed azoospermia

All females will be considered of childbearing potential unless they are postmenopausal (at least 12 months consecutive amenorrhea in the appropriate age group without other known or suspected cause) or have been sterilized surgically (eg, bilateral salpingectomy, hysterectomy, bilateral oophorectomy).

20. Lactating females are not eligible unless they have agreed not to breastfeed their infants during treatment and for a period of 1 month following completion of treatment.
21. Males who are sexually active with WOCBP and who have not had a vasectomy must agree to use a barrier method of birth control from the start of study drug administration through at least 6 months after the last dose of study drug. Males should not donate sperm from the start of study treatment through at least 6 months after the last dose of study drug.

4.2. Subject Exclusion Criteria

Subjects meeting any of the following criteria will not be eligible for enrollment:

1. Received temozolomide or DTIC at any time
2. Any other systemic anticancer therapy, including investigational agents, ≤3 weeks before initiation of study treatment. Additionally, subjects may not have received radiation ≤3 weeks before initiation of study treatment.
3. Known intolerance to DTIC or one or more of the excipients in unesbulin
4. Co-existing active infection or any co-existing medical condition likely to interfere with study procedures, including:
    a. Significant cardiovascular disease (New York Heart Association Class III or IV cardiac disease), myocardial infarction within the past 6 months, unstable angina, congestive heart failure requiring therapy, unstable arrhythmia or a need for antiarrhythmic therapy, or evidence of ischemia on ECG, marked baseline prolongation of QT/QTc (corrected QT interval) interval, eg, repeated demonstration of a QTc interval >500 msec (Long QT Syndrome [congenital])
5. Human immunodeficiency virus, hepatitis B virus, or hepatitis C virus positivity
6. History of solid organ transplantation

Therapeutics:

7. Known or suspected allergy or immediate or delayed hypersensitivity to unesbulin or DTIC, their excipients, or any agent given in this study

Gastrointestinal:

8. Bowel obstruction, malabsorption, or other contraindication to oral medication
9. Gastrointestinal disease or other conditions that could affect absorption. Active peptic ulcer disease, active gastritis, or previous history of gastric perforation within the last 2 years.

10. Inflammatory bowel disease (including ulcerative colitis and Crohn's disease), diverticulitis, cholecystitis, symptomatic cholangitis, or appendicitis

Wounds/surgery:

11. Serious non-healing wound, ulcer, or bone fractures
12. Major surgery, open biopsy, or significant traumatic injury that has not recovered, in the opinion of the investigator, within 28 days of baseline
13. Mucosal or internal bleeding

Concomitant medications:

14. Concomitant strong CYP1A2 inhibitors (such as fluoroquinolones [broad spectrum quinolone antibiotics, including enoxacin and ciprofloxacin] and selective serotonin reuptake inhibitor [SSRI] agents fluvoxamine and fluoxetine) should be avoided on the same day that DTIC or unesbulin/placebo is administered. CYP1A2 inhibitors may inhibit the conversion of DTIC to its active metabolite and may increase the exposure of unesbulin.
15. Concomitant use of moderate CYP1A2 inducers (such as phenytoin, rifampin, ritonavir, teriflunomide, and barbiturates). Chronic use of marijuana should be avoided, but irregular recreational use may be permitted at the discretion of the treating Investigator. CYP1A2 inducers may increase the conversion of DTIC to its active metabolites.
16. Coadministration of acid-reducing agents should be avoided approximately 4 hours before and after unesbulin/placebo administration.
17. Immunization with a live vaccine within 30 days before starting study drug due to the risk of serious and life-threatening infections.

Other:

18. Prior malignancies, other than LMS, that required treatment or have shown evidence of recurrence (except for non-melanoma skin cancer or adequately treated cervical carcinoma in situ, prostate cancer in situ or any other low risk malignancy that is approved by the medical monitor) during the 5 years before initiation. Cancer treated with curative intent more than 5 years previously and without evidence of recurrence is not an exclusion.
19. Known coagulopathy or bleeding diathesis. Subjects on anticoagulation should be monitored closely and International Normalized Ratio and/or activated partial thromboplastin time (APTT)/prothrombin time (PT) should be within the required range where applicable.
20. Prior or ongoing clinically significant illness, medical or psychiatric condition, medical history, physical findings, ECG findings, or laboratory abnormality that, in the investigator's opinion, could affect the safety of the subject, or alter the absorption, distribution, metabolism, or excretion of the study drugs, or could impair the assessment of study results.
21. History of brain metastases or leptomeningeal disease at any time in subject's history, including treated central nervous system (CNS) disease.

4.3. Screen Failures

Screen failures are defined as subjects who consent to participate in the clinical study but do not meet eligibility criteria. Individuals who do not meet the criteria for participation in this study due to a laboratory test result may be retested once within the screening period. Subjects who fail screening may be considered for rescreening after consultation with the PTC Medical Monitor.

5. STUDY INTERVENTION MATERIALS AND MANAGEMENT

5.1. Description of Study Drug

Unesbulin drug product is an immediate-release tablet formulation for oral use, manufactured following current GMP. Unesbulin tablets for oral administration will be provided in strengths of 50 mg (white to off-white round tablets) and 200 mg (white to off-white oval tablets). Matching placebo tablets for oral administration will also be provided. Unesbulin and placebo tablets will be provided in high-density polyethylene bottles with a heat induction seal.

DTIC for injection will be provided (by the site or the sponsor) and will be reconstituted with Sterile Water for Injection, USP. The resulting solution contains 10 mg/mL of DTIC having a pH of 3.0 to 4.0. The reconstitution step may be modified based on institutional policy and or the availability of a specific strength of the DTIC vial to obtain a resulting DTIC solution of 10 mg/mL.

5.2. Study Intervention Preparation and Administration

Unesbulin 300 mg or placebo will be administered PO BIW in combination with DTIC 1000 mg/m^2 administered IV Q21D. Participants should be instructed to not take unesbulin/placebo on the same day as DTIC administration. Additionally, unesbulin/placebo should not be dosed on consecutive days, and preferably at least 3 days (72 hours) between each unesbulin/placebo dose. If a subject misses a dose of unesbulin/placebo, then every attempt should be made to administer within 24 hours. Otherwise, the subject should skip the dose and take unesbulin/placebo at the next scheduled dosing day.

Coadministration of acid-reducing agents should be avoided approximately 4 hours before and after unesbulin/placebo administration.

Placebo tablets will be supplied and administered in a blinded manner to subjects BIW in combination with DTIC per the same dosing schedule as for unesbulin.

Based on the results from a food effect assessment in the ongoing Phase 1b Study PTC596-ONC-007-LMS, it is recommended that unesbulin/placebo be administered with food.

For subjects who cannot swallow the whole tablet, unesbulin/placebo tablets can be dispersed in water at the same dose level at which the tablets are being dosed and be administered orally. The entire dispersion is administered to the subject and taken as soon as possible (within 15 minutes of mixing), ensuring consumption of the entire content with adequate rinsing. Please refer to the Pharmacy Manual for additional details.

If a subject vomits following dosing with unesbulin/placebo, then the unesbulin/placebo tablet may be readministered if the vomiting occurs within 3 minutes of dosing and the tablet contents (white matter) can be seen in the vomitus. If these 2 conditions are not met, then unesbulin/placebo should not be readministered until the next scheduled dosing day.

After DTIC is reconstituted (Section 5.1), the calculated dose of the resulting solution is drawn into a syringe. The reconstituted solution is further diluted with 5% Dextrose Injection, USP or 0.9% Sodium Chloride, USP and administered as an IV infusion over 1 hour ±30 minutes. Within 30 minutes before DTIC administration, the following premedication regimen is recommended: fosaprepitant 150 mg IV once, dexamethasone 20 mg PO once, and ondansetron

16 mg PO once. The premedication regimen as per institutional practice is allowed. Refer to Section 3.3 for instructions on DTIC dose interruptions and dose modifications due to toxicity.

5.3. Storage, Handling, and Accountability

5.3.1. Storage

Unesbulin/placebo tablets should be stored at controlled room temperature (68°F-77°F [20°C-25°C]) with excursions allowed between 59°F and 86°F (15°C-30°C).

After reconstitution and before use, DTIC solution in the vial may be stored at 4°C for up to 72 hours or at normal room conditions (temperature and light) for up to 8 hours. If the reconstituted solution is further diluted in 5% Dextrose Injection, USP or 0.9% Sodium Chloride, USP, the resulting solution may be stored at 4°C for up to 24 hours or at normal room conditions for up to 8 hours.

5.3.2. Handling and Accountability

Study personnel must ensure that all study drug supplies are kept in a secure locked area with access limited to authorized personnel. Study drug must not be used outside the context of this protocol. Under no circumstances should the investigator or site personnel supply study drug to other investigators or clinics or allow study drug to be used other than as directed by this protocol.

The investigator is responsible for keeping accurate records of the clinical supplies received from PTC or designee including, but not limited to, the date received, lot number, amount received, amount returned, and the disposition of all study drug products. Drug accountability records that include the subject's assigned study number, date and amount of study drug dispensed, and relevant lot numbers must also be maintained by the site.

Unused clinical supplies or study drug must be returned to PTC or its designee. Records documenting the date of study drug destruction or shipping, relevant lot numbers, and amount destroyed or shipped should be kept in the investigator site study file. Study drug must be returned to PTC or its designee, except where sites are destroying study drug per their local standard operating procedures (SOPs). In order for a site to destroy study drug on site, the site must submit their SOP to PTC for review to confirm adequacy and fulfill PTC requirements.

5.4. Study Intervention and Compliance

A subject medication diary will be given to subjects to record unesbulin/placebo dosing at home and will be dispensed at Day 1 of each cycle or at other visits, as needed, to ensure compliance. Subjects should bring the diary back at the scheduled visits for review with the research staff to assess treatment compliance.

5.5. Concomitant Medications

To the extent possible, administration of any prescription or over-the-counter drug products other than study drug should be minimized during the study period and the investigator and/or staff should be informed. Subjects should be discouraged from use of street drugs, herbal remedies, self-prescribed drugs, tobacco products, or excessive use of caffeinated products and alcohol at any time during their participation in the study.

If considered necessary for the subject's well-being, drugs for concomitant medical conditions or for symptom management may be given at the discretion of the investigator. Neither approved nor guideline-recommended therapy options should be withheld from subjects if the investigator determines it is in the best interest of the subject. The decision to authorize the use of any drug other than study drug should account for subject safety, the medical need, the potential for drug interactions, the possibility for masking symptoms of a more significant underlying event, and whether use of the drug will compromise the outcome or integrity of the study.

Immunization with live vaccines should be avoided throughout treatment and at least 30 days before or after treatment due to the risk of serious and life-threatening infections.

Subjects should receive full supportive care, including antiemetics and antidiarrheals as appropriate.

Conversion of DTIC to the pharmacologically active metabolite is mainly mitigated by CYP1A2, and to a lesser degree by CYP1A1. The metabolism of unesbulin is mitigated by multiple CYP450 enzymes, mainly by CYP1A2, while CYP2D6, CYP2E1, and CYP3A4 may also be involved. Coadministration of a strong CYP1A2 inhibitor may increase unesbulin exposure. Concomitant use of strong CYP1A2 inhibitors (such as fluoroquinolones [broad spectrum quinolone antibiotics, including enoxacin and ciprofloxacin] and SSRI agents fluvoxamine and fluoxetine) should be avoided on the same day that DTIC or unesbulin/placebo is administered. Unesbulin is identified as a strong CYP1A2 inhibitor and should not be administered with DTIC at the same time. Concomitant use of moderate CYP1A2 inducers (such as phenytoin, rifampin, ritonavir, teriflunomide, and barbiturates) should be avoided. Chronic use of marijuana should be avoided, but irregular recreational use may be permitted at the discretion of the treating Investigator. CYP1A2 inducers may increase the conversion of DTIC to its active metabolites.

Coadministration of acid-reducing agents (eg, antacids, histamine H2-receptor antagonists, and proton pump inhibitors) should be avoided approximately 4 hours before and after unesbulin/placebo administration. As a reminder, subjects should not have active peptic ulcer disease, active gastritis, or previous history of gastric perforation within the last 2 years.

For orally administered medications, breast cancer resistance protein (BCRP)-mediated PK-related interactions could contribute to interactions within the gastrointestinal tract due to the expression of BCRP in that region. However, the absolute bioavailability following oral administration of unesbulin in fasted cynomolgus monkeys was estimated to be 100%. A relatively high absolute bioavailability is also expected in humans, which would limit the impact of BCRP-mediated interactions with unesbulin. This will be investigated in a human absorption, metabolism, and excretion (AME) study. Until results from the AME study are available, it is recommended that BCRP substrates, inhibitors, and inducers (such as curcumin, orange juice, apple juice, cranberry juice, and grape juice) be avoided within 4 hours before or after unesbulin/placebo administration.

Interactions of unesbulin with the substrates of the organic anion transporter polypeptide transporters, OATP1B1 and OATP2B1, have not been assessed clinically. Therefore, caution should be exercised if coadministration of unesbulin/placebo is required with OATP1B1 and OATP2B1 substrates. Investigators are encouraged to consult with PTC for further guidance.

5.6. Randomization and Blinding

Subjects will be randomized 2:1 to either unesbulin plus DTIC or placebo plus DTIC using a central randomization process. For subjects with 1 to 3 prior lines of treatment, randomization will be stratified by the number of prior systemic therapies (1 or >1), ECOG PS score (0 or 1), and histological tumor type (uterine or nonuterine LMS). For subjects with at least 4 prior lines of treatment, randomization will be stratified by ECOG PS score (0 or 1) and histological tumor type (uterine or nonuterine LMS).

The investigator and study staff (including processing lab personnel), the subjects, and the sponsor's staff (with exception of Pharmacovigilance staff for the purposes of suspected unexpected serious adverse reaction [SUSAR] reporting) will remain blinded until the primary analysis is completed. The investigational drug and its matching placebo are indistinguishable and will be packaged in the same way.

The randomization code will be kept strictly confidential. Further details are provided in the Pharmacy Manual.

5.6.1. Emergency Procedure for Unblinding

The investigator and the study staff must remain blinded to the subject's treatment assignment.

The blind may be broken by the investigator only if specific emergency treatment would be dictated by knowing the treatment assignment. The investigators have the ability to unblind via the Interactive Response Technology system.

In all cases, PTC must be informed as soon as possible before or after the code break.

Any code break must be documented in a detailed report with the date and time of the code break and signed by the investigator.

6. STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

6.1.1. Discontinuation of Study Intervention

If after appropriate consideration of study drug interruption/modification and consultation with the PTC Medical Monitor, it is not appropriate for a subject to continue with study treatment, then the study drug must be permanently discontinued. After permanent discontinuation of study drug for a safety concern, and if the initial event was reported as a serious adverse event (SAE) then a follow-up SAE report form should be completed. In the case of a treatment discontinuation due to an event that is not an SAE, the PTC Medical Monitor should be notified. In addition, details regarding the reasons for discontinuation and the AEs leading to the discontinuation should be recorded in the source documents and in the appropriate electronic case report form (eCRF).

