A Phase I, First-in-Human, Dose-Escalation Study to Evaluate the Safety of the Monoclonal Antibody PTX-35 in Patients with Advanced Solid Tumors Refractory to Standard of Care

Protocol No.:	PTX35-001
IND No.:	139925
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Protocol Version:	4.0
Replaces:	3.0
Date:	29 July 2021

CONFIDENTIAL

INVESTIGATOR'S STATEMENT

1. I have carefully read this protocol entitled "A Phase I, First-in-Human, Dose-Escalation Study to Evaluate the Safety of the Monoclonal Antibody PTX-35 in Patients with Advanced Solid Tumors Refractory to Standard Care" and agree that it contains all the necessary information required to conduct the study. I agree to conduct this study as outlined in the protocol.
2. I understand that this study will not be initiated without approval of the appropriate Institutional Review Committee/Independent Ethics Committee (IRB/IEC), and that all administrative requirements of the governing body of the Institution will be complied with fully.
3. Informed written consent will be obtained from all participants in accordance with institutional guidelines, FDA requirements as specified in Title 21 CFR, Part 50, the European Union Directive 2001/20/EC and its associated Detailed Guidances, European Union GCP Directive 2005/28/EC, the ICH Guideline for Good Clinical Practice, Section 4.8, and the terms of the Declaration of Helsinki (2013).
4. I will enroll participants who meet the protocol criteria for entry.
5. I understand that my signature on each completed Case Report Form (CRF) indicates that I have carefully reviewed the complete set of CRFs and accept full responsibility for the contents thereof.
6. I understand that the information presented in this study protocol is confidential, and I hereby assure that no information based on the conduct of the study will be released without prior consent from the Sponsor unless this requirement is superseded by the Food and Drug Administration, a Competent Authority of the European Union or another Regulatory Authority.

Protocol Version 4.0: 29 July 2021

Investigator:

Name: _____ Telephone: _____

Address: _____

_____

Signature: _____ Date: _____

Sponsor/Representative:

Signature: _____ Date: 29 Jul 2021

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CLINICAL STUDY SYNOPSIS

Name of Sponsor: Pelican Therapeutics, Inc. 8122 Datapoint Drive, Suite 445 San Antonio, TX 78229 (917) 892-9996	**Name of CRO:** Cancer Insight

Name of finished product: PTX-35

Name of active ingredient: PTX-35

Title of the study: A Phase I, First-in-Human, Dose-Escalation Study to Evaluate the Safety of the Monoclonal Antibody PTX-35 in Patients with Advanced Solid Tumors Refractory to Standard Care

Protocol number: PTX35-001

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Clinical phase: Phase I

Objectives:

Primary:

- To establish the safety profile of PTX-35 in a population of patients with advanced solid tumors.

Secondary:

- To determine the optimal immunological dose (OID), or maximum tolerated dose (MTD), of PTX-35.
- To establish the pharmacokinetic profile of PTX-35.

Exploratory:

- To study the immunological effect generated by PTX-35.
- To evaluate immunogenicity of PTX-35.
- To evaluate preliminary evidence of clinical benefit of PTX-35 in a population of patients with advanced solid tumors.
- To evaluate general immunologic expansion: The frequency and expansion of NK, CD4 and CD8 T cell subsets.
- To measure immune responses to well-characterized first-time exposure or recall antigen.
- To determine specific cytokine expression in response to treatment with PTX-35, or any other unspecific inflammatory response.

Study Overview:

This is an open-label, single arm, first-in-human, Phase I study of intravenous administration of PTX-35 to patients with advanced solid tumors refractory to, or ineligible for, or who refuse available SOC.

Patients who meet the eligibility criteria will be enrolled to receive intravenous PTX-35 every two weeks as well as a single intramuscular injection of TENIVAC vaccine (tetanus and diphtheria toxoids adsorbed) on Day 1 of Cycle 1. Upon completion of two cycles of PTX-35, in the absence of disease progression or unacceptable toxicity, patients may continue to be treated with PTX-35 at the same dose and schedule until disease progression, death, and patient withdrawal of consent, Investigator's decision to remove patient, or intolerable toxicity, whichever occurs first.

Seven escalating dose levels of PTX-35 will be explored using a traditional 3+3 design based on dose-limiting toxicities (DLTs) until optimal immunological dose (OID) or maximum tolerated dose (MTD) is established. The starting dose of PTX-35 is based on the MABEL for Treg activity (considered the most sensitive of PTX-35 pharmacodynamic markers) obtained from dose-ranging studies conducted in non-clinical animal models. PTX-35 will be administered to patients intravenously on Day 1 of each cycle. The length of each cycle is 14 days, and the DLT observation period includes the safety data obtained from the first two treatment cycles (4 weeks).

PTX-35 Dose Levels

Dose level*	PTX-35 dose (mg/kg)	PTX-35 Concentration (mg/mL)	PTX-35 Dose (mL/kg)	Number of patients
1	0.01 mg/kg	1 mg/mL	0.01 mL/kg	3-6
2	0.03 mg/kg	1 mg/mL	0.03 mL/kg	3-6
3	0.10 mg/kg	1 mg/mL	0.10 mL/kg	3-6
4	0.30 mg/kg	5 mg/mL	0.06 mL/kg	3-6
5	1.0 mg/kg	5 mg/mL	0.20 mL/kg	3-6
6	3.0 mg/kg	5 mg/mL	0.60 mL/kg	3-6
7	10 mg/kg	5 mg/mL	2.0 mL/kg	3-6

**Dose levels 1 through 3 will be administered at a concentration of 1 mg/mL, and dose levels 4 through 7 will be administered at a concentration of 5 mg/mL.*

At each dose level, 3 patients will be enrolled initially, with treatment initiation staggered by one week after the first patient only. After all 3 patients in a dose level have received the second dose of PTX-35 and completed the DLT observation period, the Safety Review Committee (SRC) will review the safety data to make a dose escalation decision. If none of these 3 patients experiences a DLT, dose escalation may proceed. If 1 of these 3 patients experiences a DLT, the dose level will be expanded to at least 6 patients at the same dose level. If $\leq$1 out of 6 patients experiences a DLT, then the SRC may recommend dose escalation to the next dose level. If a DLT occurs in 2 or more patients in a particular dose level, the maximum tolerated dose (MTD) has been exceeded and dose escalation will cease. Up to 3 additional patients will be enrolled at the next lowest dose if only 3 patients were treated at that dose level, to confirm safety of that dose.

The conduct and completion of each subsequent dose cohort will follow in similar fashion until the MTD is established. Note that if the optimal immunological dose (OID), or observed plateau of Treg activity, is determined prior to MTD, further dose escalation may be discontinued by decision of the SRC.

Visits and study examinations will be performed per Table 2: Schedule of Assessments. Safety will be assessed by frequency of treatment-emergent adverse events (TEAEs), evaluation of clinical laboratory parameters (hematology, and biochemistry), weight, vital signs, electrocardiogram (ECG), performance status, and physical exam findings. NCI CTCAE version 5.0 will be used to grade all toxicities.

A post-treatment safety visit will be conducted approximately 30 days following the last dose of PTX-35. Response to treatment will be assessed according to RECIST 1.1 (APPENDIX II) with planned evaluation performed every 8 weeks (± 1 week) from start of treatment (C1D1).

Target number of patients: Up to 22 - 34 total patients will be enrolled if all dose levels are evaluated.

Eligibility Criteria

Inclusion Criteria:
In order to participate in this study, a patient *must*:

1. Be willing and have the capacity to sign the written informed consent form.
2. Be male or female of at least 18 years of age at the time of signing informed consent.
3. Have a documented diagnosis of metastatic or advanced, unresectable solid tumor disease. Patient must have progressed or recurred following standard of care (SOC) therapies, or are ineligible for, or who refuse other safe and effective SOC therapies, and whom the Investigator believes may benefit from experimental treatment with PTX-35.
4. Have an acceptable organ function, as defined below:
    a. Albumin ≥ 2.5 g/dL
    b. Total bilirubin < 3.0 × upper limit of normal (ULN), unless patient has Gilbert's syndrome
    c. Alanine transaminase (ALT) and aspartate transaminase (AST) ≤ 3.0 × ULN, or ≤ 5 × ULN in the case of liver metastases
    d. Calculated or measured creatinine clearance > 35 mL/minute per the Cockcroft-Gault formula
    e. Absolute neutrophil count ≥ 1,500/mm^3
    f. Hemoglobin ≥ 9 g/dL
    g. Platelet count ≥ 100,000/mm^3
5. Have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2 (APPENDIX I).
6. Have life expectancy of at least three months.
7. Patients, both females and males, of childbearing/reproductive potential must agree to use adequate contraception, or continuous abstinence, while included in the trial and for six months after the last treatment with PTX-35.

Exclusion Criteria:

In order to participate in this study, a patient *must not*:

1. Have received any systemic anticancer therapy including small molecules, chemotherapy, radiation therapy, monoclonal antibodies or any other experimental drug within 4 weeks of first dose of PTX-35. Adjuvant anti-hormonal treatment(s) for prior breast cancer or prostate cancer are allowed. (Note: washout for palliative radiation therapy is 2 weeks).
2. Have clinically significant cardiac disease, including:
    a. Onset of unstable angina within 6 months of signing the Informed Consent Form (ICF).
    b. Acute myocardial infarction within 6 months of the signing the ICF.
    c. Known congestive heart failure (Grade III or IV as classified by the New York Heart Association); and/ or a known decreased cardiac ejection fraction (LVEF) of < 45%.
    d. Uncontrolled hypertension defined as systolic blood pressure ≥160 mmHg and/or diastolic blood pressure ≥ 100 mmHg, despite optimal medical management.
3. Have known or clinically suspected leptomeningeal disease. Stable, previously treated metastases in the brain or spinal cord, are allowed as long as these are considered stable (by CT or MRI), and not requiring systemic corticosteroids.
4. Have a history of ≥ Grade 3 allergic reactions, or suspected allergy or intolerance to monoclonal antibody therapies.
5. Have a history of suspected cytokine release syndrome (CRS).
6. Have any known immunodeficiency disorders (testing not required).
7. Have received prior allogeneic stem cell transplant.
8. Have ongoing or current autoimmune disease. Permanent but stable and manageable immune related adverse events (irAE) from prior therapies are permissible if prednisone equivalent corticosteroid use does not exceed 10 mg/day.
9. Have any other condition requiring concurrent systemic immunosuppressive therapy (other than allowable exceptions which do not exceed 10mg/day of prednisone/corticosteroid use).
10. Have clinically significant active viral, bacterial or fungal infection requiring:
    a. Intravenous treatment with antimicrobial therapy completed less than two weeks prior to first dose, or
    b. Oral treatment with antimicrobial therapy completed less than one week prior to first dose.

    Prophylactic treatment with antibiotics (e.g., for dental extractions) is allowed.
11. Have had major surgery (requiring general anesthesia or inpatient hospitalization) within four weeks before first administration of PTX-35.
12. Have had a known tetanus/diphtheria vaccine within the past 10 years.
13. Have known additional malignancy that is active and/or progressive requiring treatment; exceptions include basal cell or squamous cell skin cancer, *in situ* cervical cancer, or other cancer for which the patient has been disease-free for at least two years.
14. Have known previously untreated or symptomatic metastases in the brain or spinal cord requiring steroids. Patients with treated and stable CNS metastases may be enrolled after approval of the sponsor and/or Medical Monitor.

15. Have any other ongoing significant, uncontrolled medical condition in the opinion of the Investigator.
16. Have known positive serology for human immunodeficiency virus (HIV), hepatitis B, or hepatitis C (except in cases of immunity after cured infection). Testing not required.
17. Have a history of substance abuse, medical, psychological or social conditions that may interfere with the patient's participation in the trial or evaluation of the trial result.
18. Be a female patient who is pregnant or breast feeding.

Test product, dose and mode of administration:
PTX-35 is a humanized, affinity matured, IgG2 monoclonal antibody and is a functional agonist of human TNFRSF25, a member of the TNF-receptor superfamily that is expressed preferentially by activated and antigen-experienced T lymphocytes.

TENIVAC vaccine, Tetanus and Diphtheria Toxoids Adsorbed, is a sterile isotonic suspension of tetanus and diphtheria toxoids adsorbed on aluminum phosphate. Each 0.5 mL dose of TENIVAC vaccine contains the following active ingredients: Tetanus Toxoid 5 Lf (flocculation units) and Diphtheria Toxoid 2 Lf.

Each patient will be administered an intravenous infusion of PTX-35 every 2 weeks during the DLT period (2 cycles) based on the dose levels defined in the dose escalation scheme (Section 6.1.2) and may, per Investigator discretion, continue treatment every 2 weeks until disease progression or unacceptable toxicity, whichever occurs first.

Each patient will also be administered a single 0.5 mL dose of TENIVAC vaccine intramuscularly on Day 1 of Cycle 1. TENIVAC should be administered as directed in the package insert.

Dose-Limiting Toxicity (DLT):
An event will be considered a DLT per NCI CTCAE version 5.0 criteria if it occurs within the DLT reporting period and meets at least one of the criteria below:

- Hematological toxicities ≥ Grade 3
- Non-hematological toxicities ≥ Grade 3
- Cytokine release syndrome ≥ Grade 3
- Infusion reaction ≥ Grade 3
- Any ≥ Grade 3 hepatic toxicity meeting Hy's Law criteria, or Total bilirubin ≥2.0 x ULN to ≤3.0 x ULN for >7 consecutive days, ≥CTCAE G3 total bilirubin, CTCAE G3 AST or ALT for >7 consecutive days, or CTCAE G4 AST or ALT
- Any other treatment-emergent toxicity that is considered clinically significant and/or unacceptable, and that does not respond to supportive care and results in a disruption of the treatment schedule of more than 14 days.

DLT excludes:

- Hematological and non-hematological ≤ Grade 2 unless considered non-acceptable.
- Other Grade 3 self-limited or medically controllable toxicities (e.g., fever without ≥ Grade 3 neutropenia, lymphopenia, nausea, vomiting, diarrhea, fatigue). Note, such events are considered DLT if the abnormality leads to hospitalization or the abnormality persists for

>72 hours despite appropriate interventions (e.g., replacement therapy for electrolyte abnormalities, when indicated).
- Electrolyte disturbances that are managed to Grade 1 or less with supplemental therapy.

Any adverse event that is potentially related to PTX-35 and requires discontinuation of treatment should be considered a DLT. A DLT will be considered related to PTX-35 treatment unless there is a clear, well-documented, relation to intercurrent illness or primary malignant disease. AEs that meet the above criteria but occur after the DLT evaluation period will not be defined as DLTs but will be reported as AEs/Serious Adverse Events (SAEs), as applicable, and will be reviewed across all dose levels during the study to help inform dose escalation decisions.

In case of suspicion of a DLT, the Investigator must inform the Medical Monitor/Sponsor immediately. The DLT will be confirmed by the Medical Monitor/Sponsor and a decision must be taken by the Sponsor regarding if an *ad hoc* SRC meeting is required.

Patients who discontinue therapy before completion of 2 cycles (DLT period) for other reasons than dose-limiting toxicity will be replaced. Patients who are tolerating PTX-35 will not have to discontinue study treatment prematurely due to the occurrence of DLTs in another patient in the same cohort, unless decided by the SRC.

Safety Review Committee:
A Safety Review Committee (SRC) comprised of at least 3 members: one Investigator, a representative of Pelican Therapeutics, and the Medical Monitor. The SRC will evaluate the data obtained at each dose level and will recommend whether the dose should be escalated as per protocol, revised to a lower level or intermediate level, halted altogether or more patients are required at the same dose level to evaluate safety.

Duration of Treatment:
Upon completion of two cycles (4 weeks), in the absence of disease progression or unacceptable toxicity, patients may continue to be treated with PTX-35 at the same dose and schedule until disease progression, death, and patient withdrawal of consent, Investigator's decision to remove patient, or intolerable toxicity, whichever occurs first.

At the discretion of the Investigator, and in the absence of clinical deterioration, treatment with PTX-35 may continue beyond initial evidence of radiological progression as per Section 8.2.1.

Criteria for Evaluation:
Primary Endpoints
- Number of Dose-limiting toxicities (DLTs) per NCI CTCAE v5.0.
- Frequency of treatment-emergent adverse events (TEAEs) and serious adverse events (SAEs) related to PTX-35 during the trial.

Secondary Endpoints
- Optimal Immunological Dose (OID; dose where T-reg plateau is observed) or Maximum tolerated dose (MTD; highest dose level at which ≤ 1 patient of at least 6 patients experienced a DLT during the first two treatment cycles).

- Pharmacokinetic parameters will include maximum concentration (C_{max}), area under curve up to the last measurable concentration (AUC_{last}), trough observed serum concentration (C_{trough}) and terminal elimination half-life ($T_{1/2\lambda z}$).

Exploratory Analysis
Immune phenotype profiling of immune cell subsets will be conducted by flow cytometry in peripheral blood at various time-points to assess change from baseline in:

- Percentage of Treg cells (immunological effect)
- Frequency of anti-therapeutic antibody (ATA) titers to PTX-35 (immunogenicity)
- General immunologic expansion: The frequency and expansion of NK, CD4 and CD8 T cell subsets
- Specific antigen driven immune expansion: immune responses to a well-characterized first-time exposure or recall antigen based on the patient's HLA type will be measured at baseline and on study using ELISA, ELISPOT and flow cytometric methods.
- Specific cytokine expression in response to treatment with PTX-35.

Clinical benefit will be evaluated where possible for:

- Best Overall Response (BOR), Overall Survival (OS), and Progression-Free Survival (PFS) assessed by RECIST 1.1 (APPENDIX II).

Safety:
Safety will be assessed throughout the study by means of physical examination, weight, vital signs, ECOG performance status, laboratory evaluations (hematology, biochemistry and cytokines), electrocardiogram (ECG), and recording of concurrent illness/therapy and treatment-emergent adverse events. CTCAE version 5.0 will be used to grade all toxicities. Patients will also be monitored for any clinical symptoms associated with elevated cytokine levels. All related adverse events will be monitored until resolution or permanent outcome. Concomitant medications will be recorded throughout the study.

Cytokines will be monitored during cycle 1 and 2. Cytokines will only be monitored at cycle 3 and beyond if patient is symptomatic at time of visit (i.e., flu-like symptoms, fever, or allergic reactions).

Efficacy:
Clinical benefit (Best Overall Response, Progression-Free Survival, and Overall Survival) will be evaluated via radiologic assessments according to RECIST 1.1 (APPENDIX II).

Pharmacokinetics:
Plasma concentrations of PTX-35 will be measured by ELISA at the following timepoints: pre-dose; 30min (±2min), 2hr (±5min), 4hr (±5min), 24hr (±2hr), 72hr (±2hr), 144hr (±2hr), 216hr (±2hr) after end of PTX-35 infusion for Cycles 1 and 2, and pre-dose at Cycle 3 only. Non-compartmental or population pharmacokinetic methods will be used to derive PTX-35 PK parameters, which will be summarized using descriptive statistics (mean, standard deviation, median, minimum, maximum).

Immunogenicity: Blood samples will be drawn for central analysis of anti-therapeutic antibodies (ATA), which will be collected pre-dose on Day 1 of each cycle and at the 30-day follow up visit. Samples testing positive in the screening assay will undergo a confirmatory assay to demonstrate

that the ATAs are specific for the therapeutic protein product. Samples identified as positive in the confirmatory assay will be further characterized in titration and neutralization assays.

General and Specific Antigen Immune Expansion: Blood samples will be drawn to determine each patient's HLA type (collected pre-dose Cycle 1 Day 1) for exclusive use in determining the patients primary or recall response to the TENIVAC vaccine. Blood samples will also be collected pre-dose on Day 1 of each cycle and at the 30-day follow up visit to measure anti-TENIVAC antibody titer (TDAR). Blood samples will also be collected pre-dose on Day 1 and Day 7 of Cycles 1 and 2 as well as at the 30-day follow up visit to measure the general and antigen reactive expansion and activity of NK and T cell subsets.

Statistical Methods and Justification of Sample Size:
All analyses will be descriptive. Categorical variables will be presented with numbers and, if meaningful, percentages. Continuous variables will be presented by *n*, mean, median, standard deviation and range (minimum and maximum) as appropriate. Presentations will be by each dose level.

Safety Evaluable Population: All patients who received at least 1 dose of PTX-35.

DLT Evaluable Population: All patients who receive at least 1 dose of PTX-35 and experience a DLT and/or complete the DLT evaluation period.

Efficacy Evaluable Population: All patients with pre-treatment measurable disease by RECIST 1.1, who received at least 1 dose of PTX-35 and who had at least one radiological assessment during treatment.

This is an exploratory, first-in-human trial. We believe that the sample sizes will be sufficient to characterize the initial safety of PTX-35. No formal sample size calculations have been performed.

LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	Adverse Event
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
APC	Antigen-Presenting Cell
ATA	Anti-Therapeutic Antibody
AUC	Area under Concentration Time Curve
AUC_{last}	Area under Concentration Time Curve up to the Last Measurable Concentration
BOR	Best Overall Response
BUN	Blood Urea Nitrogen
CDR	Complementarity-determining regions
CFR	Code of Federal Regulations
C_{max}	Maximum Observed Serum Concentration
CR	Complete Response
CRF	Case Report Form
CRO	Contract Research Organization
CRS	Cytokine Release Syndrome
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTL	Cytotoxic T Lymphocyte
CTLA-4	Cytotoxic T Lymphocyte-Associated Protein 4
C_{trough}	Trough Observed Serum Concentration
DLT	Dose-Limiting Toxicity
DR3	Death Receptor 3
EC	Ethics Committee
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
ELISA	Enzyme-linked immunosorbent assay
FACS	Fluorescence Activated Cell Sorting
FDA	Food and Drug Administration
GCP	Good Clinical Practice Guidelines
GITR	Glucocorticoid-Induced TNFR-Related Gene
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HLA	Human Leucocyte Antigen
ICF	Informed Consent Form
ICH	International Council for Harmonization
IEC	Independent Ethics Committee
Ig	Immunoglobulin

IgSF	Immunoglobulin Superfamily
IL	Interleukin
IM	Intramuscular
IRB	Institutional Review Board
IV	Intravenous
K_D	Binding Affinity
Kg	Kilogram
Lf	Flocculation Units
LVEF	Left Ventricular Ejection Fraction
µg	Microgram
mAb	Monoclonal Antibody
MABEL	Minimum Anticipated Biological Effect Level
MedDRA	Medical Dictionary for Drug Regulatory Activities
MHC	Major Histocompatibility Complex
mL	Milliliter
MRI	Magnetic Resonance Imaging
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
NKT	Natural Killer T Cell
NOAEL	No Observable Adverse Event Level
NOEL	No Observable Effect Level
NSCLC	Non-Small Cell Lung Cancer
OS	Overall Survival
OT-I	Ovalbumin-Specific TCR Transgenic Mouse
PBMC	Peripheral Blood Mononuclear Cell
PD	Progressive Disease
PECAM	Platelet Endothelial Cell Adhesion Molecule
PETG	Polyethylene Terephthalate Glycol
PFS	Progression-Free Survival
PK	Pharmacokinetic(s)
PR	Partial Response
RECIST 1.1	Response Evaluation Criteria in Solid Tumors version 1.1
SAE	Serious Adverse Event
SAR	Suspected Adverse Reaction
SD	Stable Disease
SOC	Standard of Care
SRC	Safety Review Committee
$T_{1/2\lambda z}$	Terminal Elimination Half-Life
Tconv	Conventional T Cells
TCR	T Cell Receptor
TDAR	T Dependent Antibody Response
TEAE	Treatment-Emergent Adverse Events
T_{max}	Time to Peak Serum Concentration

TL1A	Tumor Necrosis Factor–Like Cytokine 1A
TNF	Tumor Necrosis Factor
TNFR	Tumor Necrosis Factor Receptor
TNFRSF	Tumor Necrosis Factor Receptor Superfamily
TRAMP	Trf4/Air2/Mtr4p Polyadenylation Complex
Treg	Regulatory T Cell
Tx	Treatment
ULN	Upper Limit of Normal

1.0 GENERAL INFORMATION

1.1 Protocol Number and Title of the Study

Protocol No. PTX35-001
Protocol Title: A Phase I, First-in-Human, Dose-Escalation Study to Evaluate the Safety of the Monoclonal Antibody PTX-35 in Patients with Advanced Solid Tumors

1.2 Sponsor

Pelican Therapeutics, Inc.
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1.3 Medical Monitor

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1.4 Signature Authorization

Heat Biologics will sign the protocol.