The EOT visit should be completed within 30 days after the end of treatment (Table 1) or until recovery from or stabilization of the AE, whichever comes last. For subjects requiring immediate initiation of a new treatment option, an EOT visit may be completed earlier.

6.1.2. Participant Discontinuation/Withdrawal from the Study Drug or Study

All subjects who receive study drug should remain in the study whenever possible. However:

- The subject has the right to withdraw consent and discontinue study drug or the study at any time.
- Any subject who experiences progression of disease (per RECIST 1.1) must discontinue the study drug.
- Any subject whose condition substantially changes or experiences intolerable toxicity after entering the study should be carefully evaluated by the investigator in consultation with the PTC Medical Monitor. Such subjects should be withdrawn from the study drug if continuing would place them at risk.
- Any subject who becomes pregnant must discontinue the study drug.
- Any subject who becomes significantly noncompliant with study drug administration, study procedures, or study requirements should be withdrawn from study drug.
- The investigator may withdraw the subject from study drug if, in the investigator's clinical judgment, it is not in the subject's best interest to continue.
- This study may be discontinued by the relevant regulatory authority and/or PTC at any time.

The date that study drug is discontinued and the reason for discontinuation will be recorded in the source documents and in the eCRF. The PTC Medical Monitor (or designee) should be informed via email when a subject discontinues study drug or withdraws from the study.

When study drug is discontinued, the investigator is expected to perform all evaluations required at the EOT visit and any additional evaluations that may be necessary to ensure that the subject is free of untoward effects.

6.1.3. Lost to Follow-up

A subject who repeatedly fails to return for scheduled visits and is unable to be contacted by the study site will be considered lost to follow-up.

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

- Attempt to contact the subject and reschedule the missed visit as soon as possible and counsel the subject on the importance of maintaining the assigned visit schedule and determine whether or not the subject wishes to and/or should continue in the study.
- Before a subject is deemed lost to follow-up, the investigator or designee must make every effort to contact the subject. These contact attempts should be documented in the subject's medical record.

7. STUDY ASSESSMENT AND PROCEDURES

7.1. Assessment of Efficacy

Response assessments will be performed by radiological imaging using RECIST 1.1 criteria every 6 weeks (±7 days) until disease progression. After Cycle 8, scans may be performed every 9 weeks. If any subject responds with a CR or PR at any assessment, then the subsequent confirmatory scan should be performed 4 weeks (+5 days) after the previous scan where the objective response was first evidenced. Assessment of response and the primary endpoint, PFS, will be assessed by an independent central reader who will be blinded to treatment assignment.

Subjects should be followed with the same imaging procedure throughout the study. Computed tomography scans or magnetic resonance imaging may be used as clinically indicated. If the subject withdraws for reasons other than tumor progression, an EOT scan should be performed if the last scan was ≥3 weeks earlier.

Survival follow-up will continue by phone every 3 months until the later of 184 events or 2 years after the last subject is randomized.

7.2. Assessment of Safety

7.2.1. Safety Parameters

Subjects will be monitored closely for AEs and laboratory abnormalities during the study. All AEs that occur after any subject has been enrolled, before treatment, during treatment, or within 30 days following the cessation of treatment, whether or not they are related to the study, must be recorded on forms provided by PTC.

For AEs and laboratory abnormalities, the investigator should use his/her judgment in determining whether the event or abnormality is clinically significant, whether diagnostic evaluation is warranted, and whether potential interruption of study drug therapy is appropriate. In general, life-threatening (Grade 4) or severe (Grade 3) AEs or laboratory abnormalities should be considered clinically significant, although recurrent or persistent moderate events (Grade 2) may also be considered clinically significant in certain circumstances. Reference should be made to the NCI Common Terminology Criteria for Adverse Events Version 5.0 (CTCAE V5.0) for grading the severity of AEs and laboratory abnormalities.

While specific monitoring, diagnostic testing, and supportive care measures must be instituted based on the clinical judgment of the investigator, investigators should contact the PTC Medical Monitor to obtain guidance and to ascertain whether similar events are being seen at other sites. The PTC Medical Monitor should be notified of any AE or laboratory abnormality that leads to dose interruption and should be apprised of ancillary laboratory or other diagnostic findings and the evolving data from any work-up of the initial abnormality. The PTC Medical Monitor may suggest review of the case with consultants or with other experts (either at the site or retained by PTC).

Additional safety assessments include vital signs, ECGs, physical examinations, and ECOG PS scores.

7.3. Adverse Events and Serious Adverse Events

7.3.1. Definition of Adverse Event

An AE is any untoward medical occurrence after exposure to a drug in humans, whether or not it is considered related to the drug. An AE can therefore be any unfavorable or unintended sign (including an abnormal laboratory finding), symptom, or disease in a study subject who is administered study drug in this study.

For this protocol, untoward medical occurrences that should be reported as AEs include the following:

- All AEs during treatment with study drug administration
- All AEs resulting from medication misuse, abuse, withdrawal, or overdose of study drug
- All AEs resulting from medication errors such as dispensing or administration error outside of what is described in the protocol
- Apparently unrelated illnesses, including worsening of a preexisting illness
- Injury or accidents. Note that if a medical condition is known to have caused the injury or accident (a fall secondary to dizziness), the medical condition (dizziness) and the accident (fall) should be reported as 2 separate AEs. The outcome of the accident (hip fracture secondary to the fall) should be recorded in source documents.
- Abnormalities in physiological testing or physical examination findings that require clinical intervention or further investigation (beyond ordering a repeat [confirmatory] test)
- Laboratory or ECG abnormalities that require clinical intervention or further investigation (beyond ordering a repeat [confirmatory] test) unless they are associated with an already reported clinical event. Laboratory abnormalities associated with a clinical event should be captured in the source documents. Laboratory abnormalities not requiring clinical intervention or further investigation will be captured as part of overall laboratory monitoring and should not be reported as AEs.
- A preexisting condition (eg, allergic rhinitis) must be noted on the appropriate eCRF but should not be reported as an AE unless the condition worsens, or episodes increase in frequency during the AE reporting period.
- Diagnostic and therapeutic noninvasive and invasive procedures, such as surgery, should not be reported as AEs. However, the medical condition for which the procedure was performed should be reported if it meets the definition of an AE. For example, an acute appendicitis that occurs during the treatment with study drug should be reported as the AE and the resulting appendectomy should be recorded in the source documents and eCRF. If a surgical procedure was planned before entry into the study, and the surgery is not performed because of a worsening of a baseline condition, this should not be reported as an AE. Note that, as described in Section 7.3.2, any hospitalization occurring as the consequence of an AE during the study period should be reported as an SAE.

Each AE is to be classified as serious or nonserious by the investigator using medical and scientific judgment.

7.3.2. Definition of Serious Adverse Events

An SAE is an untoward medical occurrence or effect that occurs after exposure to a study drug at any dose, regardless of whether it is considered to be related to the study drug, which results in one of the following:

- Results in death. This includes all deaths on treatment or within 30 days after last study drug administration, including deaths due to disease progression. Any death occurring later than 30 days following the last dose need not be reported as an SAE unless it is a result of an event that started within the period covered by the on-study definition. The reported AE should be the event that caused the death. In addition, any AE resulting in death that occurs subsequent to the AE reporting period and that the investigator assesses as possibly related to the study drug should also be reported as serious.
- Is life-threatening. This refers to an event in which the subject was at risk of death at the time of the event. It does not include an event that, had it occurred in a more severe form, hypothetically might have caused death.
- Requires hospitalization or prolongation of existing hospitalization (excluding hospitalizations for administration of the study drug, procedures required by the study protocol, or treatment-related diagnostic procedures; other planned hospitalizations; or hospitalizations related only to progression of disease).
- Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.
- Additionally, hospitalization should be used as an indicator of the seriousness of the AE and should only be used for situations where the AE truly fits this definition and not for hospitalizations associated with less serious events (eg, a hospital visit where subject is admitted for observation or minor treatment [eg, hydration] and released in less than 24 hours). Furthermore, hospitalization for PK sampling, if needed, is not an AE.
- Results in persistent or significant disability or incapacity or substantial disruption of the ability to conduct normal life functions, not related to cancer.
- Any other medically important event that the investigator or PTC judges to be serious or which is defined as serious by the regulatory agency in the local country. These are AEs that might not be immediately life-threatening or result in death or hospitalization but might jeopardize the subject or might require intervention to prevent one of the other outcomes listed above. Medical judgment should be

exercised in deciding whether an AE is serious based on above definition. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

- A pregnancy resulting in spontaneous abortion, stillbirth, neonatal death, or congenital anomaly (including that in an aborted fetus).

Note that any SAEs occurring from signing of consent form through 30 days after the end of the subject's participation in the study (last dose) should be reported to PTC if the investigator becomes aware of them.

7.3.2.1. Disease Progression

An event need not be reported as an SAE if it exclusively represents a relapse or an expected change or progression of the baseline cancer. In case of fatal outcome with disease progression, it should be reported in "Results in death" subsection (refer to Section 7.3.2).

7.3.3. Unexpected Adverse Events

The Reference Safety Information (RSI) will be used for assessing expectedness. The RSI for the purpose of expedited safety reporting for unesbulin is located in Section 7 of the Unesbulin IB. The RSI for DTIC may be found in local dacarbazine Product Information or Summary of Product Characteristics.

If an event is not listed in the RSI, it should be considered unexpected, or if the AE occurs at a greater severity, specificity, or frequency, it should be considered unexpected.

7.3.4. Eliciting Adverse Event Information

The investigator is to report all directly observed AEs and all AEs spontaneously reported by the subject. In addition, each study subject will be questioned about AEs at each scheduled clinic visit after study drug administration or during any telephone contact with the subject. The type of question asked should be open-ended, for example, "How have you been feeling?" or a similar type of query.

7.3.5. Recording Nonserious Adverse Events

All AEs (both serious and nonserious) that occur in subjects during the AE reporting period must be recorded, whether or not the event is considered drug related. In addition, any untoward event that occurs subsequent to the AE reporting period that the investigator assesses as possibly related to the investigational drug/product should also be recorded as an AE.

All AEs are to be recorded in the source documents and on the eCRF using concise medical terminology; whenever possible, terms contained in the Medical Dictionary for Regulatory Activities (MedDRA) should be employed. In addition, the following information should be recorded:

- Indication of whether the event is nonserious or serious (see Section 7.3.1 and Section 7.3.2, respectively)
- Relationship to study drug (see Section 7.3.6)

- Severity of the event (see Section 7.3.7)
- Onset date
- Date of resolution or date of death
- Action taken
- Outcome of the event
- Whether the AE resulted in discontinuation from the study or treatment

Classification of the event as serious or nonserious determines the reporting procedures to be followed.

7.3.6. Describing Adverse Event Relationship to Study Drug

The investigator should provide an assessment of the relationship of the AE to the study drug (ie, whether there is a reasonable possibility that the study drug caused the AE) using the considerations outlined in Table 4.

Table 4: Relationship of Study Drug to Adverse Event

Relationship Category	Description
Probable	A clinical event in which a relationship to the study drug seems probable because of such factors as consistency with known effects of the drug; a clear temporal association with the use of the drug; improvement upon withdrawal of the drug; recurrence upon rechallenge with the drug; lack of alternative explanations for the event.
Possible	A clinical event occurring coincident with administration of the study drug and which may or may not be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal or rechallenge may be lacking.
Unlikely	A clinical event with a temporal relationship to the study drug exposure that does not preclude causality but for which there is a clear alternate cause that is more likely to have caused the adverse event than study drug. Such alternatives include a concomitantly administered drug, the subject's disease state, other medical conditions, or environmental factors.
Unrelated	A clinical event, for which a relationship to the study drug seems improbable because of factors such as inconsistency with known effects of the study drug, lack of a temporal association with study drug administration, lack of association of the event with study drug withdrawal or rechallenge, and/or presence of alternative explanations for the event. Alternative explanations might include a known relationship of the adverse event to a concomitant drug, medical history of a similar event, the subject's disease state, other medical conditions, or environmental factors.

7.3.7. Grading of Severity of Adverse Event

The severity of AE will be graded using the latest version of the NCI CTCAE V5.0 (refer to Study Manual). For each episode, the highest severity grade attained should be reported.

If an NCI CTCAE V5.0 criterion does not exist, the investigator should use the grade or adjectives: Grade 1 (mild), Grade 2 (moderate), Grade 3 (severe), Grade 4 (life-threatening), or Grade 5 (fatal) to describe the maximum intensity of the AE. For purposes of consistency with the NCI CTCAE V5.0, these intensity grades are defined in Table 5.

Table 5: Grading of Adverse Events

Grade	Severity Grade	Description
1	Mild	Sign or symptom is present, but it is easily tolerated, is not expected to have a clinically significant effect on the subject's overall health and well-being, does not interfere with the subject's usual function, and is not likely to require medical attention
2	Moderate	Sign or symptom causes interference with usual activity or affects clinical status, and may require medical intervention
3	Severe	Sign or symptom is incapacitating or significantly affects clinical status and likely requires medical intervention and/or close follow-up
4	Life-threatening	Sign or symptom results in a potential threat to life
5	Fatal	Sign or symptom results in death

7.3.8. Reporting Adverse Events

Investigator site reporting requirements for AEs are summarized in Table 6.

Table 6: Investigator Site Requirements for Reporting Adverse Events

Event	Recorded in the eCRF	Reported on the SAE Report Form to PTC Pharmacovigilance Within 24 Hours of Awareness
Serious AE	All	All
Nonserious AE	All	None
Exposure to the study drug during pregnancy or breastfeeding, and occupational exposure	All (regardless of whether associated with an AE), except occupational exposure	Exposure during pregnancy, exposure via breastfeeding, occupational exposure (regardless of whether associated with an AE)

Abbreviations: AE, adverse event; eCRF, electronic case report form; SAE, serious adverse event

All AEs should be followed up by the investigator until they are resolved, or the investigator assesses them as chronic or stable. The investigator should consider protocol guidelines and use his/her discretion in ordering additional tests as necessary to monitor the resolution of such events. In the event of additional investigations, the PTC Pharmacovigilance Department or designee should be informed via email or fax. A subject withdrawn from the study because of an AE must be followed by the investigator until clinical recovery is complete and laboratory results have returned to normal, or until progression has been stabilized. Follow-up may need to continue after the subject has discontinued from the study, and additional investigations may be requested by the PTC Medical Monitoring team.

The first day of AE reporting will coincide with the date of signing of the informed consent form (ICF) and will include a minimum of 30 calendar days after the last administration of study drug.