1.5 Principal Investigator and Institution

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2.0 BACKGROUND INFORMATION

2.1 Introduction

2.1.1 T lymphocyte Activation and Regulation: Co-Stimulator Molecules

T lymphocyte recognition of specific cognate antigenic peptides presented by MHC molecules triggers T cell receptor (TCR) signaling. Co-stimulatory and co-inhibitory receptors on T cells are required for successful signaling and they play a crucial role in regulating T cell activation, subset differentiation, effector function and survival. Following recognition of cognate peptide–MHC complexes on antigen-presenting cells (APCs) by the TCR, co-signaling receptors often co-localize with TCR molecules, where they synergize with TCR signaling to

promote or inhibit T cell activation and function[1,2]. CD28 is a prototype co-stimulatory TCR and its discovery provided evidence for the two-signal model of T cell activation, according to which both TCR and co stimulatory signaling are required for full T cell activation.

A large number of co-stimulator molecules have now been identified and most of them belong to either the immunoglobulin superfamily (IgSF) or tumor necrosis factor receptor superfamily (TNFRSF). The TNFRSF co-stimulatory receptors are structurally related cell surface glycoproteins that regulate innate and adaptive immunity. A subgroup of the TNFRSF contains a conserved region within the cytoplasmic domain known as the death domain. Triggering of death domain-containing members of the TNFRSF can lead to the induction of apoptosis via activation of caspase-8 or stimulation of the MAP kinase and NF-kB signaling pathways. The T lymphocyte receptor molecules CD27-, OX40- and DR3- mediated co-stimulation promotes proliferation and survival of both $CD4^+$ and $CD8^+$ effector T cells, whereas 4-1BB- and GITR- mediated co-stimulation preferentially enhances the expansion and survival of $CD8^+$ effector T cells. The expression of many co-stimulatory and co-inhibitory molecules on the surface of T cells is induced following T cell activation and changes in the cell surface expression of many of these molecules occur in overlapping fashion as T cells proliferate and differentiate.

2.1.2 TNFRSF25 and TL1A

Tumor necrosis factor (TNF) related superfamily member 25 (TNFRSF25) (also known as TRAMP, WSL-1, Apo-3, LARD or DR3), is highly restricted to lymphocyte-rich tissues including the spleen, thymus, small intestine and peripheral blood lymphocytes. At a cellular level TNFRSF25 is expressed at low levels on naïve $CD4^+$ and at even lower levels on naive $CD8^+$ T cells but is up-regulated rapidly on both after activation with TCR engagement with antigen. TNFRSF25 is highly and constitutively expressed by $CD4^+$ $FoxP3^+$ T regulatory cells (Treg), which may be related to the persistent encounter of thymic-derived Treg with cognate 'self' antigen.

The only known ligand for TNFRSF25 is TL1A (TNFSF15) and this appears to be a monogamous receptor:ligand pair. TL1A is a type II membrane bound protein but can also be processed into a soluble cytokine. TL1A is produced by activated dendritic cells (DCs), monocytes, endothelial cells and T cells, and can also be induced by IL-1β or TNFα. TL1A is not expressed by resting $CD4^+$ or $CD8^+$ T cells, but can be induced upon activation by TCR stimulation or exposure to LPS. TL1A co-stimulates T-cell production of effector cytokines *in vitro* and enhances the accumulation of $CD4^+$ effector T cells within the inflamed tissues in autoimmune and inflammatory disease models. TL1A also promotes Treg proliferation and attenuates Treg-mediated suppression of non-regulatory $CD4^+$ T cells. In addition, TL1A has been shown to co-stimulate invariant NK Tcells and may have a role in enhancing NK cell-mediated tumor cell killing.

2.1.3 TNFRSF25 Signaling in CD4 and CD8 T Lymphocytes, and Tumor Growth Inhibition

As mentioned, TNFRSF25 is constitutively and highly expressed by $CD4^+FoxP3^+$ Tregs. By using a TNFRSF25-specific agonistic monoclonal antibody, 4C12, the effects of TNFRSF25 signaling on Tregs was studied *in vivo* in mice. Signaling through TNFRSF25 induced rapid and selective expansion of preexisting Tregs *in vivo* such that they became 30%–35% of all $CD4^+$ T cells in the peripheral blood within 4 days. TNFRSF25-induced Treg proliferation was dependent upon TCR engagement with MHC class II, IL-2 receptor, and Akt signaling, but not upon co-stimulation by CD80 or CD86; it was unaffected by rapamycin[3].

While studies have demonstrated a role for TNFRSF25 in regulating $CD4^+$ T cell responses, it also has been demonstrated that TNFRSF25 can function as a co-stimulatory receptor for $CD8^+$ T cells. Human $CD8^+$ T cells have been found to preferentially express TNFRSF25 compared to $CD4^+$ T cells, B cells, monocytes, NK cells or dendritic cells. The ectopic expression of TL1A on mouse plasmacytomas promotes elimination of tumor cells in a $CD8^+$ T-cell dependent manner and renders mice immune to a subsequent challenge with tumor cells.

TNFRSF25 triggering *in vivo* with soluble TL1A promotes the proliferation and accumulation of antigen-specific $CD8^+$ T cells as well as their differentiation into CTLs. TNFRSF25 also functions as a co-stimulatory receptor for memory $CD8^+$ T cells. Thus, TNFRSF25 triggering enhances the secondary expansion of endogenous antigen-specific memory $CD8^+$ T cells. These findings suggest that TNFRSF25 agonists, such as soluble TL1A, could potentially be used to enhance the immunogenicity of vaccines that aim to elicit human anti-tumor $CD8^+$ T cells[4].

The effect of TNFRSF25 stimulation by the agonist 4C12, a hamster monoclonal antibody (mAb), has been explored using several murine models and treatment approaches. In a study of several co-stimulatory agonists in a CT26 murine colon-carcinoma model, it was demonstrated that the stimulation of TNFRSF25 by the 4C12 mAb resulted in tumor growth inhibition of an equivalent degree as agonists targeting the GITR and OX40 co-stimulator receptors. A murine breast cancer model using 4T1 tumor cells demonstrated that following cumulative radiation of 30 Gy, similar tumor growth inhibition was achieved with the administration of the 4C12 mAb agonist or an OX40 agonist, indicating similar effects whether the TNFRSF25 or OX40 co-stimulatory receptors were engaged. The best activity was seen when treatment regimens were combined with anti-TNFRSF25/DR3 4C12 agonist antibody, demonstrating proof-of-concept for the combination of current cancer therapies with TNFRSF25/DR3 agonists.

Due to the potential of TNFRSF25/DR3 stimulation to expand regulatory T-cells in the absence of inflammation/antigen, studies were conducted to measure the systemic (spleen) and local (tumor) levels of regulatory T-cells post-4C12 treatment in tumor-bearing animals.

In mice with established Lewis Lung Carcinoma (LLC), mice were treated with 4C12 and regulatory T-cells were measured in the spleen and tumor over time. Regulatory T-cells were expanded in the spleen but not within the tumor microenvironment (TME). Neither induced (peripheral) nor natural (thymic) regulatory T-cells were expanded in the TME but were found elevated systemically.

There have been clinical explorations of this approach of T cell co-stimulation. The mechanisms of action of CD137 (4-1BB), CD134 (OX40), and glucocorticoid-induced TNFR (GITR; CD357) co-stimulators depend on a complex interplay of CTL, T-helper cells, regulatory T cells, dendritic cells, and vascular endothelium in tumors. Agonist monoclonal antibodies specific for CD137 have shown signs of objective clinical activity in patients with metastatic melanoma[5].

2.1.4 TNFRSF25: TL1A Signaling and Anti-Cancer Therapy

Pre-clinical work with the 4C12 mAb to stimulate TNFRSF25 has demonstrated antigen driven T cell proliferation, increased effector cytokine production, increased $CD8^+$ effector cell function and increased survival among murine xenograft models. TNFRSF25 agonism leads to a more pronounced 'memory' $CD8^+$ cytotoxic T cell activation and TNFRSF25 signaling in T cells appears to be dependent upon prior engagement of the TCR, implying an antigen specific T cell proliferation. These findings indicate that the incorporation of a TNFRSF25 agonist such as PTX-35 into anti-cancer therapy could complement checkpoint inhibitors and significantly reverse immune suppression in the tumor microenvironment while boosting the $CD8^+$ effector arm of the tumor immune response.

2.2 PTX-35 Investigational Product

PTX-35 is a humanized, affinity matured, IgG2 monoclonal antibody derived from the hamster antibody 4C12, a product of immunization and immortalization as a hybridoma cell line. PTX-35 is a functional agonist of human TNFRSF25 (also known as the death receptor 3, DR3), a member of the TNF-receptor superfamily that is expressed preferentially by activated and antigen-experienced T lymphocytes.

The structure of PTX-35 is shown in Figure 1.

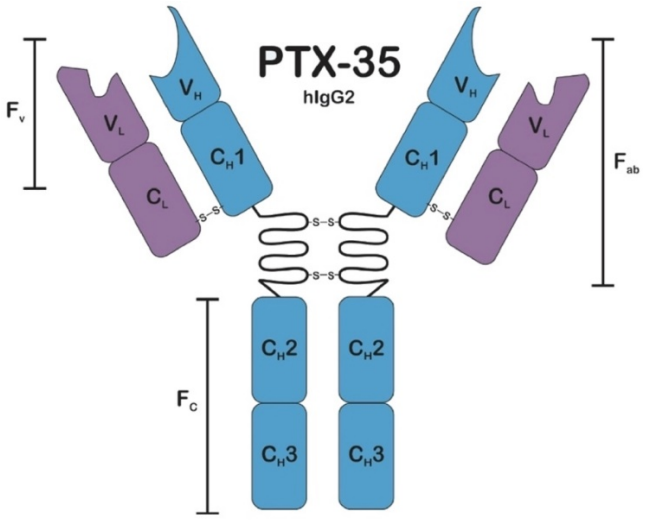

Figure 1: PTX-35 structure

PTX-35 IgG (150 kDa) consists of two heavy and light chains held together by disulfide bonds. F_v – antigen-binding variable region; F_{ab} – fragment, antigen-binding region; F_c – fragment, crystallizable region

The schematic in Figure 2 illustrates the development of the humanized monoclonal antibody, PTX-35 from the hamster monoclonal antibody, 4C12. PTX-35 was developed by a humanization process of transferring the complementarity-determining regions (CDRs) of the heavy and light chains from the hamster antibody, 4C12, onto a human framework backbone. PTX-35 was then matured by amino acid substitution to increase its affinity and strength of binding by slight modifications in the variable binding regions of the heavy and the light chains.

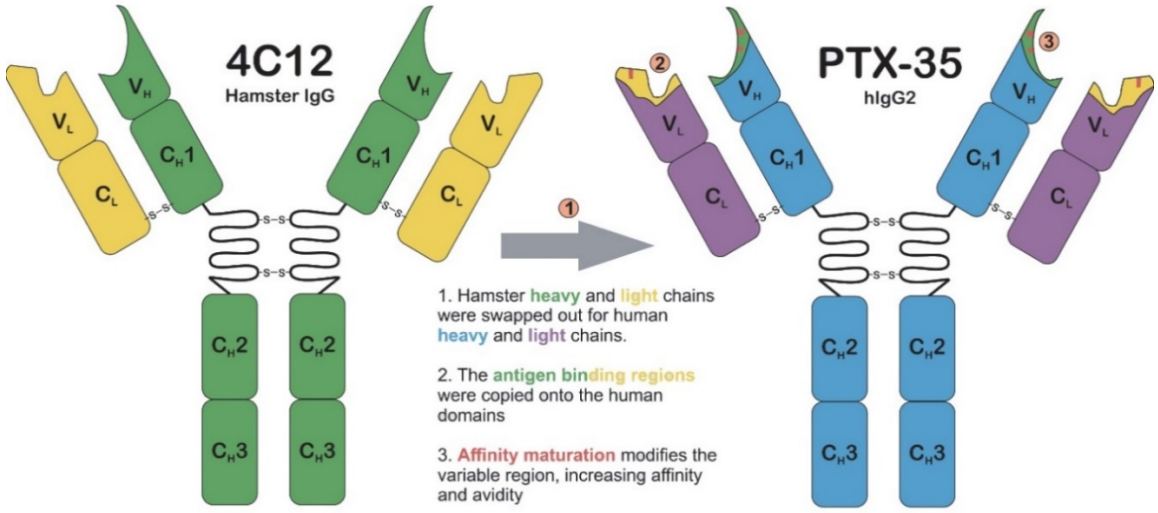

Figure 2: Schematic of PTX-35 (humanized mAb) development from 4C12 (hamster mAb)

2.3 TENIVAC Vaccine

TENIVAC vaccine, Tetanus and Diphtheria Toxoids Adsorbed, will be used in this study to assess the safety and efficacy of PTX-35 administration in the context of immune activation/re-activation against a specific set of antigens (TENIVAC) from both a humoral (B cell mediated) and cell mediated (T cell) response. TENIVAC is manufactured as a sterile isotonic suspension of tetanus and diphtheria toxoids adsorbed on aluminum phosphate. Each 0.5 mL dose of TENIVAC vaccine contains the following active ingredients: Tetanus Toxoid 5 Lf (flocculation units) and Diphtheria Toxoid 2 Lf. Other ingredients per 0.5 mL dose include 1.5 mg of aluminum phosphate (0.33 mg of aluminum) as the adjuvant and ≤5.0 mcg of residual formaldehyde.

2.4 Preclinical Studies

Key preclinical findings are summarized below. Please refer to the current Investigator's Brochure for details of these studies.

2.4.1 Primary Pharmacodynamics and Pharmacology

Since human PTX-35 lacks biological activity in mice due to the need for Fc-gamma-receptor cross-linking via the Fc portion of the molecule, a mouse-human surrogate antibody was generated by genetically splicing the affinity matured CDR variable domains of human PTX-35 onto an IgG1 mouse constant region backbone; mPTX-35. PTX-35 and its mouse surrogate mPTX-35 was tested in a series of pharmacology studies that tested target engagement and demonstrated biological proof-of-concept:

- Study of the measurement of functional affinity against human Jurkat cell lines, *in vitro*, expressing TNFRSF25/DR3
- *In vitro* study of PTX-35 co-stimulation in human PBMCs
- Study of the effect of PTX-35 on expansion of Tregs and T cell memory subsets in the peripheral blood of monkeys
- Study of mPTX-35 to identify a MABEL and NOEL in mice
- Evaluation of a hamster version of PTX-35 (4C12) in combination with radiation in a mouse metastatic breast cancer model
- Evaluation of mPTX-35 in combination with cancer cell vaccine in a mouse model of melanoma
- Evaluation of 4C12 anti-tumor activity and regulatory T-cell accumulation in a mouse model of Lewis lung carcinoma

These studies demonstrated a dependence on available antigen driven TCR engagement and synergistic activity *in vivo*, for both expansion of $CD8^+$ T cells, as well as delayed outgrowth of implanted syngeneic tumors. mPTX-35 also expanded regulatory T-cells confirming target engagement giving a pharmacodynamic based MABEL of 0.01 mg/kg and NOEL of 0.001 mg/kg. This T cell expansion was greatest for the 1 mg/kg dose of mPTX-35 with gp96-Ig cell-based vaccination and far exceeded any additive value of mPTX-35 and gp96-Ig treatment alone. This was also the best dose for vaccine combination for tumor regression, when administered simultaneously every two weeks. Additionally, the anti-tumor effects of the hamster version of PTX-35 (4C12) was not associated with an accumulation of regulatory T-cells in the tumor microenvironment.

2.4.2 Toxicity Studies and Pharmacokinetics

A PTX-35 safety pharmacology study of the effects of PTX-35 *in vitro* human PBMC cell culture demonstrated that anti-CD3 and PTX-35 stimulated human T-cells in culture does not result in the secretion of deleterious cytokines.

PTX-35 was tested in a series of toxicology safety studies in mouse and monkey:

- Tissue-cross reactivity against human, mouse and monkey
    - PTX-35 tissue cross-reactivity against mouse, human, monkey TNFRSF25/DR3 was performed to justify species selection for toxicology studies.
- 28-day toxicology study in mice with toxicokinetics
    - PTX-35 was administered via an intraperitoneal injection at doses of 0 (control article), 10, 50, 100 and 200 mg/kg to Crl:CD (ICR) mice on two occasions during a 28-day study with a 4-week recovery period.
    - No PTX-35-related adverse clinical signs, changes in hematology or clinical chemistry parameters, macroscopic findings or changes in organ weighs. No changes in myeloid:erythroid ratio.
    - NOAEL for mouse was determined to be 200 mg/kg with a combined male and female C_{max} of 2,440 µg/mL, T_{max} of 24 hours and $AUC_{(0\text{-}216\ hrs.)}$ 390,000 hr*µg/mL on day 15.
    - T_{max} of PTX-35 ranged between 8-72 hours. Systemic exposure increased with dose between 50 and 200 mg/kg [C_{max} 644 – 2,440 µg/mL and $AUC_{(0\text{-}216\ hrs.)}$ of 91,100 – 390,000 hr*µg/mL averaged for male and female animals for day 15]. Presence of ATA was observed in only one animal and this did not affect exposure.
- 2-week dose range finding toxicology study in monkey with toxicokinetics
    - Animals were dosed PTX-35 at 1, 10, 50 and 96 mg/kg, by bolus IV injection, once every 2 weeks, for a total of 2 weeks.
    - PTX-35 was well tolerated up to 96 mg/kg/dose; there were no PTX-35-related clinical observations, or changes to body weights, qualitative food consumption, clinical chemistry, coagulation, and urinalysis parameters at any dose levels evaluated.
    - NOAEL of 96 mg/kg/dose with corresponding C_{max} of 2,590 µg/mL and $AUC_{(0\text{-}t)}$ of 261,500 hr*µg/mL for male and female combined.
    - Animals in each dose group were ATA negative on day 36, except at the lowest dose level (1 mg/kg/dose), in which both animals were ATA positive and appeared to affect exposure.
- 8-week toxicology study with toxicokinetics in monkey with NOAEL of 100 mg/kg
    - PTX-35 was administered at 10, 60, 100 mg/kg by IV bolus injection once every 2 weeks over an 8-week period (total of 4 doses).

- There were no PTX-35-related clinical signs, or changes in body weights or qualitative food consumption. There were no PTX-35-related effects on eyes, electrocardiogram, coagulation, clinical chemistry, or urinalysis parameters, or bone marrow cytology; nor were there any PTX-35-related gross necropsy or histopathologic findings, or changes in absolute or relative organ weights.
- NOAEL of 100 mg/kg/dose with a C_{max} of 217 to 2,660 µg/mL and $AUC_{(0\text{-}336\ hrs.)}$ of 24,500 to 298,000 µg*hr/mL at day 43 for doses 10 to 100 mg/kg.
- Systemic exposure to PTX-35 did not appear to consistently change greater than 2-fold following repeated administration of PTX-35. Animals in the lowest dose group (10 mg/kg) were positive for ATA which influenced exposure.

The pharmacokinetics of PTX-35 drug substance (DS) and drug product (DP) when administered by slow bolus intravenous injection on a single occasion were determined for the two formulations (DS; group 1 and DP; group 2) in monkeys. Overall, these two formulations showed relatively similar pharmacokinetic values (Figure 3) suggesting the addition of polysorbate 80 to the DS has little effect on PTX-35 exposure. Apparent terminal half-life ($T_{1/2\lambda z}$) of PTX-35 drug product (Group 2) was 163 hours, with the volume of distribution at steady-state (V_{ss}) of 58.4 mL/kg.

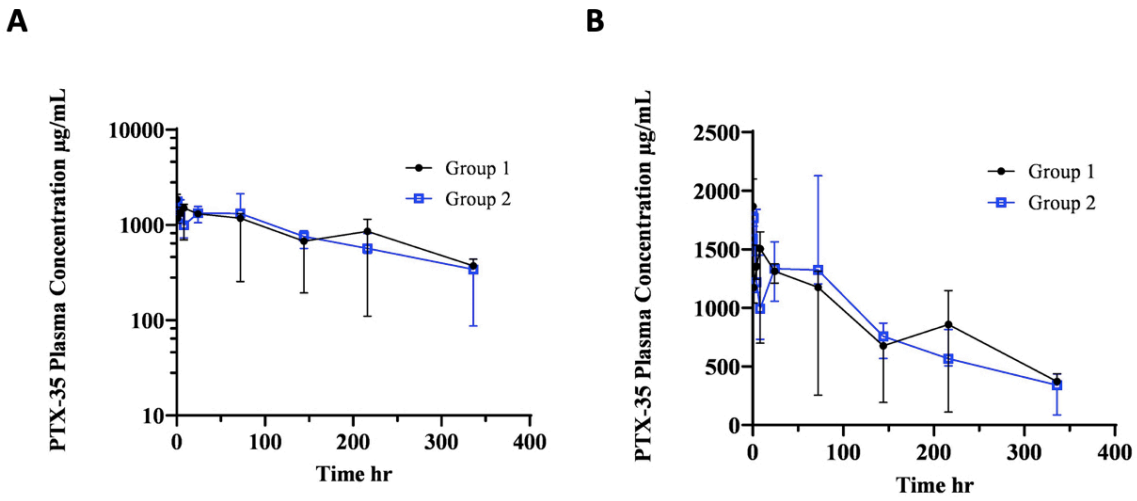

Figure 3: Median (± minimum, maximum) PTX-35 plasma concentrations vs time following single dose IV infusion (2.5 min) in cynomolgus monkeys (n=3 per group).

2.5 Rationale for Starting Dose and Treatment Schedule

The starting dose rationale for this current study is derived from non-clinical study in mice that generated a minimum anticipated biological effect level (MABEL) and no observable effect level (NOEL) for mouse-human surrogate antibody, mPTX-35. mPTX-35 was used as an alternative to human PTX-35, in mice, due to the poor binding affinity (K_D) of human IgG for mouse Fc-γ receptors. The MABEL was determined to be 0.01 mg/kg for mPTX-35 with the NOEL being 0.001 mg/kg. This study also established the pharmacologically active dose as the dose between 0.1 and 10 mg/kg, with 10 mg/kg being the highest dose given without reversing the intended effect. This 0.01 mg/kg starting dose considers the calculated MABEL in mice using a mouse-human surrogate that shares the same complementary determining region (CDR) binding as that of human PTX-35 with a functionally comparable mouse Fc. This proposed dose is 20,000-fold below the 200 mg/kg NOAEL as determined in mice, and 10,000-fold below the 100 mg/kg NOAEL as determined in monkey.

Thus, the suggested starting dose in patients is 0.01 mg/kg of PTX-35 administered every 2 weeks. This dose represents the MABEL dose in mice with a 10,000 to 20,000-fold safety factor based on monkey and mouse toxicity studies. Five dose levels of PTX-35 will be explored using half-logarithmic dose escalation increments, using a traditional 3+3 design based on DLTs until MTD is established, as described in Section 6.1.2.

2.6 Potential Risks and Benefits

As this is the first-in-man study of PTX-35, no human data regarding the safety or potential benefit of PTX-35 is available. PTX-35 is a functional agonist of human TNFRSF25 (also known as the death receptor 3, DR3), a member of the TNF-receptor superfamily that is expressed preferentially by activated and antigen-experienced T lymphocytes. No other agonistic antibody targeting TNFSRF25 has been evaluated in clinical studies previously.

Other TNFRSF agonistic antibodies, including OX-40, 4-1BB/CD137, CD27, CD40, have been evaluated in clinical studies. For example, a recent integrated safety analysis of urelumab, an agonist anti-CD137 monoclonal antibody, showed that urelumab was relatively well tolerated, with fatigue (16%) and nausea (13%) being the most common treatment-related AEs, and was associated with immunologic and pharmacodynamic activity demonstrated by the induction of IFN-inducible genes and cytokines[6]. Another anti-CD137 monoclonal antibody, utomilumab, appears well tolerated, and most common treatment-related adverse events observed in a Phase I clinical study were fatigue, pyrexia, decreased appetite, dizziness, and rash (<10% of patients). Only one (1.8%) patient experienced a Grade 3-4 treatment-related adverse event (fatigue), and no clinically relevant elevations in transaminases were noted[7]. In a Phase I randomized study of KHK4083, an anti-OX40 monoclonal antibody, in patients with mild to moderate plaque psoriasis, the most frequent treatment-related AEs observed were mild or moderate chills (9.1%), and infusion/injection site reactions (7.3%). No clinically meaningful or dose-related changes from baseline in

laboratory values, vital signs, ECG recordings or physical examinations were observed[8]. The Phase I study of varilumab, an agonistic antibody targeting CD27, the treatment-related adverse events were generally Grade 1 or 2 in severity, with 1 dose-limiting toxicity (asymptomatic Grade 3 hyponatremia), but no high-grade immune-related adverse events[9].

Cytokine release syndrome (CRS) is a potentially life-threatening toxicity that has been observed following administration of natural and bispecific antibodies and, more recently, following adoptive T-cell therapies for cancer.

No relevant toxicity of PTX-35 has been observed in a series of dedicated toxicology safety studies (mice and monkeys). Based on the experience with other antibodies, infusion reactions may occur. Manipulation of immune checkpoints may, theoretically, trigger immune-related adverse reactions and/or cytokine release syndrome but based on experience with other agonists of the TNFRSF, this risk appears limited with PTX-35.