7.3.9. Serious Adverse Events Reporting

All SAEs occurring from the signing of the ICF through 30 days after the end of the subject's participation in the study (last dose) should be reported via the SAE report form to PTC immediately but no later than 24 hours of becoming aware of the event(s). In addition, the AE portion of the eCRF must also be completed.

The SAE report form should be signed by the investigator; however, if the investigator is unable to sign at the time of the event or within 24 hours, the form should be signed by the clinical staff member reporting the SAE (eg, the study coordinator). The SAE report form must be faxed or emailed to the PTC Pharmacovigilance Department or designee and to the site Institutional Review Board (IRB) or Institutional Ethics Committee (IEC) (if required by local regulations) within 24 hours.

Follow-up information to the SAE should be clearly documented as "follow-up" in the SAE report form and must also be faxed or emailed to the same party. All follow-up SAE report forms for the event must be signed by the investigator. Any source documents (eg, progress notes, nurses' notes, laboratory and diagnostic test results, discharge summaries) provided to PTC should be redacted so that the subject's name, address, and other personal identity information are obscured. Only the subject's study number and initials are to be provided (in regions where the provision of such information is permitted). The information in the AE portion of the eCRF and the SAE report form(s) must match or be reconciled. Where the same data are collected, the forms must be completed in a consistent manner. For example, the same AE term should be used on both forms.

In the rare event that the investigator does not become aware of the occurrence of an SAE immediately (for example, if a subject initially seeks treatment elsewhere), the investigator is to report the event within 24 hours after learning of it and to document his/her first awareness of the AE.

The PTC Pharmacovigilance Department contact information for reporting SAEs is provided below. This information is also provided in the SAE report form.

PTC Therapeutics Safety Department
Attention: Pharmacovigilance
Email: Pharmacovigilance@ptcbio.com
Facsimile (USA): +1 (908) 325-0355

7.3.10. Contraception

For pregnancy testing and contraception requirements in WOCBP and contraception requirements and sperm donation limitations in males who are sexually active with WOCBP, see the corresponding inclusion criteria in Section 4.1.

7.3.11. Reporting Pregnancy

PTC should be notified if a female subject in the study, or a female partner of a male subject in the study, becomes pregnant on study or within 30 days of the last administration of study drug. The pregnancy must be reported on a Pregnancy Notification Form (see Study Manual for details).

This must be done whether or not an AE has occurred and within 24 hours of awareness of the pregnancy. The information submitted should include the anticipated date of birth or pregnancy termination.

Written consent is required before collecting and reporting any information on a female partner of a male subject in the study.

If possible, the investigator should follow the subject, or the pregnant female partner of a male subject, until completion of the pregnancy and notify the PTC Medical Monitor of the outcome within 5 days or as specified below. The investigator will provide this information as a follow-up to the initial Pregnancy Notification Form via the Pregnancy Outcome Form (see Study Manual for details).

If the outcome of the pregnancy meets the criteria for immediate classification as an SAE (ie, spontaneous abortion, stillbirth, neonatal death, or congenital anomaly [including that in an aborted fetus]), the investigator should follow the procedures for reporting SAEs, ie, report the event to the PTC Pharmacovigilance Department or designee and follow up by submission of appropriate AE eCRFs (see Section 7.3.9).

7.3.12. PTC Safety Reporting Requirement

In compliance with local requirements, PTC shall immediately notify the investigators of any new or emerging safety information that impacts the benefit-risk ratio of the drug, once confirmed by the executive safety review board. This will include any urgent measures that need to be taken with respect to the protocol.

As the sponsor of the study, PTC is responsible for reporting certain safety information, particularly SUSARs and other significant findings, as appropriate per local reporting requirements, to each investigator in an expedited manner. If PTC is notified of a SUSAR requiring expedited reporting to investigators, PTC or its designated representative will contact each investigational site participating in this study by email, fax, and/or overnight mail such that the investigator can promptly notify the site IRB/IEC per their local requirements. The initial expedited safety report will be provided as required according to local regulations (eg, within 15 days) after the earliest date PTC or an agent of PTC (eg, a site monitor) becomes aware of an SAE. This awareness date is the date the regulatory reporting clock begins, and the date is considered Day 0.

7.4. Pharmacokinetics

For the subset of subjects who provide consent, blood samples per PK sample interval will be collected for the determination of DTIC, AIC, and unesbulin. PK sample collection, processing, storage, and shipment will be performed according to instructions outlined in the laboratory manual separate from this protocol. The collected PK samples may also be used for further exploratory analysis of unesbulin and DTIC metabolism.

Approximately 12 subjects will be assessed for PK of unesbulin, DTIC, and AIC. Blood samples will be collected according to the schedule shown in Table 1.

Plasma samples may be used to explore metabolism and additional biomarkers post-PK analysis if remaining volume of sample is sufficient. If such exploratory analyses are to proceed, then the study will be conducted at a designated laboratory, and a separate report may be issued.

7.5. Patient-Reported Outcomes

HRQoL will be assessed using the EORTC QLQ-C30 and the EQ-5D-5L according to the schedule outlined in Table 1.

The EORTC QLQ-C30 is a widely used, validated 30-item, quality-of-life questionnaire developed by the European Organisation for Research and Treatment of Cancer (Aaronson 1993, Giesinger 2016). This health-related questionnaire encompasses 15 outcomes in total: 5 functional scales (physical, role, cognitive, emotional, and social functioning), a global quality-of-life scale, 3 symptom scales (fatigue, nausea and vomiting, and pain), and 6 single items (appetite loss, diarrhea, dyspnea, constipation, insomnia, and financial impact).

The EQ-5D-5L is a frequently used, standardized patient-reported HRQoL that measures 5 dimensions of health: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression (Herdman 2011). Each dimension has 5 levels: no problems, slight problems, moderate problems, severe problems, and extreme problems.

7.6. Biomarkers

Blood samples (10 mL) will be collected at screening (predose) and prior to dosing on Day 1 of Cycle 3 and at the time of disease progression or EOT for all subjects for analyzing ccfDNA. By targeted DNA sequencing, the presence of ccfDNA containing known mutations associated with LMS will be assessed. Samples will be retained for no more than 5 years after collection.

8. STATISTICS

A SAP will be prepared and approved before the interim analysis to provide a more detailed description of the nature of the analyses and the manner in which results will be compiled. The SAP will include details of the statistical models to be used and the test statistics to be employed.

8.1. Statistical Hypotheses

The null hypothesis for the primary efficacy endpoint is that there is no difference in PFS between unesbulin plus DTIC versus placebo plus DTIC. The alternative hypothesis is that unesbulin plus DTIC increases PFS. The null hypothesis corresponding to the key secondary endpoint is that there is no difference in OS between subjects treated with unesbulin plus DTIC when compared to those treated with placebo plus DTIC. The alternative hypothesis is that unesbulin plus DTIC increases OS.

8.2. Sample Size Determination

The sample size for the primary analysis was computed based on the comparison of PFS between subjects randomized to unesbulin plus DTIC and subjects randomized to placebo plus DTIC. The median PFS was assumed to be approximately 1.5 to 2.5 months for the placebo plus DTIC group and more than 4 months for the unesbulin plus DTIC group. With an overall one-sided alpha level of 0.025, a total of 245 events are required to achieve at least 90% power at a hazard ratio of 0.5. Assuming 19 months of enrollment time, a minimum follow-up period of 6 months for the primary endpoint, and approximately 11.5% uniform dropout rate, a total of 300 subjects are planned to be randomized.

One interim analysis will be performed when approximately 36% of the PFS events occur (approximately 88 events). The study may stop for both efficacy and futility. The study may stop for efficacy if the observed hazard ratio is less than 0.447, and the study may stop for futility if the observed hazard ratio is more than 0.67.

The alpha allocation will be controlled separately at the interim and the final analysis timepoints by using the Lan-DeMets spending function that approximates the O'Brien-Fleming approach. The family-wise significance level is 0.025, with 0.000184 allocated at the interim analysis and 0.024816 at the final analysis.

For the key secondary endpoint OS, sample size is based on detecting a hazard ratio of 0.625 (median OS of 10 months versus 16 months, for placebo plus DTIC vs unesbulin plus DTIC, respectively) and a minimum 15-month follow-up. With 184 events (among 265 subjects) a 1-sided 0.025 level log-rank test will have approximately 84% power.

A separate group sequential design and monitoring will be performed for OS, with one interim analysis performed when at least 60% of the OS events occur and a final OS analysis when 184 events occur. The alpha allocation will be controlled by using the Lan-DeMets spending function that approximates the O'Brien-Fleming approach.

Data from the additional 45 subjects enrolled with at least 4 prior lines of therapy will not be included in the primary analysis; rather, the efficacy analysis of data from these subjects will be considered exploratory and included as a sensitivity analysis only.

8.3. Analysis Populations

Intent-to-treat (ITT) population: will include all randomized subjects. Subjects will be grouped based on the group they are randomized to. If a subject receives treatment in the group different from the one to which he/she was randomized, the subject's efficacy data will be analyzed "as randomized." The ITT population will be used as sensitivity analysis for all efficacy analysis.

Modified Intent-to-treat (mITT) population: will include randomized subjects with 3 or less line of therapy. The mITT population will be used in all the efficacy analysis, including analyses of the primary and secondary endpoints. Subjects will be grouped based on the group they are randomized to. If a subject receives treatment in the group different from the one to which he/she was randomized, the subject's efficacy data will be analyzed "as randomized."

Safety population: will include all subjects who received at least one dose of study drug (unesbulin/placebo or DTIC). If a subject receives treatment in the group different from the one to which he/she was randomized, the subject's safety data will be analyzed "as treated." The Safety population will be used in the statistical analyses for safety.

PK population: will include safety population subjects who have at least one plasma concentration of unesbulin, DTIC, or AIC.

8.4. Statistical Analyses

8.4.1. General Approach

Summary tables for continuous variables will contain the following statistics: N, mean, median, standard deviation, standard error, minimum, maximum, and 95% CIs as appropriate. Summary tables for categorical variables will include N and percentage in each category. Graphical techniques will be used when such methods are appropriate and informative. By-subject listings will be created.

Transformations of the data may be explored if warranted by the distribution of the data.

8.4.2. Analysis of Primary Efficacy Endpoints

The primary endpoint in this study is PFS. PFS is defined as the time from the randomization date to the date of the first documented tumor progression as determined by the independent, blinded radiologist using RECIST 1.1 or death due to any cause, whichever occurs first. Clinical deterioration in the absence of unequivocal evidence of progression (per RECIST 1.1) is not considered progression for purposes of determining PFS. Subjects who die without a reported prior progression will be considered to have progressed on the date of their death. Subjects who did not progress or die will be censored on the date of their last evaluable tumor assessment if they did not receive subsequent anticancer therapy (including on-treatment palliative radiotherapy of non-target bone lesions, skin lesions, or CNS lesions). Subjects who received any subsequent anticancer therapy without a prior reported progression will be censored at the last evaluable tumor assessment before or on the date of initiation of the subsequent anticancer therapy.

All hypotheses testing will be one-sided based on a significance level of 0.025 based on the mITT population.

The distribution of PFS will be compared in the unesbulin and placebo groups via a log-rank test. For subjects with 1 to 3 prior lines of systemic therapy, this will be stratified by prior lines of therapy (1 versus >1), ECOG PS score (0 versus 1), and LMS origin (uterine versus nonuterine). For subjects with at least 4 prior lines of systemic therapy, this will be stratified by ECOG PS score (0 versus 1) and LMS origin (uterine versus nonuterine). The hazard ratio and the corresponding 95% CI will be estimated in a stratified Cox proportional hazards model using randomized group as the single covariate. The PFS curves for each randomized group will be estimated using the Kaplan-Meier (KM) product-limit method. Two-sided, 95% CIs for median PFS will be computed by Brookmeyer and Crowley method (using log-log transformation). PFS rates at 3, 6, 12, 18, 24, and 30 months will also be estimated using KM estimates on the PFS curve for each randomized group. Associated two-sided 95% CIs will be calculated using the Greenwood formula.

8.4.3. Analysis of Key Secondary Efficacy Endpoint

The key secondary endpoint is OS. OS is defined as the time from the randomization date to the date of death from any cause. For subjects who are alive, survival time will be censored at the date of last contact ("last known alive date").

OS will be compared between the two treatment groups, provided the primary endpoint PFS is statistically significant favoring the test treatment arm. A hierarchical testing procedure will be utilized.

Two analyses are planned for OS: the first (interim) at the time of the final analysis for PFS (provided PFS is significant), at which point the information fraction for OS will be at least 60% and a second (final) analysis when 184 events have accumulated. Details will be provided in the study SAP.

8.4.4. Analysis of Other Secondary Efficacy Endpoints

Other secondary efficacy endpoints include ORR, DCR or CBR, and DoR.

ORR is defined as the proportion of subjects who achieve a confirmed best response of CR or PR using RECIST 1.1 as per independent radiologist assessment. BOR is defined as the best response designation recorded between randomization date and the date of objectively documented progression per RECIST 1.1 or the date of initiation of subsequent therapy or palliative local therapy, whichever occurs first. For subjects without documented progression or subsequent therapy or palliative local therapy, all available response designations will contribute to the BOR determination.

DCR or CBR is defined as the proportion of subjects with BOR of CR, PR, or at least 3 months of SD.

DoR is defined as the time from the date of first confirmed response to the date of the first documented tumor progression (per RECIST 1.1) or death due to any cause, whichever occurs first. Subjects who neither progress nor die will be censored on the date of their last evaluable tumor assessment. Subjects who started any subsequent anticancer therapy without a prior reported progression will be censored at the last evaluable tumor assessment before or on the date of initiation of the subsequent anticancer therapy. DoR will be evaluated for responders only.

The same statistical models applied to the analysis of PFS will be applied to the analysis of OS (Section 8.4.2).

BOR will be summarized by response category for each treatment group. ORR will be computed in each treatment group along with the exact 95% CI using Clopper-Pearson method. An estimate of the difference in ORRs and corresponding 95% CI will be calculated using Cochran-Mantel-Haenszel methodology and adjusted by the same stratification factors as in primary analysis of PFS.

DCR will be analyzed the same way as ORR.

DoR in each treatment group will be estimated using KM product-limit method for subjects who achieve PR or CR. Median values along with two-sided 95% CI will be calculated.

8.4.5. Safety Analyses

The safety analysis will be performed for all treated subjects. Descriptive statistics of safety will be presented using NCI CTCAE V5.0 by treatment group. All TEAEs will be tabulated by system organ class and MedDRA preferred term. On-study laboratory abnormalities including hematology and chemistry will be summarized using worst grade per NCI CTCAE V5.0 criteria.

8.4.5.1. Adverse Events

AEs will be tabulated using the MedDRA classification system. The frequency of subjects experiencing a specific AE will be tabulated by treatment group, system organ class, preferred term, seriousness (nonserious versus serious), worst severity, outcome, and relationship to study drug. Subject with AEs leading to death or to discontinuation from treatment, and SAEs will be listed.