3.0 TRIAL OBJECTIVES AND PURPOSE

Primary:

- To establish the safety profile of PTX-35 in a population of patients with advanced solid tumors.

Secondary:

- To determine the optimal immunological dose (OID), or maximum tolerated dose (MTD) of PTX-35.
- To establish the pharmacokinetic profile of PTX-35.

Exploratory:

- To study the immunological effect generated by PTX-35.
- To evaluate immunogenicity of PTX-35.
- To evaluate preliminary evidence of clinical benefit of PTX-35 in a population of patients with advanced solid tumors.
- To evaluate general immunologic expansion: The frequency and expansion of NK, CD4 and CD8 T cell subsets.
- To measure immune responses to well-characterized first-time exposure or recall antigen.
- To determine specific cytokine expression in response to treatment with PTX-35, or any other unspecific inflammatory response.

4.0 TRIAL DESIGN

4.1 Characteristics of a Well-Conducted Trial

The following characteristics of an adequate and well-conducted trial will be implemented:

1. The Investigators will be well qualified by scientific training and experience.
2. Detailed Case Report Forms (CRFs) will be completed for every patient.
3. Requirements for institutional ethics review as set forth by the appropriate Institutional Review Board/Independent Ethics Committee (IRB/IEC), Title 21 Code of Federal Regulations (CFR) Part 56, the European Union Directive 2001/20/EC and its associated Detailed Guidances, European Union GCP Directive 2005/28/EC, the ICH Guideline for Good Clinical Practice, Sections 3 and 4, and the terms of the Declaration of Helsinki (2013), will be followed.
4. Requirements for informed consent in accordance with institutional guidelines, FDA requirements as specified in Title 21 CFR, Part 50, the European Union Directive 2001/20/EC and its associated Detailed Guidances, European Union GCP Directive 2005/28/EC, the ICH Guideline for Good Clinical Practice, Section 4.8, and the terms of the Declaration of Helsinki (2013), will be followed.
5. Safety data will be recorded and evaluated.
6. Routine monitoring visits will be conducted by the Sponsor's representative to ensure data accuracy.
7. Drug accountability will be strictly maintained.
8. This trial will be conducted according to Good Clinical Practice (GCP), the protocol and applicable regulatory requirements.

4.2 Overview of Trial Design

This is an open-label, single arm, first-in-human, Phase I study of the intravenous administration of PTX-35 to patients with advanced solid tumors refractory to, or ineligible for, or who refuse, SOC therapy.

Patients who meet the eligibility criteria will be enrolled to receive intravenous PTX-35 every two weeks as well as a single intramuscular injection of TENIVAC vaccine on Day 1 of Cycle 1. Upon completion of two cycles of PTX-35, in the absence of disease progression or unacceptable toxicity, patients may continue to be treated with PTX-35 at the same dose and schedule until disease progression, death, and patient withdrawal of consent, Investigator's decision to remove patient, or intolerable toxicity, whichever occurs first. At the discretion of the Investigator, and in the absence of clinical deterioration, treatment with PTX-35 may continue beyond initial evidence of radiological progression as per Section 8.2.1.

Seven escalating dose levels of PTX-35 will be explored using a traditional 3+3 design based on DLTs (dose-limiting toxicities) until optimal immunological dose (OID) or maximum tolerated dose (MTD) is established. The starting dose of PTX-35 is based on the MABEL for Treg activity (considered the most sensitive of PTX-35 pharmacodynamic markers) obtained from dose-ranging studies conducted in non-clinical animal models. PTX-35 will be administered to patients intravenously on Day 1 of each cycle. The length of each cycle is 14 days, and the DLT period includes the safety data obtained from the first two treatment cycles (4 weeks).

4.3 Patient Population

This study will enroll adult male and female patients of age ≥ 18 years with metastatic or advanced, unresectable solid tumor types, who have progressed or recurred following standard of care (SOC) therapies, or are ineligible for, or refuse, other safe and effective SOC treatment.

4.4 End of Study

The end of the study is defined as the date of the last visit of the last patient participating in the trial.

4.5 Duration of Therapy

Upon completion of the first two treatment cycles (i.e., 4 weeks, with at least 2 doses of PTX-35), in the absence of disease progression and unacceptable toxicity, patients may continue to be treated with PTX-35 at the same dose and schedule until disease progression, death, patient's withdrawal of consent, Investigator's decision to remove patient, or intolerable toxicity, whichever occurs first.

At the discretion of the Investigator, and in the absence of clinical deterioration, treatment with PTX-35 may continue beyond initial evidence of radiological progression as per Section 8.2.1.

4.6 Trial Discontinuation

For reasonable cause, either the Investigator or the Sponsor may terminate this study prematurely. Written notification of the termination is required. Conditions that may warrant termination include, but are not limited to:

- The discovery of an unexpected, significant, or unacceptable risk to the patients enrolled in the study.
- Failure of the Investigator to enter patients at an acceptable rate.
- Insufficient adherence to protocol requirements (non-compliance).
- Lack of evaluable and/or complete data.
- Decision to modify the developmental plan of the drug.
- A decision on the part of the Sponsor to suspend or discontinue development of the drug.

Section 5.5 Study Stopping Criteria lists events that warrants immediate stop of treatment until reviewed by the Safety Review Committee. This assessment may also result in trial discontinuation (equivalent to first bullet above).

4.7 Investigational Product Accountability/Disposition of Clinical Trial Supplies

Investigational Product (IP) accountability records will be maintained for all clinical trial supplies.

All unused clinical trial supplies will be destroyed per the institution's standard operating procedure, or returned to the Sponsor or designee. Destruction and/or return of IP and trial supplies must be documented, and the documentation will be reviewed by/sent to the Sponsor or their Designee.

5.0 SELECTION AND WITHDRAWAL OF PATIENTS

5.1 Inclusion Criteria

Patients **must** meet all of the following inclusion criteria before they will be allowed to participate in the trial:

1. Be willing and have the capacity to sign the written informed consent form.

2. Be male or female of at least 18 years of age at the time of signing informed consent.

3. Have a documented diagnosis of metastatic or advanced, unresectable solid tumor disease. Patient must have progressed or recurred following standard of care (SOC) therapies, or are ineligible for, or who refuse, other safe and effective SOC therapies, and whom the Investigator believes may benefit from experimental treatment with PTX-35.

4. Have an acceptable organ function, as defined below:

    a. Albumin ≥ 2.5 g/dL
    b. Total bilirubin < 3.0 × upper limit of normal (ULN) unless patient has Gilbert's syndrome.
    c. Alanine transaminase (ALT) and aspartate transaminase (AST) ≤ 3.0 × ULN, or ≤ 5 × ULN in the case of liver metastases.
    d. Calculated or measured creatinine clearance > 35 mL/minute per the Cockcroft-Gault formula.
    e. Absolute neutrophil count ≥ 1,500/mm^3
    f. Hemoglobin ≥ 9 g/dL.
    g. Platelet count ≥ 100,000/mm^3

5. Have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2 (APPENDIX I).

6. Have life expectancy of at least three months.

7. Patients, both females and males, of childbearing/reproductive potential must agree to use adequate contraception, or continuous abstinence, while included in the trial and for six months after the last treatment with PTX-35.

5.2 Exclusion Criteria

Patients **MUST NOT** enter the trial if they meet any of the following exclusion criteria:

1. Have received any systemic anticancer therapy including small molecules, chemotherapy, radiation therapy, monoclonal antibodies or any other experimental drug within 4 weeks of first dose of PTX-35. Adjuvant anti-hormonal treatment(s) for prior breast cancer or prostate cancer are allowed. (Note: washout for palliative radiation therapy is 2 weeks).

2. Have clinically significant cardiac disease, including:

    a. Onset of unstable angina within 6 months of signing the Informed Consent Form (ICF).

    b. Acute myocardial infarction within 6 months of the signing the ICF.

    c. Known congestive heart failure (Grade III or IV as classified by the New York Heart Association); and/or a known decreased cardiac ejection fraction (LVEF) of < 45%.

    d. Uncontrolled hypertension defined as systolic blood pressure ≥160 mmHg and/or diastolic blood pressure ≥ 100 mmHg, despite optimal medical management.

3. Known or clinically suspected leptomeningeal disease. Stable, previously treated metastases in the brain or spinal cord, are allowed as long as these are considered stable (by CT or MRI), and not requiring systemic corticosteroids.

4. Have a history of ≥ Grade 3 allergic reactions, or suspected allergy or intolerance to monoclonal antibody therapies.

5. Have a history of suspected cytokine release syndrome (CRS).

6. Have any known immunodeficiency disorders (testing not required).

7. Have received prior allogeneic stem cell transplant.

8. Have ongoing or current autoimmune disease. Permanent but stable and manageable immune related adverse events (irAE) from prior therapies are permissible if prednisone equivalent corticosteroid use does not exceed 10 mg/day.

9. Have any other condition requiring concurrent systemic immunosuppressive therapy (other than allowable exceptions which do not exceed 10mg/day of prednisone/corticosteroid use).

10. Have clinically significant active viral, bacterial or fungal infection requiring:

    a. Intravenous treatment with antimicrobial therapy completed less than two weeks prior to first dose, or

    b. Oral treatment with antimicrobial therapy completed less than one week prior to first dose.

    Prophylactic treatment with antibiotics (e.g., for dental extractions) is allowed.

11. Have had major surgery (requiring general anesthesia or inpatient hospitalization) within four weeks before first administration of PTX-35.

12. Have had a known tetanus/diphtheria vaccine within the past 10 years.

13. Have known additional malignancy that is active and/or progressive requiring treatment; exceptions include basal cell or squamous cell skin cancer, *in situ* cervical cancer, or other cancer for which the patient has been disease-free for at least two years.

14. Have known previously untreated or symptomatic metastases in the brain or spinal cord requiring steroids. Patients with treated and stable CNS metastases may be enrolled after approval of the sponsor and/or Medical Monitor.

15. Have any other ongoing significant, uncontrolled medical condition in the opinion of the Investigator.

16. Have known positive serology for human immunodeficiency virus (HIV), hepatitis B, or hepatitis C (except in cases of immunity after cured infection). Testing not required.

17. Have a history of substance abuse, medical, psychological or social conditions that may interfere with the patient's participation in the trial or evaluation of the trial result.

18. Be a female patient who is pregnant or breast feeding.

5.3 Inclusion of Women, Minorities and Children

Both men and women and members of all races and ethnic groups are eligible for this study. Children are not eligible for this study because the safety and tolerability of the proposed administration schedule has not been determined in adults.

5.4 Withdrawal Criteria

Protocol therapy will be discontinued at any time if any of the following situations occur:

1. Progressive disease (not including unconfirmed radiographic PD if Investigator opts to continue treatment as outlined in section 8.2.1).
2. Adverse event.
   - The development of toxicity which, in the Investigator's judgment, precludes further therapy.
   - Unacceptable adverse event(s).
   - Intercurrent illness that prevents further administration of treatment.
3. Withdrawal by subject.
4. Lost to follow-up.
5. Non-compliance.
6. At the discretion of the Investigator/physician decision.

7. Death.
8. Pregnancy.
9. Study termination.

5.4.1 Withdrawn Patients

When a patient is removed from the study, the Investigator will clearly document the reason in the medical record and complete the appropriate case report form page describing the reason for discontinuation. In addition, every effort should be made to complete the appropriate assessments listed in Section 7.4.

Patients lost to follow-up will be withdrawn from the study. Patients will be declared "lost to follow-up" if the last contact date has exceeded two years, and/or the site has documented three attempts to contact the patient by telephone and/or certified letter to last known address.

5.4.2 Replacement of Subjects

Patients who discontinue due to toxicity (related to PTX-35) during the 4-week DLT window of observation of Cycle 1 and Cycle 2, or who do not receive all doses due to toxicity in Cycle 1 and Cycle 2, will not be replaced. Patients who discontinue or who do not receive at least two doses of PTX-35 for any reason other than toxicity during Cycle 1 and 2 will be replaced. Note, that all patients who receive PTX-35 will be included in assessment of DLT per Section 6.1.3.

5.5 Study Stopping Criteria

If any of the following occur, administration of PTX-35 will be temporarily or permanently stopped, pending a review by the Safety Review Committee. During that time, no drug can be administered to any patient, until a decision is made.

- Any Death (other than progressive disease) that is at least possibly related to the study agent(s)
- Occurrence of two or more grade 4 events that are at least possibly related to the study agent(s)

Conditions that may warrant trial termination are listed in Section 4.6

6.0 TREATMENT OF PATIENTS

6.1 Investigational Product Preparation and Administration

6.1.1 Drug Products

6.1.1.1 PTX-35

PTX-35 is a humanized, affinity matured, IgG2 monoclonal antibody and is a functional agonist of human TNFRSF25, a member of the TNF-receptor superfamily that is expressed preferentially by activated and antigen-experienced T lymphocytes.

The drug substance is manufactured by KBI Biopharma (Durham, NC) and formulated in 20 mM histidine (pH 6.5) and 250 mM sorbitol at a target of 25 mg/mL and stored frozen at -80°C in PETG bottles.

The final drug product is reformulated and filled at Integrity Bio (Camarillo, CA) at a final concentration of 10 mg/mL in 20 mM histidine (pH 6.0), 250 mM sorbitol and 0.01% polysorbate 80 and stored at 2–8°C.

PTX-35 will be administered via intravenous infusion according to the dose levels in the dose escalation scheme (Section 6.1.2) on Day 1 of each cycle. The dose will be calculated using the weight obtained during Screening. The weight obtained at each dosing visit is to be reviewed prior to dispensing IP. If the patient's weight changes by >10% during the course of the study, the drug dose should be recalculated. Recalculation of drug dose on each treatment day regardless of percentage of body weight change is also allowed, if required per institutional dosing policy. The dosing solution should include additional volume to account for dose losses due to administration set priming. PTX-35 should be diluted with 0.9% sodium chloride for injection under aseptic conditions to the appropriate concentration according Table 1: PTX-35 Dose Levels. Dose levels 1 through 3 will be administered at a concentration of 1 mg/mL, and dose levels 4 through 7 will be administered at a concentration of 5 mg/mL. Diluted Investigational Product should be infused with a MedFusion 3500 syringe pump (or equivalent) with compatible polypropylene syringe connected to Smith's Medical FS116 60" PVC tubing with 0.22 micron in-line filter and 0.7 mL priming volume. [Note that the total priming volume to be used during administration will be 1.0 mL to ensure filter saturation.] The rate of infusion should be approximately 1 mL per minute. Please refer to the study Investigational Product Manual for full administration details.

Each patient must remain in clinic for a 6-hour safety observation period after the first cycle of PTX-35 infusion. Afterwards, the Investigator may reduce the observation period to 2 hours for the remaining cycles according to the following criteria:

- Absence of any significant safety concerns following a complete review of all clinical and laboratory data for all treated subjects at the given dose level and the preceding dose levels,
- Absence of any late-onset (>2 hours post dose) infusion-related reactions or IRR symptoms that continue beyond 2 hours post-dose, and lack of any severe or serious IRRs,

6.1.1.2 TENIVAC Vaccine

TENIVAC vaccine is a suspension available in 0.5 mL single-dose for injection. TENIVAC vaccine is a sterile isotonic suspension of tetanus and diphtheria toxoids adsorbed on aluminum phosphate. Each 0.5 mL dose of TENIVAC vaccine contains the following active ingredients: Tetanus Toxoid 5 Lf (flocculation units) and Diphtheria Toxoid 2 Lf. Other ingredients per 0.5 mL dose include 1.5 mg of aluminum phosphate (0.33 mg of aluminum) as the adjuvant and ≤5.0 mcg of residual formaldehyde.

TENIVAC will be administered via intramuscular injection on Day 1 of Cycle 1 only and will be administered per the current product package insert.

If any serious adverse reaction occurs these may be treated per institutional practice, as noted in Section 6.4 Concomitant Treatment.

6.1.2 Dose Escalation Scheme

Escalating doses of PTX-35 will be administered to patients via intravenous infusion on Day 1 of each cycle.

Upon completion of two cycles (4 weeks), in the absence of disease progression or unacceptable toxicity, patients may continue to be treated with PTX-35 at the same dose and schedule until disease progression, death, and patient withdrawal of consent, Investigator's decision to remove patient, or intolerable toxicity, whichever occurs first. At the discretion of the Investigator, treatment with PTX-35 may continue beyond initial evidence of radiological progression as per Section 8.2.1.

Seven escalating dose levels of PTX-35 will be explored using a traditional 3+3 design based on DLTs (dose-limiting toxicities) until MTD (maximum tolerated dose) or optimal immunological dose (OID) is established. The starting dose of PTX-35 is based on the MABEL for Treg activity (considered the most sensitive of PTX-35 pharmacodynamic markers) obtained from dose-ranging studies conducted in non-clinical animal models. PTX-35 will be administered to patients intravenously on Day 1 of each cycle. The length of each cycle is

14 days, and the DLT observation period includes the safety data obtained from the first two treatment cycles (4 weeks).

Table 1: PTX-35 Dose Levels

Dose level*	PTX-35 dose (mg/kg)	PTX-35 Concentration (mg/mL)	PTX-35 Dose (mL/kg)	Number of patients
1	0.01 mg/kg	1 mg/mL	0.01 mL/kg	3-6
2	0.03 mg/kg	1 mg/mL	0.03 mL/kg	3-6
3	0.10 mg/kg	1 mg/mL	0.10 mL/kg	3-6
4	0.30 mg/kg	5 mg/mL	0.06 mL/kg	3-6
5	1.0 mg/kg	5 mg/mL	0.20 mL/kg	3-6
6	3.0 mg/kg	5 mg/mL	0.60 mL/kg	3-6
7	10 mg/kg	5 mg/mL	2.0 mL/kg	3-6

**Dose levels 1 through 3 will be administered at a concentration of 1 mg/mL, and dose levels 4 through 7 will be administered at a concentration of 5 mg/mL.*

At each dose level, 3 patients will be enrolled, with a minimum waiting period of one week after treatment initiation of the first patient only. The remaining subjects in the dose level will be treated with no stagger. After all 3 patients in a dose level have received the second dose of PTX-35 and completed the DLT observation period, the Safety Review Committee (SRC) will review the safety data to make a dose escalation decision. If none of these 3 patients experiences a DLT, dose escalation may proceed. If 1 of these 3 patients experiences a DLT, the dose level will be expanded to at least 6 patients at the same dose level. If $\leq$1 out of 6 patients experiences a DLT, then the SRC may recommend dose escalation to the next dose level. If a DLT occurs in 2 or more patients in a particular dose level, the maximum tolerated dose (MTD) has been exceeded and dose escalation will cease. Up to 3 additional patients will be enrolled at the next lowest dose if only 3 patients were treated at that dose level, to confirm safety of that dose.

The conduct and completion of each subsequent dose cohort will follow in similar fashion until the MTD is established. Note that if the optimal immunological dose (OID), or observed plateau of Treg activity, is determined prior to MTD, further dose escalation may be discontinued by decision of the SRC.

Visits and study examinations will be performed per Table 2: Schedule of Assessments. Safety will be assessed by frequency of treatment-emergent adverse events (TEAEs), evaluation of clinical laboratory parameters (hematology and biochemistry), weight, vital signs, electrocardiogram (ECG), performance status, and physical exam findings. NCI CTCAE version 5.0 will be used to grade all toxicities.

A post-treatment safety visit will be conducted approximately 30 days following the last dose of PTX-35. Response to treatment will be assessed according to RECIST 1.1 with planned evaluation performed every 8 weeks (± 1 week) from the start of treatment (C1D1).

6.1.3 Dose-Limiting Toxicity

An event will be considered a DLT per NCI CTCAE version 5.0 criteria if it occurs within the DLT reporting period during Cycle 1 and Cycle 2 (i.e., 4 week evaluation period following the initial administration of PTX-35) and meets at least one of the criteria below:

- Hematological toxicities ≥ Grade 3
- Non-hematological toxicities ≥ Grade 3
- Cytokine release syndrome ≥ Grade 3
- Infusion reaction ≥ Grade 3
- Any ≥ Grade 3 hepatic toxicity meeting Hy's Law criteria, or total bilirubin ≥2.0 x ULN to ≤3.0 x ULN for >7 consecutive days, ≥CTCAE G3 total bilirubin, CTCAE G3 AST or ALT for >7 consecutive days, or CTCAE G4 AST or ALT
- Any other treatment-emergent toxicity that is considered clinically significant and/or unacceptable, and that does not respond to supportive care and results in a disruption of the treatment schedule of more than 14 days.

<u>DLT excludes:</u>

- Hematological and non-hematological ≤ Grade 2 unless considered non-acceptable.
- Other Grade 3 self-limited or medically controllable toxicities (e.g., fever without ≥ Grade 3 neutropenia, lymphopenia, nausea, vomiting, diarrhea, fatigue). Note, such events are considered DLT if the abnormality leads to hospitalization or the abnormality persists for >72 hours despite appropriate interventions (e.g., replacement therapy for electrolyte abnormalities, when indicated).
- Electrolyte disturbances that are managed to Grade 1 or less with supplemental therapy.

Any adverse event that is potentially related to PTX-35 and requires discontinuation of treatment should be considered a DLT. A DLT will be considered related to PTX-35 treatment unless there is a clear, well-documented relation to intercurrent illness or primary malignant disease. AEs that meet the above criteria but occur after the DLT evaluation period will not be defined as DLTs but will be reported as AEs/Serious Adverse Events (SAEs), as applicable, and will be reviewed across all cohorts during the study to help inform dose escalation decisions.

In case of suspicion of a DLT, the Investigator must inform the Medical Monitor/Sponsor immediately. The DLT will be confirmed by the Medical Monitor/Sponsor and a decision must be taken by the Sponsor regarding if an *ad hoc* SRC meeting is required.

Patients who discontinue therapy before completion of 2 cycles (DLT period) for other reasons than dose-limiting toxicity will be replaced. Patients who are tolerating PTX-35 will not have to discontinue study treatment prematurely due to the occurrence of DLTs in another patient in the same cohort, unless decided by the SRC.

6.1.4 Maximum Tolerated Dose and Optimal Immunological Dose

The MTD will be defined as the highest dose level at which ≤ 1 patient of at least 6 patients experienced a DLT during the first two treatment cycles. The OID will be defined as the dose level where increasing doses of PTX-35 do not result in increases in Treg activity (i.e., a plateau of Treg activity is observed). If the OID is reached prior to the MTD, further dose escalations may be discontinued by decision of the SRC.

6.2 Dose Interruptions/Withholding

Investigational Product may be withheld from a patient based on the Investigator's decision in the event of intercurrent illness, adverse event, administrative reasons, or other reasons. If the patient's condition subsequently improves, or the situation that resulted in withholding study drug rectifies itself, the Investigator may resume study treatment as soon as possible, unless the delay is more than 4 weeks.

Dosing should be delayed for any DLT-equivalent toxicity and possible CTCAE > Grade 2 adverse events considered related to study medication. At the Investigator's discretion, study treatment may recommence when the toxicity has resolved to Grade 2 or less. Immune related reactions or allergy should resolve to ≤ Grade 1.

Treatment should be discontinued if a TEAE has not resolved (to acceptable grade) after ≤ 4 weeks.

6.3 Dose Modification and Management of Immune-Related Adverse Events, Infusion Reactions, and Cytokine Release Syndrome

One level dose reduction per the dose escalation scheme, for toxicity is allowed. If the toxicity is equivalent to a DLT (see Section 6.1.3) then continued treatment at a lower dose level needs approval by the sponsor and/or SRC.

If immune related adverse events occur, these should be managed according to the ASCO2018 guideline for immune checkpoint inhibitors[10], and therapy should be continued with close monitoring for Grade 1 toxicities, with the exception of some neurologic, hematologic, and cardiac toxicities. For Grade 2 toxicities, corticosteroids may be administered, and dose should be held until resolution of the AE. Grade 3-4 toxicities treatment with PTX-35 should be discontinued and treatment with high-dose corticosteroids initiated.

All patients should be observed for infusion-related reactions (e.g., back pain, fever and/or shaking chills, flushing and/or itching, alterations in heart rate and blood pressure, dyspnea or chest discomfort, skin rashes, or anaphylaxis). For Grade 1-2 reactions, the infusion should be temporarily interrupted and then resumed when symptoms are resolved. If symptoms have not resolved in 15 minutes treatment with acetaminophen and/or antihistamines may be administered per institutional standard at the discretion of the investigator. Premedication prior to subsequent doses is allowed and should be considered. Grade 3 or 4 infusion related reactions should be managed per institutional standards (e.g., IM epinephrine, followed by IV diphenhydramine and ranitidine, and IV glucocorticoid), and treatment with PTX-35 should be permanently discontinued.