An AE will be defined as treatment emergent if one of the following criteria is met:

- A new AE has an onset date on or after the date that study drug (unesbulin/placebo or DTIC) is first administered through 30 days after the last dose of study drug.
- A preexisting AE worsens in severity on or after the date that study drug is first administered through 30 days after the last dose of study drug.

8.4.5.2. Laboratory Abnormalities

The severity of laboratory abnormalities will be graded using the NCI CTCAE V5.0 whenever possible. The frequency of subjects experiencing a specific laboratory abnormality will be tabulated by treatment group and worst severity. In addition, the number and percentage of subjects experiencing a specific laboratory abnormality will be tabulated similarly.

8.4.5.3. Other Safety Assessments

The results of vital sign measurements, body weight assessments, ECG assessments, ECOG PS score determinations, and screening/baseline physical examination will be summarized by treatment group by using appropriate descriptive statistics.

8.4.6. Baseline Descriptive Statistics

8.4.6.1. Disposition

Subjects who did not complete the treatment or study will be summarized. Reasons for treatment/study discontinuation will be summarized.

8.4.6.2. Demographics and Baseline Characteristics

Subject demographics and baseline characteristics at study entry will be summarized with frequency tables for categorical variables and descriptive statistics such as the mean, standard deviation, median, and range, as appropriate, for quantitative variables.

8.4.6.3. Medical History and Prior Medication

Medical history and prior medication information will be summarized by treatment group.

8.4.7. Extent of Exposure, Treatment Compliance, and Concomitant Medications

Exposure to study drug will be summarized descriptively by treatment group.

Treatment compliance will be assessed in terms of the percentage of drug actually taken relative to the amount that should have been taken during the study.

Concomitant medication information will be summarized by treatment group.

8.4.8. Exploratory Analyses

8.4.8.1. Pharmacokinetics

Unesbulin, DTIC, and AIC plasma concentrations will be determined by the bioanalytical laboratory at ICON, Lenexa, Kansas, USA, using validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) methods.

PK parameters for unesbulin, DTIC, and AIC will be calculated using the actual sample collection times. PK variables will be calculated from the plasma concentration data using standard compartmental or noncompartmental methods, as appropriate for the data. Variables of interest include T_{max}, C_{max}, area under the concentration-time curve (AUC), $T_{½}$ for all compounds (ie, unesbulin, DTIC, and AIC), and clearance (CL) and volume of distribution (Vz) for DTIC only. Plasma concentrations and PK parameter results will be presented in tabular and graphic form by cycle and dose level.

8.4.8.2. Patient-Reported Outcomes

HRQoL will be assessed using the EORTC QLQ-C30 and the EQ-5D-5L. PROs will be summarized descriptively.

8.4.8.3. Biomarkers

Biomarkers will be summarized descriptively by treatment group. Relationship of biomarkers with PFS and OS will be explored.

8.4.9. Planned Interim Analysis

One interim analysis is planned when approximately 36% of PFS events occur (approximately 88 events). For additional details, refer to Section 8.2.

8.4.10. Subgroup Analyses

The primary endpoint (PFS), key secondary endpoint (OS), and ORR will be summarized by subgroups of the stratification factors using the mITT population.

Efficacy endpoints for the subgroup of subjects with at least 4 prior lines of treatment will be analyzed separately.

9. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

9.1. Regulatory, Ethical, and Study Oversight Considerations

9.1.1. Ethics

9.1.1.1. Ethical Conduct of the Study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/GCP, applicable regulatory requirements, and the PTC's policy on Bioethics.

9.1.1.2. Institutional Review Board

The final study protocol, including the final version of the ICF, must be approved or given a favorable opinion in writing by an IRB or IEC as appropriate. In addition, the IRB or IEC must approve all advertising used to recruit patients for the study. The investigator must submit written approval to PTC before he or she can enroll any subject into the study. All materials approved by the IRB for this study including the ICF and recruitment materials must be maintained by the investigator and made available for inspection.

The investigator is responsible for informing the IRB or IEC of any amendment to the protocol in accordance with local requirements. The protocol must be re-approved by the IRB or IEC upon receipt of amendments and annually, as local regulations require.

The investigator is also responsible for providing the IRB with reports of any reportable serious adverse drug reactions from any other study conducted with the investigational product. PTC will provide this information to the investigator.

Progress reports and notifications of serious adverse drug reactions will be provided to the IRB or IEC according to local regulations and guidelines.

9.1.1.3. Informed Consent

By signing the protocol, the investigator assures that informed consent will be obtained from each subject before study entry and that the informed consent will be obtained in accordance with current regulations.

The investigator or site delegate will give each subject full and adequate verbal and written information regarding the objectives and procedures of the study and the possible risks involved. An informed consent document will be provided to each subject in a language in which the subject is fluent. This information must be provided to the subject before undertaking any study-related procedure. Adequate time should be provided for the subject to read the informed consent, to understand the risks and benefits of participating in the study, and to ask any questions that the subject may have about the study. The subject should be able to ask additional questions as and when needed during the conduct of the study. The subject's signature on the ICF should be obtained at the investigator site in the presence of the investigator or a qualified representative (eg, site delegate).

Each subject will be given a copy of the signed consent form. The original signed ICFs will be retained by the investigator with the study records.

The written subject information must not be changed without prior approval by PTC and the IRB.

9.1.2. Study Discontinuation and Closure

PTC reserves the right to discontinue the study before inclusion of the intended number of subjects. The investigator, after consultation with the PTC Medical Monitor, reserves the right to discontinue the study at the investigator site for safety reasons at any time.

After a decision to terminate the study, investigators must contact all subjects who are continuing their participation in the study and must do so within a period set by PTC. As directed by PTC, all study materials must be collected, and all electronic data entry forms completed to the greatest extent possible.

9.1.3. Confidentiality and Privacy

Research records will be collected and stored in a manner that protects the confidentiality of subject information. The names and identities of all research subjects will be kept in strict confidence and will not appear on eCRFs or other records provided to or retained by PTC (or its authorized designee). The names and identities of the subjects need not be divulged; however, the records must nevertheless be inspected. This will be accomplished by blanking out the subject's name and replacing the name with the subject's study identification number on any record provided to or retained by PTC. The ICF must include appropriate statements explaining these requirements.

By signing this protocol, the investigator affirms to PTC that the investigator will maintain, in confidence, information furnished by PTC and will divulge such information to the IRB under an appropriate understanding of confidentiality with such board.

9.1.4. Data Monitoring Committee

External oversight for this study will be provided by a DMC. The primary responsibility of the DMC is to protect the safety and welfare of subjects participating in this clinical study and to ensure the integrity of the clinical study. To maintain the blinding and integrity of the study, procedures will be implemented to ensure the DMC and independent statistician have sole access to unblinded safety data.

Specifically, for this study, the DMC will be responsible for:

- Examining accumulated safety data and compliance data to make recommendations concerning continuation, termination, or modification of the study based on the safety of the interventions under study
- Reviewing the general progress of the study including accrual, protocol violations, and study conduct

The DMC will review the interim efficacy and safety data as described in Section 8.2. The DMC may review the efficacy and safety data at other times as warranted by emerging results. Based on review of the efficacy and safety data, the DMC can recommend continuation of the study unchanged, study interruption, study termination, modification of the study, or alteration. Full details of the DMC procedures, including primary responsibilities, will be documented in a DMC Charter.

9.1.5. Data Handling and Recordkeeping

To enable evaluations and/or audits from regulatory authorities or PTC, the investigator agrees to keep accurate and complete records, including the identity of all participating subjects (sufficient information to link eCRFs and clinic records/source documents), all original signed ICFs, electronic copies (ie, CD-ROM, USB, etc.) or paper copies of the data that have been captured in the eCRF for each subject, and detailed records of study drug disposition. All records and documents pertaining to the study will be maintained by the investigator until notification is received from PTC that the records no longer need to be retained.

The investigator must obtain written permission from PTC before disposing of any records. The investigator will promptly notify PTC in the event of accidental loss or destruction of any study records. If the investigator relocates, retires, or for any reason withdraws from the study, the study records may be transferred to an acceptable designee, such as another investigator, another institution, or to PTC, as applicable.

If it becomes necessary for PTC or the Regulatory Authority to review any documentation relating to the study, the investigator must permit access to such records.

9.1.6. Direct Access to Source Data/Documents

9.1.6.1. Study Monitoring

In accordance with 21 Code of Federal Regulations Part 312.56 and/or relevant ICH guidelines, PTC or a designee will periodically inspect all eCRFs, study documents, research facilities, and clinical laboratory facilities associated with this study at mutually convenient times, before, during, and after completion of the study. As required by applicable regulations (Responsibilities of Sponsors and Investigators), the monitoring visits provide PTC with the opportunity to evaluate the progress of the study; verify the accuracy and completeness of data in the eCRFs; ensure that all protocol requirements, relevant regulations, and investigator's obligations are being fulfilled; and resolve any inconsistencies in the study records. This includes inspection of all documents and records required to be maintained by the investigator, including but not limited to medical records (office, clinic, or hospital) for the subjects in this study. The names and identities of all research subjects will be kept in strict confidence and will not appear on eCRFs or other records provided to or retained by PTC.

The investigator/institution guarantees direct access to source documents, the drug storage area, drug accountability records, and other study-related records and areas by PTC and appropriate regulatory authorities. It is important that the investigator and relevant institutional personnel are available during the monitoring visits and possible audits or inspections, and that sufficient time is devoted to the process.

9.1.6.2. Audits and Inspections

To ensure compliance with GCP and all applicable regulatory requirements, PTC, PTC's representatives, a regulatory authority, or IRB may conduct a quality assurance audit. Reasons for quality assurance audit may include but are not limited to random selection, geographic proximity, suspected GCP violation, high enrolling site, recurring protocol deviations, etc. The purpose of a PTC audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, GCP guidelines of the ICH, and any applicable regulatory requirements. The investigator should contact PTC immediately if contacted by a regulatory agency about an inspection.

9.1.7. Future Use of Stored Specimens and Data

The ICF will contain a separate section that addresses the use of remaining mandatory samples for exploratory research. The investigator or authorized designee will explain to each subject the objectives of the exploratory research and make clear that their participation is optional.

PTC will have access to de-identified subject data regarding mutations that are identified through genetic sequencing of tumor biopsy(ies).

9.1.8. Quality Control and Quality Assurance

To ensure compliance with GCP and all applicable regulatory requirements, PTC may conduct a quality assurance audit. Please see Section 9.1.6.2 for more details regarding the audit process.

9.1.9. Protocol Deviations

A protocol deviation is defined as any intentional or unintentional change to, or noncompliance with, the approved protocol procedures or requirements. Deviations may result from the action or inaction of the subject, investigator, or site staff. Examples of deviations include, but are not limited to:

- Failure to adhere to study exclusion and inclusion criteria
- Failure to comply with dispensing or dosing requirements
- Use of medications that are specifically prohibited in the protocol
- Missed or out-of-window visits
- Drug dosing not administered within the time frame specified in the protocol
- Failure to adhere to test requirements, including vital signs, laboratory tests, physical examinations, PK blood draws, medical history, etc—either tests not done, incorrect tests done, or not done within the time frame specified in the protocol
- Procedural deviations such as incorrect storage of study drug, failure to update the ICF when new risks become known, or failure to obtain IRB approvals for the protocol and ICF revisions

At the outset of the study, a process for defining and handling protocol deviations will be established with the site. This will include determining which deviations will be designated significant; thus, requiring immediate notification to the PTC Medical Monitor and/or other PTC designee.

Prospective deviations (eg, protocol waivers) are prohibited per PTC policy.

Significant deviations are any deviations that impact subject eligibility (ie, protocol inclusion/exclusion violations), subject safety, or a subject's ability to continue in the clinical study.

The investigator is responsible for seeing that any known protocol deviations are recorded and handled as agreed.

9.1.10. Publication Policy

The information developed during the conduct of this clinical study is considered confidential by PTC. This information may be disclosed as deemed necessary by PTC.

PTC intends that the data from this study will be presented and published. The PTC staff under the direction of the PTC or designee in collaboration with the investigator will be responsible for writing presentations and manuscripts for publication. Investigators will not be allowed to publish or present the data from this study without prior agreement with PTC.

The investigator is obliged to provide PTC with complete test results and all study data. During the study, only PTC may make study information available to other study investigators or to regulatory agencies, except as required by law or regulation. Except as otherwise allowable in the Clinical Study Site Agreement, any public disclosure (including publicly accessible websites) related to the protocol or study results, other than study recruitment materials and/or advertisements, is the sole responsibility of PTC.

PTC may publish any data and information from the study (including data and information generated by the investigator) without the consent of the investigator. Manuscript authorship for any peer-reviewed publication will appropriately reflect contributions to the production and review of the document. All publications and presentations must be prepared in accordance with this section and the Clinical Study Site Agreement. In the event of any discrepancy between the protocol and the Clinical Study Site Agreement, the Clinical Study Site Agreement will prevail.

Data from all sites participating in the study will be pooled and analyzed by PTC or PTC's designee. The first publication of the study results shall be made in conjunction with the results from other study sites as a multicenter publication. If a multicenter publication is not forthcoming within 24 months of completion of the study at all sites, the investigator may publish or present the results generated at his or her site.

The investigator will provide PTC with a copy of any proposed publication or presentation for review and comment at least 60 days before such presentation or submission for publication. PTC shall inform the investigator in writing of any changes or deletions in such presentation or publication required to protect PTC's confidential and proprietary technical information and to address inaccurate data or inappropriate interpretations in the context of any pooled multicenter results. At the expiration of such 60-day period, the investigator may proceed with the presentation or submission for publication unless PTC has notified the institution or the investigator in writing that such proposed publication or presentation discloses PTC's confidential and proprietary technical information. Further, upon the request of PTC, the investigator will delay the publication or presentation for an additional 90 days to permit PTC to take necessary actions to protect its intellectual property interests.

9.2. Protocol Amendment History

Original Protocol Issued: 20 December 2021

Version 2.0: 31 January 2022

Version 3.0: 28 April 2023

Version 4.0: 28 November 2023

9.2.1. Version 2.0: 31 January 2022

Overall reason for Version 2.0: The overall reason for Version 1.0 of the protocol was to modify the timing and description of the interim analysis for futility and efficacy evaluation.

Table 7: Summary of Changes for Global Amendment Version 1.0 to Version 2.0

Item No.	Protocol Section	Summary of Change	Reason/ Rationale
1	Protocol	The version number and date were updated throughout. The synopsis was updated to be consistent with the changes in the protocol.	Update
2	Signature Page	The company title was updated for a Responsible Medical Officer.	Update
2	Schedule of Assessments	A "X" was added in the Screening column to indicate that the ECOG assessment is to be done as part of Screening.	Clarification
3	Table 2	The lower limit of Grade 1 thrombocytopenia platelet count was changed from 750000 to 75000.	Clarification
4	Synopsis, Section 8.2 Section 8.4.9 Section 9.1.4	The timing and description of the interim analysis for futility and efficacy evaluation were modified.	Update

Abbreviations: ECOG, Eastern Cooperative Oncology Group

9.2.2. Version 3.0: 28 April 2023

Overall reason for Version 3.0: The overall reason for Version 3.0 of the protocol is to incorporate comprehensively all modifications requested by country-specific health authorities and Ethics Committees in response to the Clinical Trial Application. Additional updates were made for improved clarity and study conduct. A summary of changes from global Version 2.0 to Version 3.0 is presented below, along with country-specific summaries of changes from the latest local amendment to Version 3.0.

Table 8: Summary of Changes From Global Protocol Version 2.0 to Version 3.0

Item No.	Protocol Section	Summary of Change	Reason/ Rationale
1	Protocol	The version number and date were updated throughout. The synopsis was updated to be consistent with the changes in the protocol. The title page was modified to conform with the new clinical trial protocol template.	Update
2	Global Update	Minor editorial changes were made throughout for grammar and clarity.	Update
3	Global Update	Considering the study is blinded, “placebo” was added to statements pertaining to the administration and handling of both unesbulin and placebo, where applicable.	Update/ clarification
4	Protocol Identifiers and Study Personnel	Changes in PTC study staff and contact information.	Update
5	Synopsis, Section 2.5, Section 8.4.3, and Section 8.4.10	It was specified that OS is the key secondary endpoint. Additional details describing the analysis of this key secondary endpoint were added to the Synopsis, Section 8.4.3, and Section 8.4.10.	Health authority request
6	Synopsis, Section 2.5, and Section 8.4.4	Clarified that at least 3 months of stable disease was required in the definition of DCR and CBR.	Clarification
7	Synopsis, Section 3.1, and Section 7.4	Clarified that approximately 12 subjects will be assessed for PK.	Clarification
8	Synopsis and Section 3.1	The DMC will not review the interim data for sample size re-evaluation; this has been clarified. The timing for the DMC meetings was removed and replaced with a reference to the DMC charter. It was clarified that the interim efficacy analysis will be performed by an external vendor.	Update and clarification
9	Synopsis, Schedule of Assessments, and Section 3.3	It was clarified that no dose reduction of unesbulin/placebo is permitted. For subjects who have interruptions in DTIC due to toxicities, detailed instructions on timing of assessments relative to continued unesbulin/placebo dosing were added to the protocol.	Clarification
10	Synopsis and Section 4.1	An exception to Inclusion Criterion #10 was added for patients with Gilbert’s syndrome who have elevated bilirubin values.	Update based on feedback from investigators
11	Synopsis and Section 4.1	Inclusion Criterion #11 was modified to increase the eligibility limit for AST and ALT from 1.5 to 3 times the ULN.	Update based on feedback from investigators
12	Synopsis and Section 4.1	It was clarified in Inclusion Criterion #16 that prior lines of treatment may include but are not limited to single-agent doxorubicin or other anthracycline, doxorubicin plus ifosfamide, trabectedin, pazopanib, or gemcitabine with or without docetaxel.	Health authority request

Item No.	Protocol Section	Summary of Change	Reason/ Rationale
13	Synopsis Inclusion Criterion #19, Section 4.1, and Section 7.3.11	Contraception or abstinence was extended from 90 days to 6 months for WOCBP as per CTFG guidance, Section 2.2.2, for products with demonstrated or suspected human teratogenicity/fetotoxicity. Pregnancy reporting was updated accordingly.	Align with CTFG guidelines and DTIC Technical Data Sheet
14	Synopsis Inclusion Criterion #21, Section 4.1, and Section 7.3.11	Contraception, including avoidance of sperm donation, was extended from 90 days to 6 months for men as per the Technical Data Sheet for DTIC. Pregnancy reporting for female partners of male subjects were updated accordingly.	Align with DTIC Technical Data Sheet
15	Synopsis and Section 4.2	Exclusion Criterion #5: Removed “known” prior to human immunodeficiency virus, hepatitis B virus, or hepatitis C virus positivity because viral testing will be required for all subjects at screening.	Clarification
16	Synopsis, Section 4.2, and Section 5.5	The criterion excluding the concomitant use of NSAIDs was deleted because the potential risk for gastrointestinal perforation was mitigated by adding gastritis to Exclusion Criterion #9. A statement was added to the Concomitant Medication Section 5.5 as a reminder that subjects should not have active peptic ulcer disease, active gastritis, or previous history of gastric perforation within the last 2 years.	Update based on feedback from investigators
17	Synopsis, Section 4.2, and Section 5.5	Exclusion Criterion #15 was modified to provide clarifications on the chronic use of marijuana.	Clarification
18	Synopsis, Section 4.2, and Section 5.5	Administration of live vaccines was added as an Exclusion Criterion (#17) and as a concomitant medication.	Health authority request
19	Synopsis, Section 4.2	APTT/PT assessments were added to Exclusion Criterion #19 to monitor subjects on anticoagulation where applicable. It was clarified that coagulation tests should fall within the required range (instead of “normal”) considering the patient disease and demographic characteristics.	Health authority request and investigator feedback
20	Synopsis, Section 3.1, and Section 5.2	Based on the results from a food effect assessment in the ongoing Phase 1b Study PTC596-ONC-007-LMS, it is recommended that unesbulin/placebo be administered with food.	Update
21	Synopsis and Section 5.1	PTC may provide DTIC to sites that are unable to procure it locally.	Procedural change
22	Synopsis and Section 5.1	Instructions to reconstitute DTIC were added to accommodate variation in institutional policies.	Update
23	Synopsis and Section 5.2	The window to administer DTIC was widened from 1 hour ± 5 minutes to 1 hour ± 30 minutes to allow sites more flexibility to administer this study drug.	Procedural change
24	Synopsis and Section 7.1	To align with the Schedule of Assessments, clarified that after Cycle 8, scans may be performed every 9 weeks.	Clarification

Item No.	Protocol Section	Summary of Change	Reason/ Rationale
25	Synopsis, Schedule of Assessments (footnote “k”), and Section 7.1	It was clarified that either MRI or CT could be used as clinically indicated.	Health authority request
26	Schedule of Assessments, Figure 1, Section 3.1, and Section 6.1.1	To accommodate patient management and care and AE stabilization (as applicable), a more flexible timeframe for the EOT visit was provided.	Update
27	Schedule of Assessments	For urinalysis, an “X” was added to EOT.	Update
28	Schedule of Assessments	Footnote “f”: It was clarified that weight must be assessed on the day of DTIC administration.	Clarification
29	Schedule of Assessments	Footnote “g”: It was clarified that local laboratory testing is acceptable. The window of clinical laboratory testing on Day 1 of Cycle 1 prior to the first dose of study drug was extended to up to 72 hours to allow sites flexibility over weekends. After Cycle 3, monthly clinical laboratory testing will be adjusted in the protocol so that sampling will be performed once every cycle instead of on a weekly basis. The urinalysis assessment in the footnote was an error; this was replaced with a CMP to be performed at the same frequency as the CBC assessments. Instructions for performing laboratory assessments were added in case DTIC is interrupted/held due to toxicity.	Request by participating sites to decrease subject and staff burden
30	Schedule of Assessments	For serum pregnancy testing, an “X” was deleted from Day 5 of Cycle 2 and added to Day 1 of Cycle 2. A pregnancy test prior is required within 24 hours or dosing on C1D1, and this may be assessed with a urine test. In footnote “h”, it was also clarified that a urine or serum pregnancy test should be performed 24 hours prior to dosing on Day 1 of Cycle 2 and continuing every 2 cycles beginning with Cycle 4.	Clarification and health authority request
31	Schedule of Assessments	Footnotes “i” and “j”: Instructions were added for performing the single ECG in cases if/when DTIC is interrupted or held. ECGs will not be required after Cycle 3.	Clarification and procedural change based on cumulative safety data in the clinical program
32	Schedule of Assessments	The following text in footnote “j” was deleted: “On Day 1 of all other cycles and at the time of collection for sparse PK samples, approximately 2 hours after DTIC infusion.” and was replaced with “Approximately 2 hours after DTIC infusion on Cycle 2.”	Clarification

Item No.	Protocol Section	Summary of Change	Reason/ Rationale
33	Synopsis, Schedule of Assessments (footnote “m”), and Section 7.6	Timing and mandatory collection of biomarker samples was clarified. Footnote “m” was updated as follows: “Blood samples (10 mL) to assess circulating biomarkers will be collected at screening (predose) and prior to dosing on Day 1 of Cycle 3 and at the time of disease progression or EOT. Samples will be processed, stored, and shipped according to instructions in the laboratory manual.”	Clarification
34	Schedule of Assessments	Footnote “n”: Instructions for performing PK sampling in case of DTIC interruption/hold were added.	Clarification
35	Schedule of Assessments (footnote “o”) and Section 5.4	Subjects are encouraged to bring back the patient diary at all scheduled visits.	Procedural change
36	Schedule of Assessments and Section 5.2	Footnote “p”: Reference to Section 3.3 was added for instructions on DTIC dosing in case of toxicity.	Clarification
37	Schedule of Assessments	Footnote “q”: Instructions were added for administering unesbulin/placebo in case of DTIC interruption.	Clarification
38	Section 1.1	A reference to the ESMO-EURACAN-GENTURIS (European Society for Medical Oncology; European Reference Network for Rare Adult Solid Cancers; European Reference Network for Genetic Tumour Risk Syndromes) Clinical Practice Guidelines by Gronchi et al was added.	Health authority request
39	Section 1.3	A statement was added in Section 1.3 referring investigators to the Investigator’s Brochure for additional information and guidance on mitigating potential drug-drug interactions.	Health authority request
40	Schedule of Assessments and Section 3.3	Procedural guidelines for DTIC interruptions were added. Instructions for modifying dose of DTIC in the case of nonhematological toxicities were added. It was clarified that the dose of unesbulin/placebo should not be reduced or withheld.	Clarification and Health authority request
41	Section 3.4	A definition of the end of study was added to the protocol.	Health authority request
42	Section 5.2	Instructions were added for subjects who cannot swallow the whole tablet. Specifically, tablets can be dispersed in water at the same dose level at which the tablets are being dosed and be administered orally. Instructions were added to provide guidance in cases of a subject who misses a dose of unesbulin/placebo or vomits after unesbulin/placebo dosing. It was clarified that coadministration of acid-reducing agents should be avoided approximately 4 hours before and after unesbulin/placebo administration to make instructions in this section consistent with other sections.	Clarifications and process updates

Item No.	Protocol Section	Summary of Change	Reason/ Rationale
43	Section 5.5	It was added that neither approved nor guideline-recommended therapy options should be withheld from subjects if the investigator determines it is in the best interest of the subject. Immunization with live vaccines should be avoided. Updated guidelines were added for mitigating potential BCRP, OATP1B1, and OATP2B2 interactions with unesbulin. Clarifications on chronic use of marijuana was added, along with a reminder on enrolling subjects with active peptic ulcer disease, active gastritis, or history of gastric perforation within the last 2 years.	Update based on new data and Health authority request
44	Section 5.6.1	The following statement was removed: “Before unblinding, the investigator must make every attempt to discuss the intended code break with PTC.”	Health authority request
45	Synopsis and Section 7.1	It was clarified that survival follow-up will continue every 3 months as per the Schedule of Assessments.	Clarification
46	Section 7.3.1 and Section 7.3.2	AE and SAE definitions revised as per PTC template language; revisions had no effect on reporting.	Update
47	Section 7.3.3	The locations of the Reference Safety Information for unesbulin and DTIC were clarified.	Health authority request
48	Section 7.3.9	Serious Adverse Events Reporting: the existing “within 24 hours” was updated to “immediately but no later than 24 hours”	Health authority request
49	Section 8.4.4	Content that was previously in Section 8.4.3 was moved to this new section and entitled “Analysis of Other Secondary Efficacy Endpoints.”	Update
50	Section 8.4.5.1	Treatment-emergent adverse events were defined.	Update
51	Section 9.1.1.3	It was clarified that the informed consent may be delegated to a site delegate and not limited to a subinvestigator.	Clarification
52	Section 9.1.7	It was clarified that a subject’s participation in the exploratory part of the study is optional.	Clarification

Abbreviations: AE, adverse event; ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; BCRP, breast cancer resistance protein; CBC, complete blood count; CBR, clinical benefit rate; CMP, complete metabolic panel; CT, computed tomography; CTFG, Clinical Trial Facilitation and Coordination Group; DCR, disease control rate; DMC, data monitoring committee; DTIC, dacarbazine; ECG, electrocardiogram; EOT, end of treatment; MRI, magnetic resonance imaging; NSAID, nonsteroidal anti-inflammatory drug; OATP1B1 and OATP1B2, organic anion transporter polypeptide transporters; OS, overall survival; PK, pharmacokinetic; PT, prothrombin time; SAE, serious adverse event; ULN, upper limit of normal; WOCBP, women of childbearing potential

Table 9: Germany: Summary of Changes From Local Amendment Version 2.3 to Global Amendment Version 3.0

Item No.	Protocol Section	Summary of Change	Reason/ Rationale
1	Protocol	The version number and date were updated throughout. The synopsis was updated to be consistent with the changes in the protocol. The title page was modified to conform with the new clinical trial protocol template.	Update
2	Global Update	Minor editorial changes were made throughout for grammar and clarity.	Update
3	Global Update	Considering the study is blinded, “placebo” was added to statements pertaining to the administration and handling of both unesbulin and placebo, where applicable.	Update/ clarification
4	Protocol Identifiers and Study Personnel	Changes in PTC study staff and contact information	Update
5	Synopsis, Section 2.5, Section 8.4.3, and Section 8.4.10	It was specified that OS is the key secondary endpoint. Additional details describing the analysis of this key secondary endpoint were added to the Synopsis, Section 8.4.3, and Section 8.4.10.	Health authority request
6	Synopsis, Section 2.5, and Section 8.4.3	Clarified that at least 3 months of stable disease was required in the definition of DCR and CBR.	Clarification
7	Synopsis, Section 3.1, and Section 7.4	Clarified that approximately 12 subjects will be assessed for PK.	Clarification
8	Synopsis and Section 3.1	The DMC will not review the interim data for sample size re-evaluation; this has been clarified. The timing for the DMC meetings was removed and replaced with a reference to the DMC charter. It was clarified that the interim efficacy analysis will be performed by an external vendor.	Update and clarification
9	Synopsis, Schedule of Assessments, and Section 3.3	It was clarified that no dose reduction of unesbulin/placebo is permitted. For subjects who have interruptions in DTIC due to toxicities, detailed instructions on timing of assessments relative to continued unesbulin/placebo dosing were added to the protocol.	Clarification
10	Synopsis and Section 4.1	An exception to Inclusion Criterion #10 was added for patients with Gilbert’s syndrome who have elevated bilirubin values.	Update based on feedback from investigators
11	Synopsis and Section 4.1	Inclusion Criterion #11 was modified to increase the eligibility limit for AST and ALT from 1.5 to 3 times the ULN.	Update based on feedback from investigators
12	Synopsis Exclusion Criterion #5 and Section 4.2	Removed “known” prior to human immunodeficiency virus, hepatitis B virus, or hepatitis C virus positivity because all subjects will be required for viral testing at screening.	Clarification