Cytokine release syndrome (CRS) is associated with elevated circulating levels of several cytokines including interleukin (IL)-6 and interferon γ, and uncontrolled studies demonstrate that immunosuppression using tocilizumab, an anti-IL-6 receptor antibody, with or without corticosteroids, can reverse the syndrome. However, because early and aggressive immunosuppression could limit the efficacy of the immunotherapy, current approaches seek to limit administration of immunosuppressive therapy to patients at risk for life-threatening consequences of the syndrome. The treatment algorithm suggested by Lee et al[11] outlined in Figure 4 should be used to manage such reactions.

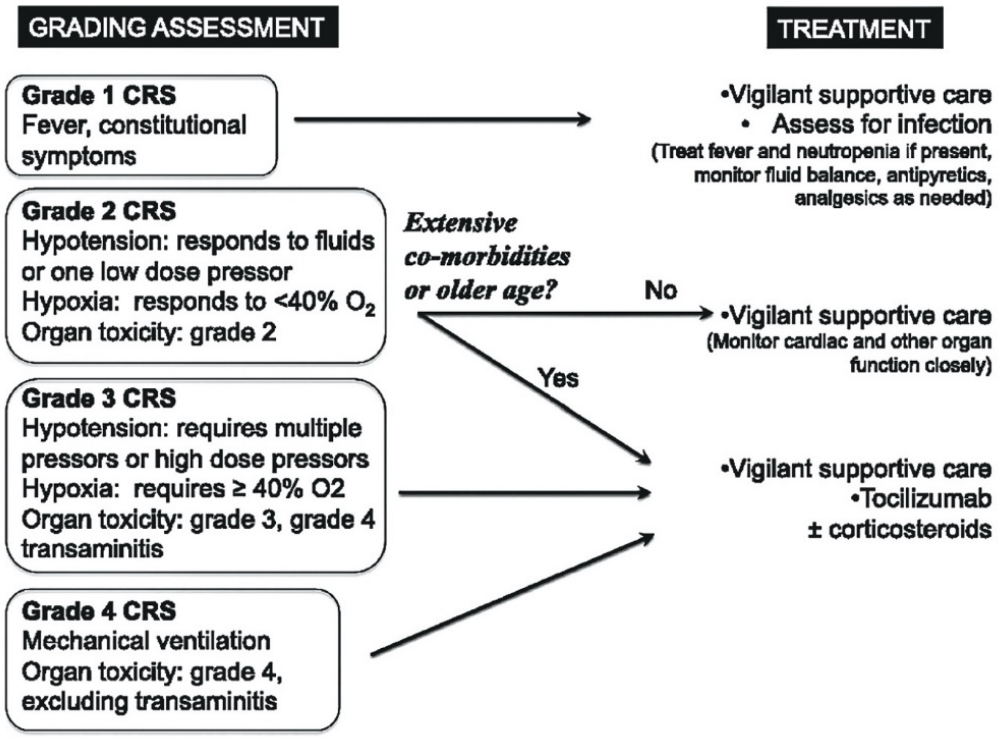

Figure 4: Current concepts in the diagnosis and management of cytokine release syndrome

6.4 Concomitant Treatment

Patients must not receive any other concurrent anti-cancer therapy, including investigational agents while on study treatment.

Unexpected serious adverse reactions, including for example severe allergic reactions, may be treated according to standard-of-care and institutional practice without limitations, including antihistamines, corticosteroids, cytokine antagonists, antipyretics and analgesics.

While on treatment in the study, use of the type of medications specified below should be avoided. PTX-35 will be temporarily stopped for the period of time these medications are used.

- Immunosuppressive agents (i.e., methotrexate, azathioprine, and TNFα blockers), except to treat a PTX-35-related adverse event
- Systemic corticosteroids > 10 mg daily prednisone equivalent

Ocular, intra-articular, intranasal, and inhalational corticosteroids are allowed.

For patients with bone metastases requiring medication for treatment and/or prevention of skeletal-related events bisphosphonates are permissible but RANK-ligand inhibitors, such as Denosumab are prohibited throughout the trial.

Supportive treatment may include anti-emetic, anti-diarrheal, anti-pyretic, anti-histamines, analgesics, antibiotics, and blood products. At the discretion of the treating physician, patients may receive anti-histamine prophylaxis according to the standard of care in clinical practice.

6.5 Monitoring Patient Compliance

This study will be monitored by Pelican Therapeutics or its designee according to ICH E6 guidelines of GCP. The study site monitor will regularly visit the study sites to ensure that the study is conducted according to the protocol and GCP principles.

7.0 STUDY EVALUATIONS

7.1 Schedule of Assessments

Study visit and assessment schedule are summarized in Table 2 and described in Sections 7.2 through 7.6.

Table 2: Schedule of Assessments

Study procedures	Pre-Treatment	Study Treatment											Follow-Up		
		Cycle 1					Cycle 2					Cycle ≥3 D1	30-Day Safety Follow-Up	Post-Treatment Follow-Up (q8 wks)	Survival Follow-Up (q12 wks)
		D1	D2	D4	D7	D10	D1	D2	D4	D7	D10				
Informed consent	×[a]														
Medical history	×[a]														
Physical exam	×[a]	×[c]					×					×	×		
Weight	×[a]	×[c]					×					×	×		
Vital signs	×[a]	×[d]					×[d]					×[d]	×		
ECOG performance status	×[b]	×[c]					×					×	×		
ECG[e]	×[a]	×[c]					×					×			
Tumor measurement[f]	×[a]	Every 8 weeks (± 1 week) from C1D1 until PD or change in cancer treatment													N/A
Hematology[g]	×[b]	×[c]					×					×	×		
Serum chemistry[h]	×[b]	×[c]					×					×	×		
Pregnancy test [i]	×[b]														
Confirmation of eligibility	×	×													
PTX-35 administration[j]		×					×					×			
TENIVAC administration		×													
Blood sample for HLA[k]		×													
Blood sample for PK[l]		×	×	×	×	×	×	×	×	×	×	×[l]			
Blood sample for cytokines		×[m]					×[m]					×[m]			
Blood sample for ATA and anti-TENIVAC titer [n]		×					×					×	×		
Blood sample for immunological effect[o]		×			×		×			×			×		
Prior/concomitant medications review	×[a]	×	×	×	×	×	×	×	×	×	×	×	×	×[p]	×[p]
Adverse Event assessment		×	×	×	×	×	×	×	×	×	×	×	×	×[q]	×[q]
Survival Status															×[r]

Footnotes to Schedule of Assessments

a. Within 28 days prior to study treatment initiation.
b. Within 7 days prior to study treatment initiation.
c. If performed within 3 days of Cycle 1 Day 1, these tests do not need to be repeated unless clinically indicated.
d. Vital signs, including blood pressure, heart rate, respiration rate, and temperature. During Cycle 1 and 2: prior to PTX-35 administration and at 30 min (±5 min) and 1h (±5 min) after end of PTX-35 infusion. Other cycles: only prior to PTX-35 administration.
e. 12-lead ECGs (in triplicate) to be performed with patient in a semi-recumbent position at screening and on Day 1 of each cycle prior to PTX-35 administration.
f. Tumor measurement per RECIST 1.1. Baseline tumor assessment should be performed within 28 days prior to study treatment initiation. Patients will be evaluated every 8 weeks (±1 week) from C1D1 until disease progression, regardless of dose delays.
g. Hematology, including hemoglobin, WBC with differential, and platelet count (Approximately 5 mL blood per draw).
h. Serum chemistry including: sodium, potassium, chloride, CO_2, BUN, creatinine, glucose, calcium, albumin, total protein, total bilirubin, AST, ALT, alkaline phosphatase, TSH (Approximately 5 mL blood per draw).
i. For women of childbearing potential only, a negative serum or urine β-hCG must be documented within 7 days prior to first dose of PTX-35.
j. PTX-35 is administered as an IV infusion. Each patient must remain in clinic for a 6-hour safety observation period after the end of the PTX-35 infusion unless the SRC reduces the required observation period.
k. HLA sample will be collected pre-dose at Cycle 1 only.
l. PK samples will be collected at the following timepoints: pre-dose; 30min (±5min), 2hr (±5min), 4hr (±5min), 24hr (±2hr), 72hr (±2hr), 144hr (±2hr), 216hr (±2hr) after end of PTX-35 infusion for Cycles 1 and 2, and pre-dose at Cycle 3 only.
m. Cytokine blood sample collection at the following timepoints during Cycle 1 and 2: prior to PTX-35 administration and at 2 hr (±5min) after end of PTX-35 infusion. Collection at cycle 3 and beyond is only required if the patient is symptomatic (i.e., flu-like or allergic reactions) prior to PTX-35 infusion and at 2 hr (±5min) after end of PTX-35 infusion.
n. Pre-dose blood sample collection to monitor development of anti-therapeutic antibodies (ATA) and anti-TENIVAC antibody titer.
o. Blood sample collection for immunological effect; Day 1 (prior to PTX-35 administration) and Day 7 for Cycle 1 and Cycle 2, and 30-Day Safety Follow-Up only.
p. In post-treatment and survival follow-up, concomitant medications limited to subsequent anti-cancer therapy.
q. If > 30 days since last dose, only record AEs if deemed related to Investigational Product
r. Survival status and subsequent anti-cancer therapy information will be collected approximately every 12 weeks in a clinic visit or by telephone/electronic communication, from the time of disease progression or initiation of new anti-cancer treatment until death.

7.2 Pre-treatment

Within 28 days from C1D1:

- Sign written informed consent
- Medical history including prior cancer treatments, prior surgeries (requiring an overnight hospitalization), and pre-existing clinical signs and symptoms
- Record prior medications (taken within 30 days of C1D1)
- Complete physical exam
- Weight
- Vital signs (blood pressure, heart rate, respiratory rate, and temperature)
- ECG (a standard 12-lead ECG in triplicate, approximately 2 to 5 minutes apart) is taken while patient is in a semi-recumbent position)
- Imaging for tumor measurements by RECIST 1.1 (APPENDIX II)
- Confirmation of eligibility

Within 7 days from C1D1:

- ECOG performance status (APPENDIX I)
- Blood draw for hematology
- Blood draw for serum chemistry
- Pregnancy test for women of childbearing potential

7.3 During Treatment

7.3.1 Day 1 of Cycle 1

- Eligibility must be re-verified before dosing on Day 1
- Physical exam (may be done within 3 days prior)
- Weight (may be done within 3 days prior)
- Vital signs (blood pressure, heart rate, respiratory rate, and temperature) at 3 timepoints: pre-dose, at 30 min (±5 min), and at 1hr (±5 min) after end of PTX-35 infusion
- ECG (a standard 12-lead ECG in triplicate, approximately 2 to 5 minutes apart) taken while patient is in a semi-recumbent position (may be done within 3 days prior)
- ECOG performance status (may be done within 3 days prior)
- Blood draw for hematology (may be done within 3 days prior)
- Blood draw for serum chemistry tests (may be done within 3 days prior)
- Blood sample for pharmacokinetics at 4 timepoints: pre-dose; 30min (±5 min), 2hr (±5 min) and 4hr (±5 min) after end of PTX-35 infusion
- Blood sample for cytokines at 2 timepoints: pre-dose, and 2hr (± 5 minutes) after end of PTX-35 infusion
- Blood sample for anti-therapeutic antibodies (ATA) and anti-TENIVAC antibody titer (pre-dose)
- Blood sample for immunological effect (pre-dose)

- Blood sample for HLA (pre-dose)
- PTX-35 administration and observation period
- TENIVAC administration
- Concomitant medications review
- Adverse event assessment

7.3.2 Days 2, 4, 7, and 10 of Cycle 1 and Cycle 2

- Blood sample for pharmacokinetics at the following post-dose time points: Day 2 - 24hr (±2hr), Day 4 – 72hr (±2hr), Day 7 – 144hr (±2hr) and Day 10 – 216hr (±2hr)
- Blood sample for immunological effect (Day 7 only)
- Concomitant medications review
- Adverse event assessment

7.3.3 Day 1 of Cycle 2 (+ 2 days)

- Physical exam
- Weight
- Vital signs (blood pressure, heart rate, respiratory rate, and temperature): pre-dose; 30 min (±5 min) and 1hr (±5 min) after end of PTX-35 infusion
- ECG (a standard 12-lead ECG in triplicate (approximately 2 to 5 minutes apart) taken while patient is in a semi-recumbent position
- ECOG Performance status
- Blood draw for hematology
- Blood draw for serum chemistry tests
- Blood sample for pharmacokinetics at 4 timepoints: pre-dose; 30min (±5 min), 2hr (±5 min) and 4hr (±5 min) after end of PTX-35 infusion
- Blood sample for cytokines at 2 time points: pre-dose and 2hr (±5 min) after end of PTX-35 infusion
- Blood sample for anti-therapeutic antibodies (ATA) and anti-TENIVAC antibody titer (pre-dose)
- Blood sample for immunological effect (pre-dose)
- PTX-35 administration and observation period
- Concomitant medications review
- Adverse event assessment