Item No.	Protocol Section	Summary of Change	Reason/ Rationale
13	Synopsis, Section 4.2, and Section 5.5	The criterion excluding the concomitant use of NSAIDs was deleted because the potential risk for gastrointestinal perforation was mitigated by adding gastritis to Exclusion Criterion #9. A statement was added to the Concomitant Medication Section 5.5 as a reminder that subjects should not have active peptic ulcer disease, active gastritis, or previous history of gastric perforation within the last 2 years.	Update based on feedback from investigators
14	Synopsis, Section 4.2, and Section 5.5	Exclusion Criterion #15 was modified to provide clarifications on the chronic use of marijuana.	Clarification
15	Synopsis and Section 4.2	It was clarified in Exclusion Criterion #19 that coagulation tests should fall within the required range (instead of “normal”) considering the patient disease and demographic characteristics.	Investigator feedback
16	Synopsis, Section 3.1, and Section 5.2	Based on the results from a food effect assessment in the ongoing Phase 1b Study PTC596-ONC-007-LMS, it is recommended that unesbulin/placebo be administered with food.	Update
17	Synopsis and Section 5.1	PTC will provide DTIC to sites that are unable to procure it locally.	Procedural change
18	Synopsis and Section 5.1	Instructions to reconstitute DTIC were added to accommodate variation in institutional policies.	Update
19	Synopsis and Section 5.2	The window to administer DTIC was widened from 1 hour ± 5 minutes to 1 hour ± 30 minutes to allow sites more flexibility to administer this study drug.	Procedural change
20	Synopsis and Section 7.1	To align with the Schedule of Assessments, clarified that after Cycle 8, scans may be performed every 9 weeks.	Clarification
21	Synopsis, Schedule of Assessments (footnote “k”), and Section 7.1	It was clarified that either MRI or CT could be used as clinically indicated.	Health authority request
22	Synopsis, Schedule of Assessments (footnote “m”), and Section 7.6	Timing and mandatory collection of biomarker samples was clarified. Footnote “m” was updated as follows: “Blood samples (10 mL) to assess circulating biomarkers will be collected at screening (predose) and prior to dosing on Day 1 of Cycle 3 and at the time of disease progression or EOT. Samples will be processed, stored, and shipped according to instructions in the laboratory manual.”	Clarification
23	Schedule of Assessments Figure 1, Section 3.1, and Section 6.1.1	To accommodate patient management and care and AE stabilization (as applicable), a more flexible timeframe for the EOT visit was provided.	Update
24	Schedule of Assessments	For urinalysis, an “X” was added to EOT and deleted from Day 1 of Cycle 2.	Update
25	Schedule of Assessments	Footnote “f”: It was clarified that weight must be assessed on the day of DTIC administration.	Clarification

Item No.	Protocol Section	Summary of Change	Reason/ Rationale
26	Schedule of Assessments	Footnote “g”: It was clarified that local laboratory testing is acceptable. The window of clinical laboratory testing on Day 1 of Cycle 1 prior to the first dose of study drug was extended to up to 72 hours to allow sites flexibility over weekends. After Cycle 3, monthly clinical laboratory testing will be adjusted in the protocol so that sampling will be performed once every cycle instead of on a weekly basis. The urinalysis assessment in the footnote was an error; this was replaced with a CMP to be performed at the same frequency as the CBC assessments. Instructions for performing laboratory assessments were added in case DTIC is interrupted/held due to toxicity.	Request by participating sites to decrease subject and staff burden
27	Schedule of Assessments	A pregnancy test prior is required within 24 hours of dosing on Day 1 of Cycle 1, and this may be assessed with a urine pregnancy test. In footnote “h”, it was also clarified that a urine or serum pregnancy test should be performed 24 hours prior to dosing on Day 1 of Cycle 2 and continuing every 2 cycles beginning with Cycle 4.	Clarification and health authority request
28	Schedule of Assessments	Footnotes “i” and “j”: Instructions were added for performing the single ECG in cases if/when DTIC is interrupted or held. ECGs will not be required after Cycle 3.	Clarification and procedural change based on cumulative safety data in the clinical program
29	Schedule of Assessments	The following text in footnote “j” was deleted: “On Day 1 of all other cycles and at the time of collection for sparse PK samples, approximately 2 hours after DTIC infusion.” and was replaced with “Approximately 2 hours after DTIC infusion on Cycle 2.”	Clarification
30	Schedule of Assessments	Footnote “n”: Instructions for performing PK sampling in case of DTIC interruption/hold were added.	Clarification
31	Schedule of Assessments (footnote “o”) and Section 5.4	Subjects are encouraged to bring back the patient diary at all scheduled visits.	Procedural change
32	Schedule of Assessments and Section 5.2	Footnote “p”: Reference to Section 3.3 was added for instructions on DTIC dosing in case of toxicity.	Clarification
33	Schedule of Assessments	Footnote “q”: Instructions were added for administering unesbulin/placebo in case of DTIC interruption.	Clarification
34	Section 1.3	A statement was added in Section 1.3 referring investigators to the Investigator’s Brochure for additional information and guidance on mitigating potential drug-drug interactions.	Health authority request

Item No.	Protocol Section	Summary of Change	Reason/ Rationale
35	Schedule of Assessments and Section 3.3	Procedural guidelines for DTIC interruptions were added. Instructions for modifying dose of DTIC in the case of nonhematological toxicities were added. It was clarified that the dose of unesbulin/placebo should not be reduced or withheld.	Clarification and Health authority request
36	Section 5.2	Instructions were added for subjects who cannot swallow the whole tablet. Specifically, tablets can be dispersed in water at the same dose level at which the tablets are being dosed and be administered orally. Instructions were added to provide guidance in cases of a subject who misses a dose of unesbulin/placebo or vomits after unesbulin/placebo dosing. It was clarified that coadministration of acid-reducing agents should be avoided approximately 4 hours before and after unesbulin/placebo administration to make instructions in this section consistent with other sections.	Clarifications and process updates
37	Synopsis and Section 7.1	It was clarified that survival follow-up will continue every 3 months as per the Schedule of Assessments.	Clarification
38	Section 7.3.1 and Section 7.3.2	AE and SAE definitions revised as per PTC template language; revisions had no effect on reporting.	Update
39	Section 8.4.4	Content that was previously in Section 8.4.3 was moved to this new section and entitled “Analysis of Other Secondary Efficacy Endpoints.”	Update
40	Section 8.4.5.1	Treatment-emergent adverse events were defined.	Update
41	Section 9.1.1.3	It was clarified that the informed consent may be delegated to a site delegate and not limited to a subinvestigator	Clarification
42	Section 9.1.7	It was clarified that a subject’s participation in the exploratory part of the study is optional.	Clarification

Abbreviations: AE, adverse event; ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; CBC, complete blood count; CBR, clinical benefit rate; CMP, complete metabolic panel; CT, computed tomography; CTFG, Clinical Trial Facilitation and Coordination Group DCR, disease control rate; DMC, data monitoring committee; DTIC, dacarbazine; ECG, electrocardiogram; EOT, end of treatment; MRI, magnetic resonance imaging; NSAID, nonsteroidal anti-inflammatory drug; OS, overall survival; PK, pharmacokinetic; PT, prothrombin time; SAE, serious adverse event; ULN, upper limit of normal; WOCBP, women of childbearing potential

Table 10: Italy: Summary of Changes From Local Amendment Version 2.1 to Global Amendment Version 3.0

Item No.	Protocol Section	Summary of Change	Reason/ Rationale
1	Protocol	The version number and date were updated throughout. The synopsis was updated to be consistent with the changes in the protocol. The title page was modified to conform with the new clinical trial protocol template.	Update
2	Global Update	Minor editorial changes were made throughout for grammar and clarity.	Update

Item No.	Protocol Section	Summary of Change	Reason/ Rationale
3	Global Update	Considering the study is blinded, “placebo” was added to statements pertaining to the administration and handling of both unesbulin and placebo, where applicable.	Update/ clarification
4	Protocol Identifiers and Study Personnel	Changes in PTC study staff and contact information	Update
5	Synopsis, Section 2.5, Section 8.4.3, , and Section 8.4.10	It was specified that OS is the key secondary endpoint. Additional details describing the analysis of this key secondary endpoint were added to the Synopsis, Section 8.4.3, and Section 8.4.10.	Health authority request
6	Synopsis, Section 2.5, and Section 8.4.4	Clarified that at least 3 months of stable disease was required in the definition of DCR and CBR.	Clarification
7	Synopsis, Section 3.1, and Section 7.4	Clarified that approximately 12 subjects will be assessed for PK.	Clarification
8	Synopsis and Section 3.1	The DMC will not review the interim data for sample size re-evaluation; this has been clarified. The timing for the DMC meetings was removed and replaced with a reference to the DMC charter. It was clarified that the interim efficacy analysis will be performed by an external vendor.	Update and clarification
9	Synopsis, Schedule of Assessments, and Section 3.3	It was clarified that no dose reduction of unesbulin/placebo is permitted. For subjects who have interruptions in DTIC due to toxicities, detailed instructions on timing of assessments relative to continued unesbulin/placebo dosing were added to the protocol.	Clarification
10	Synopsis and Section 4.1	An exception to Inclusion Criterion #10 was added for patients with Gilbert’s syndrome who have elevated bilirubin values.	Update based on feedback from investigators
11	Synopsis and Section 4.1	Inclusion Criterion #11 was modified to increase the eligibility limit for AST and ALT from 1.5 to 3 times the ULN.	Update based on feedback from investigators
12	Synopsis Exclusion Criterion #5 and Section 4.2	Removed “known” prior to human immunodeficiency virus, hepatitis B virus, or hepatitis C virus positivity because all subjects will be required for viral testing at screening.	Clarification
13	Synopsis, Section 4.2, and Section 5.5	The criterion excluding the concomitant use of NSAIDs was deleted because the potential risk for gastrointestinal perforation was mitigated by adding gastritis to Exclusion Criterion #9. A statement was added to the Concomitant Medication Section 5.5 as a reminder that subjects should not have active peptic ulcer disease, active gastritis, or previous history of gastric perforation within the last 2 years.	Update based on feedback from investigators

Item No.	Protocol Section	Summary of Change	Reason/ Rationale
14	Synopsis, Section 4.2, and Section 5.5	Exclusion Criterion #15 was modified to provide clarifications on the chronic use of marijuana.	Clarification
15	Synopsis, Section 4.2	Added APTT/PT assessments to Exclusion Criterion #19 to monitor subjects on anticoagulation where applicable. It was clarified that coagulation tests should fall within the required range (instead of "normal") considering the patient disease and demographic characteristics.	Health authority request
16	Synopsis, Section 3.1, and Section 5.2	Based on the results from a food effect assessment in the ongoing Phase 1b Study PTC596-ONC-007-LMS, it is recommended that unesbulin/placebo be administered with food.	Update
17	Synopsis and Section 5.1	PTC may provide DTIC to sites that are unable to procure it locally.	Procedural change
18	Synopsis and Section 5.1	Instructions to reconstitute DTIC were added to accommodate variation in institutional policies.	Update
19	Synopsis and Section 5.2	The window to administer DTIC was widened from 1 hour ± 5 minutes to 1 hour ± 30 minutes to allow sites more flexibility to administer this study drug.	Procedural change
20	Synopsis and Section 7.1	To align with the Schedule of Assessments, clarified that after Cycle 8, scans may be performed every 9 weeks.	Clarification
21	Synopsis, Schedule of Assessments (footnote "k"), and Section 7.1	It was clarified that either MRI or CT could be used as clinically indicated.	Health authority request
22	Synopsis, Schedule of Assessments (footnote "m"), and Section 7.6	Timing and mandatory collection of biomarker samples was clarified. Footnote "m" was updated as follows: "Blood samples (10 mL) to assess circulating biomarkers will be collected at screening (predose) and prior to dosing on Day 1 of Cycle 3 and at the time of disease progression or EOT. Samples will be processed, stored, and shipped according to instructions in the laboratory manual."	Clarification
23	Schedule of Assessments, Figure 1, Section 3.1, and Section 6.1.1	To accommodate patient management and care and AE stabilization (as applicable), a more flexible timeframe for the EOT visit was provided.	Update
24	Schedule of Assessments	For urinalysis, an "X" was added to EOT and deleted for Day 1 of Cycle 2.	Update
25	Schedule of Assessments	A pregnancy test prior is required within 24 hours of dosing on Day 1 of Cycle 1, and this may be assessed with a urine pregnancy test. In footnote "h", it was also clarified that a urine or serum pregnancy test should be performed 24 hours prior to dosing on Day 1 of Cycle 2 and continuing every 2 cycles beginning with Cycle 4.	Clarification and health authority request

Item No.	Protocol Section	Summary of Change	Reason/ Rationale
26	Schedule of Assessments	Footnote “f”: It was clarified that weight must be assessed on the day of DTIC administration.	Clarification
27	Schedule of Assessments	Footnote “g”: It was clarified that local laboratory testing is acceptable. The window of clinical laboratory testing on C1D1 prior to the first dose of study drug was extended to up to 72 hours to allow sites flexibility over weekends. After Cycle 3, monthly clinical laboratory testing will be adjusted in the protocol so that sampling will be performed once every cycle instead of on a weekly basis. The urinalysis assessment in the footnote was an error; this was replaced with a CMP to be performed at the same frequency as the CBC assessments. Instructions for performing laboratory assessments were added in case DTIC is interrupted/held due to toxicity.	Request by participating sites to decrease subject and staff burden
28	Schedule of Assessments	Footnotes “i” and “j”: Instructions were added for performing the single ECG in cases if/when DTIC is interrupted or held. ECGs will not be required after Cycle 3.	Clarification and procedural change based on cumulative safety data in the clinical program
29	Schedule of Assessments	The following text in footnote “j” was deleted: “On Day 1 of all other cycles and at the time of collection for sparse PK samples, approximately 2 hours after DTIC infusion.” and was replaced with “Approximately 2 hours after DTIC infusion on Cycle 2.”	Clarification
30	Schedule of Assessments	Footnote “n”: Instructions for performing PK sampling in case of DTIC interruption/hold were added.	Clarification
31	Schedule of Assessments (footnote “o”) and Section 5.4	Subjects are encouraged to bring back the patient diary at all scheduled visits.	Procedural change
32	Schedule of Assessments and Section 5.2	Footnote “p”: Reference to Section 3.3 was added for instructions on DTIC dosing in case of toxicity.	Clarification
33	Schedule of Assessments	Footnote “q”: Instructions were added for administering unesbulin/placebo in case of DTIC interruption.	Clarification
34	Section 1.3	A statement was added in Section 1.3 referring investigators to the Investigator’s Brochure for additional information and guidance on mitigating potential drug-drug interactions.	Health authority request
35	Schedule of Assessments and Section 3.3	Procedural guidelines for DTIC interruptions were added. Instructions for modifying dose of DTIC in the case of nonhematological toxicities were added. It was clarified that the dose of unesbulin/placebo should not be reduced or withheld.	Clarification and Health authority request

Item No.	Protocol Section	Summary of Change	Reason/ Rationale
36	Section 5.2	Instructions were added for subjects who cannot swallow the whole tablet. Specifically, tablets can be dispersed in water at the same dose level at which the tablets are being dosed and be administered orally. Instructions were added to provide guidance in cases of a subject who misses a dose of unesbulin/placebo or vomits after unesbulin/placebo dosing. It was clarified that coadministration of acid-reducing agents should be avoided approximately 4 hours before and after unesbulin/placebo administration to make instructions in this section consistent with other sections.	Clarifications and process updates
37	Section 5.5	Updated guidelines were added for mitigating potential BCRP, OATP1B1, and OATP2B2 interactions with unesbulin. Clarifications on chronic use of marijuana was added, along with a reminder on enrolling subjects with active peptic ulcer disease, active gastritis, or history of gastric perforation within the last 2 years.	Update based on new data and Health authority request
38	Synopsis and Section 7.1	It was clarified that survival follow-up will continue every 3 months as per the Schedule of Assessments.	Clarification
39	Section 7.3.1 and Section 7.3.2	AE and SAE definitions revised as per PTC template language; revisions had no effect on reporting.	Update
40	Section 8.4.4	Content that was previously in Section 8.4.3 was moved to this new section and entitled “Analysis of Other Secondary Efficacy Endpoints.”	Update
41	Section 8.4.5.1	Treatment-emergent adverse events were defined.	Update
42	Section 9.1.1.3	It was clarified that the informed consent may be delegated to a site delegate and not limited to a subinvestigator	Clarification
43	Section 9.1.7	It was clarified that a subject’s participation in the exploratory part of the study is optional.	Clarification

Abbreviations: AE, adverse event; ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; BCRP, breast cancer resistance protein; CBC, complete blood count; CBR, clinical benefit rate; CMP, complete metabolic panel; CT, computed tomography; CTFG, Clinical Trial Facilitation and Coordination Group DCR, disease control rate; DMC, data monitoring committee; DTIC, dacarbazine; ECG, electrocardiogram; EOT, end of treatment; MRI, magnetic resonance imaging; NSAID, nonsteroidal anti-inflammatory drug; OATP1B1 and OATP1B2, organic anion transporter polypeptide transporters; OS, overall survival; PK, pharmacokinetic; PT, prothrombin time; SAE, serious adverse event; ULN, upper limit of normal; WOCBP, women of childbearing potential

Table 11: Spain: Summary of Changes From Local Amendment Version 2.1 to Global Amendment Version 3.0

Item No.	Protocol Section	Summary of Change	Reason/ Rationale
1	Protocol	The version number and date were updated throughout. The synopsis was updated to be consistent with the changes in the protocol. The title page was modified to conform with the new clinical trial protocol template.	Update

Item No.	Protocol Section	Summary of Change	Reason/ Rationale
2	Global Update	Minor editorial changes were made throughout for grammar and clarity.	Update
3	Global Update	Considering the study is blinded, “placebo” was added to statements pertaining to the administration and handling of both unesbulin and placebo, where applicable.	Update/ clarification
4	Protocol Identifiers and Study Personnel	Changes in PTC study staff and contact information	Update
5	Synopsis, Section 2.5, Section 8.4.3, and Section 8.4.10	It was specified that OS is the key secondary endpoint. Additional details describing the analysis of this key secondary endpoint were added to the Synopsis, Section 8.4.3, and Section 8.4.10.	Health authority request
6	Synopsis, Section 2.5, and Section 8.4.4	Content that was previously in Section 8.4.3 was moved to this new section and entitled “Analysis of Other Secondary Efficacy Endpoints.”	Update
7	Synopsis, Section 2.5, and Section 8.4.4	Clarified that at least 3 months of stable disease was required in the definition of DCR and CBR.	Clarification
8	Synopsis, Section 3.1, and Section 7.4	Clarified that approximately 12 subjects will be assessed for PK.	Clarification
9	Synopsis and Section 3.1	The DMC will not review the interim data for sample size re-evaluation; this has been clarified. The timing for the DMC meetings was removed and replaced with a reference to the DMC charter. It was clarified that the interim efficacy analysis will be performed by an external vendor.	Update and clarification
10	Synopsis, Schedule of Assessments, and Section 3.3	It was clarified that no dose reduction of unesbulin/placebo is permitted. For subjects who have interruptions in DTIC due to toxicities, detailed instructions on timing of assessments relative to continued unesbulin/placebo dosing were added to the protocol.	Clarification
11	Synopsis and Section 4.1	An exception to Inclusion Criterion #10 was added for patients with Gilbert’s syndrome who have elevated bilirubin values.	Update based on feedback from investigators
12	Synopsis and Section 4.1	Inclusion Criterion #11 was modified to increase the eligibility limit for AST and ALT from 1.5 to 3 times the ULN.	Update based on feedback from investigators
13	Synopsis Exclusion Criterion #5 and Section 4.2	Removed “known” prior to human immunodeficiency virus, hepatitis B virus, or hepatitis C virus positivity because all subjects will be required for viral testing at screening.	Clarification

Item No.	Protocol Section	Summary of Change	Reason/ Rationale
14	Synopsis, Section 4.2, and Section 5.5	The criterion excluding the concomitant use of NSAIDs was deleted because the potential risk for gastrointestinal perforation was mitigated by adding gastritis to Exclusion Criterion #9. A statement was added to the Concomitant Medication Section 5.5 as a reminder that subjects should not have active peptic ulcer disease, active gastritis, or previous history of gastric perforation within the last 2 years.	Update based on feedback from investigators
15	Synopsis, Section 4.2, and Section 5.5	Exclusion Criterion #15 was modified to provide clarifications on the chronic use of marijuana.	Clarification
16	Synopsis, Section 4.2	Added that APTT/PT assessments to Exclusion Criterion #19 to monitor subjects on anticoagulation where applicable. It was clarified that coagulation tests should fall within the required range (instead of "normal") considering the patient disease and demographic characteristics.	Health authority request and investigator feedback
17	Synopsis, Section 3.1, and Section 5.2	Based on the results from a food effect assessment in the ongoing Phase 1b Study PTC596-ONC-007-LMS, it is recommended that unesbulin/placebo be administered with food.	Update
18	Synopsis and Section 5.1	PTC may provide DTIC to sites that are unable to procure it locally.	Procedural change
19	Synopsis and Section 5.1	Instructions to reconstitute DTIC were added to accommodate variation in institutional policies.	Update
20	Synopsis and Section 5.2	The window to administer DTIC was widened from 1 hour ± 5 minutes to 1 hour ± 30 minutes to allow sites more flexibility to administer this study drug.	Procedural change
21	Synopsis, Schedule of Assessments (footnote "k"), and Section 7.1	It was clarified that either MRI or CT could be used as clinically indicated.	Health authority request
22	Synopsis, Schedule of Assessments (footnote "m"), and Section 7.6	Timing and mandatory collection of biomarker samples was clarified. Footnote "m" was updated as follows: "Blood samples (10 mL) to assess circulating biomarkers will be collected at screening (predose) and prior to dosing on Day 1 of Cycle 3 and at the time of disease progression or EOT. Samples will be processed, stored, and shipped according to instructions in the laboratory manual."	Clarification
23	Schedule of Assessments, Figure 1, Section 3.1, and Section 6.1.1	To accommodate patient management and care and AE stabilization (as applicable), a more flexible timeframe for the EOT visit was provided.	Update
24	Schedule of Assessments	For urinalysis, an "X" was added to EOT and deleted from Day 1 of Cycle 2.	Update

Item No.	Protocol Section	Summary of Change	Reason/ Rationale
25	Schedule of Assessments	A pregnancy test prior is required within 24 hours of dosing on Day 1 of Cycle 1, and this may be assessed with a urine pregnancy test. In footnote "h", it was also clarified that a urine or serum pregnancy test should be performed 24 hours prior to dosing on Day 1 of Cycle 2 and continuing every 2 cycles beginning with Cycle 4.	Clarification and health authority request
26	Schedule of Assessments	Footnote "f": It was clarified that weight must be assessed on the day of DTIC administration.	Clarification
27	Schedule of Assessments	Footnote "g": It was clarified that local laboratory testing is acceptable. The window of clinical laboratory testing on Day 1 of Cycle 1 prior to the first dose of study drug was extended to up to 72 hours to allow sites flexibility over weekends. After Cycle 3, monthly clinical laboratory testing will be adjusted in the protocol so that sampling will be performed once every cycle instead of on a weekly basis. The urinalysis assessment in the footnote was an error; this was replaced with a CMP to be performed at the same frequency as the CBC assessments. Instructions for performing laboratory assessments were added in case DTIC is interrupted/held due to toxicity.	Request by participating sites to decrease subject and staff burden
28	Schedule of Assessments	Footnotes "i" and "j": Instructions were added for performing the single ECG in cases if/when DTIC is interrupted or held. ECGs will not be required after Cycle 3.	Clarification and procedural change based on cumulative safety data in the clinical program
29	Schedule of Assessments	The following text in footnote "j" was deleted: "On Day 1 of all other cycles and at the time of collection for sparse PK samples, approximately 2 hours after DTIC infusion." and was replaced with "Approximately 2 hours after DTIC infusion on Cycle 2."	
30	Schedule of Assessments	Footnote "n": Instructions for performing PK sampling in case of DTIC interruption/hold were added.	Clarification
31	Schedule of Assessments (footnote "o") and Section 5.4	Subjects are encouraged to bring back the patient diary at all scheduled visits.	Procedural change
32	Schedule of Assessments and Section 5.2	Footnote "p": Reference to Section 3.3 was added for instructions on DTIC dosing in case of toxicity.	Clarification
33	Schedule of Assessments	Footnote "q": Instructions were added for administering unesbulin/placebo in case of DTIC interruption.	Clarification

Item No.	Protocol Section	Summary of Change	Reason/ Rationale
34	Section 1.1	A reference to the ESMO-EURACAN-GENTURIS (European Society for Medical Oncology; European Reference Network for Rare Adult Solid Cancers; European Reference Network for Genetic Tumour Risk Syndromes) Clinical Practice Guidelines by Gronchi et al was added.	Health authority request
35	Section 1.3	A statement was added in Section 1.3 referring investigators to the Investigator's Brochure for additional information and guidance on mitigating potential drug-drug interactions.	Health authority request
36	Schedule of Assessments and Section 3.3	Procedural guidelines for DTIC interruptions were added. Instructions for modifying dose of DTIC in the case of nonhematological toxicities were added. It was clarified that the dose of unesbulin/placebo should not be reduced or withheld.	Clarification and Health authority request
37	Section 3.4	A definition of the end of study was added to the protocol.	Health authority request
38	Section 5.2	Instructions were added for subjects who cannot swallow the whole tablet. Specifically, tablets can be dispersed in water at the same dose level at which the tablets are being dosed and be administered orally. Instructions were added to provide guidance in cases of a subject who misses a dose of unesbulin/placebo or vomits after unesbulin/placebo dosing. It was clarified that coadministration of acid-reducing agents should be avoided approximately 4 hours before and after unesbulin/placebo administration to make instructions in this section consistent with other sections.	Clarifications and process updates
39	Section 5.5	It was added that neither approved nor guideline-recommended therapy options should be withheld from subjects if the investigator determines it is in the best interest of the subject. Updated guidelines were added for mitigating potential BCRP, OATP1B1, and OATP2B2 interactions with unesbulin. Clarifications on chronic use of marijuana was added, along with a reminder on enrolling subjects with active peptic ulcer disease, active gastritis, or history of gastric perforation within the last 2 years.	Update based on new data and Health authority request
40	Section 5.6.1	The following statement was removed: "Before unblinding, the investigator must make every attempt to discuss the intended code break with PTC."	Health authority request
41	Synopsis and Section 7.1	It was clarified that survival follow-up will continue every 3 months as per the Schedule of Assessments.	Clarification
42	Section 7.3.1 and Section 7.3.2	AE and SAE definitions revised as per PTC template language; revisions had no effect on reporting.	Update
43	Section 7.3.9	Serious Adverse Events Reporting: the existing "within 24 hours" was updated to "immediately but no later than 24 hours."	Health authority request

Item No.	Protocol Section	Summary of Change	Reason/ Rationale
44	Section 8.4.4	Content that was previously in Section 8.4.3 was moved to this new section and entitled “Analysis of Other Secondary Efficacy Endpoints.”	Update
45	Section 8.4.5.1	Treatment-emergent adverse events were defined.	Update
46	Section 9.1.1.3	It was clarified that the informed consent may be delegated to a site delegate and not limited to a subinvestigator	Clarification
47	Section 9.1.7	It was clarified that a subject’s participation in the exploratory part of the study is optional.	Clarification

Abbreviations: AE, adverse event; ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; BCRP, breast cancer resistance protein; CBC, complete blood count; CBR, clinical benefit rate; CMP, complete metabolic panel; CT, computed tomography; CTFG, Clinical Trial Facilitation and Coordination Group DCR, disease control rate; DMC, data monitoring committee; DTIC, dacarbazine; ECG, electrocardiogram; EOT, end of treatment; MRI, magnetic resonance imaging; NSAID, nonsteroidal anti-inflammatory drug; OATP1B1 and OATP1B2, organic anion transporter polypeptide transporters; OS, overall survival; PK, pharmacokinetic; PT, prothrombin time; SAE, serious adverse event; ULN, upper limit of normal; WOCBP, women of childbearing potential

Table 12: UK: Summary of Changes From Local Amendment Version 2.1 to Global Amendment Version 3.0

Item No.	Protocol Section	Summary of Change	Reason/ Rationale
1	Protocol	The version number and date were updated throughout. The synopsis was updated to be consistent with the changes in the protocol. The title page was modified to conform with the new clinical trial protocol template.	Update
2	Global Update	Minor editorial changes were made throughout for grammar and clarity.	Update
3	Global Update	Considering the study is blinded, “placebo” was added to statements pertaining to the administration and handling of both unesbulin and placebo, where applicable.	Update/ clarification
4	Protocol Identifiers and Study Personnel	Changes in PTC study staff and contact information	Update
5	Synopsis, Section 2.5, Section 8.4.3, and Section 8.4.10	It was specified that OS is the key secondary endpoint. Additional details describing the analysis of this key secondary endpoint were added to the Synopsis, Section 8.4.3, and Section 8.4.10.	Health authority request
6	Synopsis, Section 2.5, and Section 8.4.4	Clarified that at least 3 months of stable disease was required in the definition of DCR and CBR.	Clarification
7	Synopsis, Section 3.1, and Section 7.4	Clarified that approximately 12 subjects will be assessed for PK.	Clarification
8	Synopsis and Section 3.1	The DMC will not review the interim data for sample size re-evaluation; this has been clarified. The timing for the DMC meetings was removed and replaced with a reference to the DMC charter. It was clarified that the interim efficacy analysis will be performed by an external vendor.	Update and clarification

Item No.	Protocol Section	Summary of Change	Reason/ Rationale
9	Synopsis, Schedule of Assessments, and Section 3.3	It was clarified that no dose reduction of unesbulin/placebo is permitted. For subjects who have interruptions in DTIC due to toxicities, detailed instructions on timing of assessments relative to continued unesbulin/placebo dosing were added to the protocol.	Clarification
10	Synopsis and Section 4.1	An exception to Inclusion Criterion #10 was added for patients with Gilbert's syndrome who have elevated bilirubin values.	Update based on feedback from investigators
11	Synopsis and Section 4.1	Inclusion Criterion #11 was modified to increase the eligibility limit for AST and ALT from 1.5 to 3 times the ULN.	Update based on feedback from investigators
12	Synopsis and Section 4.1	It was clarified in Inclusion Criterion #16 that prior lines of treatment may include but are not limited to single-agent doxorubicin or other anthracycline, doxorubicin plus ifosfamide, trabectedin, pazopanib, or gemcitabine with or without docetaxel.	Health authority request
13	Synopsis Exclusion Criterion #5 and Section 4.2	Removed "known" prior to human immunodeficiency virus, hepatitis B virus, or hepatitis C virus positivity because viral testing will be required for all subjects at screening.	Clarification
14	Synopsis, Section 4.2, and Section 5.5	The criterion excluding the concomitant use of NSAIDs was deleted because the potential risk for gastrointestinal perforation was mitigated by adding gastritis to Exclusion Criterion #9. A statement was added to the Concomitant Medication Section 5.5 as a reminder that subjects should not have active peptic ulcer disease, active gastritis, or previous history of gastric perforation within the last 2 years.	Update based on feedback from investigators
15	Synopsis, Section 4.2, and Section 5.5	Exclusion Criterion #15 was modified to provide clarifications on the chronic use of marijuana.	Clarification
16	Synopsis, Section 4.2, and Section 5.5	Administration of live vaccines was added as an Exclusion Criterion (#17) and as a concomitant medication.	Health authority request
17	Synopsis, Section 4.2	Added that APTT/PT assessments to monitor subjects on anticoagulation where applicable. It was clarified that coagulation tests should fall within the required range (instead of "normal") considering the patient disease and demographic characteristics.	Health authority request and investigator feedback
18	Synopsis, Section 3.1, and Section 5.2	Based on the results from a food effect assessment in the ongoing Phase 1b Study PTC596-ONC-007-LMS, it is recommended that unesbulin/placebo be administered with food.	Update
19	Synopsis and Section 5.1	PTC may provide DTIC to sites that are unable to procure it locally.	Procedural change
20	Synopsis and Section 5.1	Instructions to reconstitute DTIC were added to accommodate variation in institutional policies.	Update

Item No.	Protocol Section	Summary of Change	Reason/ Rationale
21	Synopsis and Section 5.2	The window to administer DTIC was widened from 1 hour ± 5 minutes to 1 hour ± 30 minutes to allow sites more flexibility to administer this study drug.	Procedural change
22	Synopsis and Section 7.1	To align with the Schedule of Assessments, clarified that after Cycle 8, scans may be performed every 9 weeks.	Clarification
23	Synopsis, Schedule of Assessments (footnote “k”), and Section 7.1	It was clarified that either MRI or CT could be used as clinically indicated.	Health authority request
24	Synopsis, Schedule of Assessments (footnote “m”), and Section 7.6	Timing and mandatory collection of biomarker samples was clarified. Footnote “m” was updated as follows: “Blood samples (10 mL) to assess circulating biomarkers will be collected at screening (predose) and prior to dosing on Day 1 of Cycle 3 and at the time of disease progression or EOT. Samples will be processed, stored, and shipped according to instructions in the laboratory manual.”	Clarification
25	Schedule of Assessments, Figure 1, Section 3.1, and Section 6.1.1	To accommodate patient management and care and AE stabilization (as applicable), a more flexible timeframe for the EOT visit was provided.	Update
26	Schedule of Assessments	For urinalysis, an “X” was added to EOT and deleted from Day 1 of Cycle 2.	Update
27	Schedule of Assessments	Footnote “f”: It was clarified that weight must be assessed on the day of DTIC administration.	Clarification
28	Schedule of Assessments	Footnote “g”: It was clarified that local laboratory testing is acceptable. The window of clinical laboratory testing on Day 1 of Cycle 1 prior to the first dose of study drug was extended to up to 72 hours to allow sites flexibility over weekends. After Cycle 3, monthly clinical laboratory testing will be adjusted in the protocol so that sampling will be performed once every cycle instead of on a weekly basis. The urinalysis assessment in the footnote was an error; this was replaced with a complete metabolic panel (CMP) to be performed at the same frequency as the CBC assessments. Instructions for performing laboratory assessments were added in case DTIC is interrupted/held due to toxicity.	Request by participating sites to decrease subject and staff burden
29	Schedule of Assessments	A pregnancy test prior is required within 24 hours or dosing on Day 1 of Cycle 1, and this may be assessed with a urine test. In footnote “h”, it was also clarified that a urine or serum pregnancy test should be performed 24 hours prior to dosing on Day 1 of Cycle 2 and continuing every 2 cycles beginning with Cycle 4.	Health authority feedback

Item No.	Protocol Section	Summary of Change	Reason/ Rationale
30	Schedule of Assessments	Footnotes “i” and “j”: Instructions were added for performing the single ECG in cases if/when DTIC is interrupted or held. ECGs will not be required after Cycle 3.	Clarification and procedural change based on cumulative safety data in the clinical program
31	Schedule of Assessments	The following text in footnote “j” was deleted: “On Day 1 of all other cycles and at the time of collection for sparse PK samples, approximately 2 hours after DTIC infusion.” and was replaced with “Approximately 2 hours after DTIC infusion on Cycle 2.”	Clarification
32	Schedule of Assessments	Footnote “n”: Instructions for performing PK sampling in case of DTIC interruption/hold were added.	Clarification
33	Schedule of Assessments (footnote “o”) and Section 5.4	Subjects are encouraged to bring back the patient diary at all scheduled visits.	Procedural change
34	Schedule of Assessments and Section 5.2	Footnote “p”: Reference to Section 3.3 was added for instructions on DTIC dosing in case of toxicity.	Clarification
35	Schedule of Assessments	Footnote “q”: Instructions were added for administering unesbulin/placebo in case of DTIC interruption.	Clarification
36	Section 1.1	A reference to the ESMO-EURACAN-GENTURIS (European Society for Medical Oncology; European Reference Network for Rare Adult Solid Cancers; European Reference Network for Genetic Tumour Risk Syndromes) Clinical Practice Guidelines by Gronchi et al was added.	Health authority request
37	Section 1.3	A statement was added in Section 1.3 referring investigators to the Investigator’s Brochure for additional information and guidance on mitigating potential drug-drug interactions.	Health authority request
38	Schedule of Assessments and Section 3.3	Procedural guidelines for DTIC interruptions were added. Instructions for modifying dose of DTIC in the case of nonhematological toxicities were added. It was clarified that the dose of unesbulin/placebo should not be reduced or withheld.	Clarification and Health authority request
39	Section 3.4	A definition of the end of study was added to the protocol.	Health authority request

Item No.	Protocol Section	Summary of Change	Reason/ Rationale
40	Section 5.2	Instructions were added for subjects who cannot swallow the whole tablet. Specifically, tablets can be dispersed in water at the same dose level at which the tablets are being dosed and be administered orally. Instructions were added to provide guidance in cases of a subject who misses a dose of unesbulin/placebo or vomits after unesbulin/placebo dosing. It was clarified that coadministration of acid-reducing agents should be avoided approximately 4 hours before and after unesbulin/placebo administration to make instructions in this section consistent with other sections.	Clarifications and process updates
41	Section 5.5	It was added that neither approved nor guideline-recommended therapy options should be withheld from subjects if the investigator determines it is in the best interest of the subject. Immunization with live vaccines should be avoided. Updated guidelines were added for mitigating potential BCRP, OATP1B1, and OATP2B2 interactions with unesbulin. Clarifications on chronic use of marijuana was added, along with a reminder on enrolling subjects with active peptic ulcer disease, active gastritis, or history of gastric perforation within the last 2 years.	Update based on new data and Health authority request
42	Synopsis and Section 7.1	It was clarified that survival follow-up will continue every 3 months as per the Schedule of Assessments.	Clarification
43	Section 7.3.1 and Section 7.3.2	AE and SAE definitions revised as per PTC template language; revisions had no effect on reporting.	Update
44	Section 7.3.9	Serious Adverse Events Reporting: the existing “within 24 hours” was updated to “immediately but no later than 24 hours”	Health authority request
45	Section 8.4.4	Content that was previously in Section 8.4.3 was moved to this new section and entitled “Analysis of Other Secondary Efficacy Endpoints.”	Update
46	Section 8.4.5.1	Treatment-emergent adverse events were defined.	Update
47	Section 9.1.1.3	It was clarified that the informed consent may be delegated to a site delegate and not limited to a subinvestigator	Clarification
48	Section 9.1.7	It was clarified that a subject’s participation in the exploratory part of the study is optional.	Clarification

Abbreviations: AE, adverse event; ALT, alanine aminotransferase; APTT, activated partial thromboplastin time; AST, aspartate aminotransferase; BCRP, breast cancer resistance protein; CBC, complete blood count; CBR, clinical benefit rate; CMP, complete metabolic panel; CT, computed tomography; CTFG, Clinical Trial Facilitation and Coordination Group DCR, disease control rate; DMC, data monitoring committee; DTIC, dacarbazine; ECG, electrocardiogram; EOT, end of treatment; MRI, magnetic resonance imaging; NSAID, nonsteroidal anti-inflammatory drug; OATP1B1 and OATP1B2, organic anion transporter polypeptide transporters; OS, overall survival; PK, pharmacokinetic; PT, prothrombin time; SAE, serious adverse event; ULN, upper limit of normal; WOCBP, women of childbearing potential

9.2.3. Version 4.0: 28 November 2023

Overall reason for Version 4.0: The main reasons for Version 4.0 were to clarify study procedures in cases of DTIC interruption and revise the analysis of the key secondary endpoint (OS). Additional updates were made to reflect changes in study staff, improved clarity, and to address clerical errors. A summary of changes from global Version 3.0 to Version 4.0 is presented below.

Table 13: Summary of Changes From Global Protocol Version 3.0 to 4.0

Item No.	Protocol Section	Summary of Change	Reason/ Rationale
1	Title Page	ClinicalTrials.gov study number was revised.	The ClinicalTrials.gov identifier number was corrected.
2	Signature Page	Titles of PTC signatories were updated.	Change in signatory titles.
3	Protocol Identifiers and Study Personnel	Study personnel was updated	Change in PTC study staff.
4	Synopsis, Section 8.2, Section 8.4.3	It was clarified that a hierarchical testing procedure will be utilized. The power calculation and analyses of OS were revised to specify that the interim analysis will occur when at least 60% of the events have accumulated and the final analysis will occur when 184 events have accumulated.	Revisions were made to address the FDA comments and to provide more flexibility in the timing of the interim OS analysis, which is planned to be done at the time of the final efficacy analysis for PFS.
5	Synopsis, Section 4.1	Inclusion criterion #11 revised as follows: Old version: "AST **or** ALT <3 times the ULN" New version: "AST **and** ALT <3 times the ULN."	Correction of clerical error
6	Synopsis, and Section 5.1	The 200 mg/vial DTIC strength was removed, along with volume of Sterile Water for reconstitution. **New text**: DTIC for injection will be provided (by the site or by the sponsor) and will be reconstituted with Sterile Water for Injection, USP.	Text revised to allow for site- and country-specific variations in specific strengths of DTIC.
7	Synopsis, Footnote "a" in Schedule of Assessments, Figure 1 footnote, Section 3.1, Section 7.1	New text added (indicated in bold): Survival follow-up will continue every 3 months **until the later of 184 events** or 2 years after the last subject is randomized	Revision made to ensure that sufficient events are achieved for analysis of OS.
8	Schedule of assessments, Footnote b	"Window" was substituted with "span" to clarify that procedures performed on Cycle 1 Day 1 and Cycle 2 Day 1 can be done over a 48-hour period.	Clarification

Item No.	Protocol Section	Summary of Change	Reason/ Rationale
9	Schedule of assessments, Footnote g	For subjects who have DTIC interrupted or held, it was added that clinical laboratory assessments will be required weekly for the first 3 weeks after DTIC reintroduction. For subsequent cycles, the clinical laboratory assessments may be performed at a minimum of once per cycle or more frequently per the investigator's discretion to ensure that the subject meets the protocol-defined safety criteria.	Improved safety monitoring for subjects who require interruptions in DTIC
10	Schedule of assessments, Footnote q	In footnote q, the bolded text was added: If DTIC is interrupted in Cycle 3 then PK sampling should be performed **upon reintroduction of DTIC** as described in footnote "n"	Clarification
11	Section 3.3	The following statement was added: DTIC should be reintroduced at least 48 hours after unesbulin dosing	Additional instructions for clarity
12	Section 4.3	The following statement was added: Subjects who fail screening may be considered for rescreening after consultation with the PTC Medical Monitor.	Procedural change
13	Section 5.6	Bolded text added: The investigator and study staff (including processing laboratory personnel), the subjects, and the sponsor's staff **(with exception of Pharmacovigilance staff for the purposes of SUSAR reporting)** will remain blinded **until the primary analysis is completed.**	Clarified that study will remain blinded until the primary analysis is completed rather than study closure, which could be later than the primary analysis. It was also clarified that certain Pharmacovigilance staff at PTC will unblind some subjects for SUSAR reporting purposes.
14	Section 8.1	Null and alternative hypotheses for the key secondary endpoint were added.	Clarification

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; DTIC, dacarbazine; OS, overall survival; PFS, progression-free survival; PK, pharmacokinetic; SUSAR, suspected unexpected serious adverse reaction; ULN, upper limit of normal

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