7.3.4 Day 1 of Cycle 3 and Beyond (+ 2 days)

- Physical exam
- Weight
- Vital signs (blood pressure, heart rate, respiratory rate, and temperature): prior to PTX-35 infusion

- ECG (a standard 12-lead ECG in triplicate, approximately 2 to 5 minutes apart) taken while patient is in a semi-recumbent position
- ECOG Performance status
- Blood draw for hematology
- Blood draw for serum chemistry tests
- Blood draw for pharmacokinetics (C3D1 pre-dose only)
- Blood sample for cytokines (ONLY IF PATIENT IS SYMPTOMATIC) at 2 time points: pre-dose and 2hr (±5 min) after end of PTX-35 infusion
- Blood sample for anti-therapeutic antibodies (ATA) and anti-TENIVAC antibody titer (pre-dose)
- PTX-35 administration and observation period
- Concomitant medications review
- Adverse event assessment
- Imaging for tumor measurements by RECIST 1.1 via CT scans or MRI (every 8 weeks ± 1 week from C1D1 until disease progression)

7.4 30-Day Safety Follow up Visit

The following assessments will be performed at 30 days ± 3 days of the last administration of PTX-35 for all patients who are off study treatment:

- Physical exam
- Weight
- Vital signs (blood pressure, heart rate, respiratory rate, and temperature)
- ECOG Performance status
- Blood draw for hematology
- Blood draw for serum chemistry tests
- Blood draw for immunological effect
- Blood sample for anti-therapeutic antibodies (ATA) and anti-TENIVAC antibody titer
- Concomitant medications review
- Adverse event assessment

7.5 Post-Treatment Follow-Up

Any patient that stops treatment (or starts new anti-cancer treatment) for any reason other than progressive disease will enter the Post-Treatment Follow-Up period, which refers to the time between last dose of PTX-35 and disease progression. The following assessments will be performed every 8 weeks from the date of last scan (±1 week) during the Post-Treatment Follow-Up period:

- Imaging for RECIST 1.1 tumor measurements via CT scan or MRI every 8 weeks (±1 week)
- Concomitant medications review, limited to subsequent anti-cancer therapy

- Adverse event assessment (only if deemed related to PTX-35)

7.6 Survival Follow-up

The Survival Follow-Up period refers to the time between disease progression (or initiation of anti-cancer treatment) and patient death. Survival status and subsequent anti-cancer therapy will be collected approximately every 12 weeks in a clinic visit, or by telephone/electronic communication. Patients who withdraw consent for study procedures should still be followed for survival and/or public records searched as per FDA guidance issued October 2008 entitled "Guidance for Sponsors, Clinical Investigators, and IRBs: Data Retention When Subjects Withdraw from FDA-Regulated Clinical Trials".

8.0 STUDY ASSESSMENTS

8.1 Safety Assessments

8.1.1 Safety Analysis

Safety data will be summarized for the safety evaluable population. These data will include Treatment emergent adverse events (TEAEs) and laboratory parameters. Adverse event terms will be coded using the most current version of Medical Dictionary for Drug Regulatory Activities (MedDRA).

8.1.2 Reporting of Adverse Events

8.1.2.1 Adverse Events

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product that does not necessarily have a causal relationship with the treatment. An adverse event can be any unfavorable and unintended sign (including a laboratory finding), symptom or disease temporally associated with the use of an Investigational Product, whether or not related to the Investigational Product.

The AE reporting period starts on Cycle 1 Day 1 after the first dose of PTX-35. If an AE occurs before the first dose of study drug it will be captured as baseline and considered part of medical history unless it directly related to a study procedure (in which case it may be reported as an AE). At each evaluation, patients should be interviewed in a non-directed manner to elicit potential adverse reactions from the patient. The occurrence of an adverse event will be based on changes in the patient's physical examination, laboratory results, and/or signs and symptoms.

All adverse events (except Grade 1 and 2 laboratory abnormalities that do not require an intervention), regardless of causal relationship, are to be recorded in the case report form and source documentation. The Investigator must determine the intensity of any adverse events according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 and their causal relationship. Those AEs not covered by these criteria will be graded as follows:

1. Mild: Discomfort noticed, but no disruption of normal daily activity. Prescription drug not ordinarily needed for relief of symptom but may be given because of personality of patient.

2. Moderate: Discomfort sufficient to reduce or affect normal daily activity. Patient is able to continue in study; treatment for symptom may be needed.

3. Severe: Incapacitating, severe discomfort with inability to work or to perform normal daily activity. Severity may cause cessation of treatment with test drug; treatment for symptom may be given and/or patient hospitalized.

4. Life-Threatening: Symptom(s) place the patient at immediate risk of death from the reaction as it occurred; it does not include a reaction that, had it occurred in a more serious from, might have caused death.

5. Fatal: Event caused the death of the patient.

Adverse events will be followed until resolution or stabilization while the patient remains on-study. Once the patient is removed from study, events thought to be related to the Investigational Product will be followed until resolution or stabilization, unless, in the Investigator's opinion the event is unlikely to resolve due to the patient's underlying disease, or until the patient starts a new treatment regimen or the patient is lost to follow-up.

8.1.2.2 Attribution Definitions

An adverse event is considered to be associated with the use of the Investigational Product if the attribution is determined as possible or definite. Attribution of AEs will be recorded in the CRF as:

- Unrelated: The AE is clearly *not* related to the study treatment.
- Possible: The AE may be related to the study treatment.
- Definite: The AE is clearly related to the study treatment.

8.1.2.3 Definition of an Unexpected Adverse Event

An unexpected adverse event is defined as any adverse drug experience, the specificity or severity of which is not consistent with the current Investigator Brochure; or, if an Investigator Brochure is not required or available, the specificity or severity of which is not consistent with the risk information described in this protocol or in the regulatory agency study authorization application.

Unexpected, as used in this definition, refers to an adverse drug experience that has not been previously observed (e.g., included in the Investigator's Brochure) rather than from the perspective of such experience not being anticipated from the pharmacological properties of the pharmaceutical product.

8.1.2.4 Serious Adverse Event (SAE)

A serious adverse event is defined as any untoward medical occurrence that at any dose:

1. Results in death,

2. Is life-threatening (i.e., the patient was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it was more severe),

3. Requires in-patient hospitalization or prolongation of existing hospitalization excluding that for pain management, disease staging/re-staging procedures, or catheter placement unless associated with other serious events,

4. Results in persistent or significant disability/incapacity, or

5. Is a congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious adverse drug events when, based on appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

Disease Progression, or death as a result of disease progression, **are not considered to be SAEs**. However, if the progression of the underlying disease is greater than what would normally be expected as part of the natural course of the disease under study for the patient, or if the Investigator considers that there was a causal relationship between treatment with study drug or protocol design/procedures and the disease progression, then it must be reported as an SAE.

8.1.2.5 Pregnancy

Any pregnancy detected during the study, or that occurs within 30 days after stopping study medication, must be reported immediately to the Investigator. Pregnancy, in and of itself, is not regarded as an adverse event, unless there is suspicion that study medication may have interfered with the effectiveness of a contraceptive medication. If the patient becomes pregnant while on-study, the study drug should be immediately discontinued. Pregnancy information about a female patient or a female partner of a male patient should be reported immediately from the time the Investigator first becomes aware of a pregnancy or its outcome. This will be performed by the Investigator per instructions from the Sponsor or its designee.

Any pregnancy complication, spontaneous abortion, elective termination of a pregnancy for medical reasons, outcome of stillbirth, congenital anomaly/birth defect, or serious adverse event in the mother will be recorded as an SAE and will be reported as described in Section 8.1.2.6.

8.1.2.6 Reporting of Serious Adverse Events

Adverse events classified as serious require expeditious handling and reporting to Sponsor or its designee to comply with regulatory requirements.

For any serious adverse event (SAE) that occurs while a patient is on-study; within 30 days of the last study drug administration, regardless of any opinion as to the relationship of the SAE to the study drug; or if any SAE that the Investigator feels is related to the study drug occurs later than 30 days after the last study drug administration, the Sponsor or its designee must be notified immediately (within 24 hours of becoming aware of the event).

8.1.2.7 Safety Data Review

8.1.2.7.1 Safety Review Committee

A Safety Review Committee (SRC) comprised of at least three members – one Investigator, a representative from Pelican Therapeutics and the Medical Monitor will evaluate the data obtained at each dose level including a review of all adverse events (serious and non-serious adverse events) as they are reported by the study site. The SRC will recommend whether the dose should be escalated as per protocol, revised to a lower level or intermediate level, halted altogether or more patients are required at the same dose level to evaluate safety.

Ad-hoc SRC meetings may be called for by both the SRC and Sponsor any time during the study if DLTs are observed and/or new safety data warrants immediate action to the conduct of the trial (including events defined by the Study Stopping Criteria in Section 5.5).

The conclusion of the SRC meeting will be documented in meeting minutes. The outcome of the SRC meeting will be communicated to all Investigators.

8.2 Efficacy Assessments

Patient's disease status will be monitored by clinical and radiological assessment (CT scans or MRI) as per RECIST 1.1 criteria where applicable. Patient response per RECIST 1.1 will be evaluated as complete response, partial response, stable disease, or progressive disease.

For the purpose of this study, patients will be evaluated after every 8 weeks ± 1-week from C1D1, regardless of any delays in treatment cycles. In the event objective response (PR or CR) is noted, changes in tumor measurements must be confirmed by repeat assessments that should be performed at least 4 weeks after the criteria for response are first met. For stable disease (SD), follow-up measurements must meet the SD criteria at least 6 weeks after study entry.

8.2.1 RECIST 1.1 Criteria

Response and progression will be evaluated in this study using the international criteria (version 1.1) proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee[12] (see APPENDIX II).

Immune response may be a delayed effect, and in immuno-oncology, treatment beyond first progression is commonly used in situations where clinical progression is asymptomatic and/or is not likely to result in life-threatening complications with further progression. Therefore, at the discretion of the Investigator the treatment with PTX-35 may continue despite evidence of radiologic disease progression, and in the absence of clinical deterioration, if the investigator considers this of a potential clinical benefit for the patient, and if the following terms are met: i) no decline in performance status, ii) absence of rapid disease progression or threat to vital organs or critical anatomical sites (e.g., CNS metastasis, respiratory failure due to tumor compression, spinal cord compression) requiring urgent alternative medical intervention, and iii) no significant, unacceptable or irreversible toxicities related to study treatment.

8.3 Pharmacokinetics

Plasma concentrations of PTX-35 will be measured by ELISA at the following timepoints: pre-dose; 30min (±5min), 2hr (±5min), 4hr (±5min), 24hr (±2hr), 72hr (±2hr), 144hr (±2hr), and 216hr (±2hr) after end of PTX-35 infusion for Cycles 1 and 2, and pre-dose at Cycle 3 only. An approximate 1 mL sample will be collected for each timepoint.

8.4 Analysis of Immunological Effect

Immune-phenotyping of patient's peripheral blood sample will be performed by flow cytometry, which may include but is not limited to surface and intracellular markers that define naïve T cells (CD4 and CD8), central memory T cells (CD4 and CD8), effector T cells (CD4 and CD8), memory T cells (CD4 and CD8), senescent T cells (cytotoxic and helper), terminal differentiated senescent T cells (cytotoxic and helper), natural killer (NK) cells, natural killer T cells (NKT), T regulatory cells, proliferative T regulatory cells, activated T cells (helper and cytotoxic), proliferative T cells (helper and cytotoxic), T cell markers of exhaustion (helper and cytotoxic; CTLA-4, PD-1, TIGIT, TIM3).

Immune phenotype profiling to determine proportions of NK and T cell subsets for levels of antigen activation, memory and exhaustion will be performed from blood samples (approximately 23 mL) collected on Day 1 (prior to PTX-35 administration) and Day 7 for Cycle 1 and Cycle 2, and at the 30-Day Safety Follow-Up visit.

8.5 Analysis of Immunogenicity

Approximately 2 mL of blood samples will be drawn for central analysis of anti-therapeutic antibodies (ATA), at the following time-points:

- All Cycles (pre-dose)
- 30-Day Safety Follow-Up Visit

Samples testing positive in the screening assay will undergo a confirmatory assay to demonstrate that the ATAs are specific for the therapeutic protein product. Samples identified as positive in the confirmatory assay will be further characterized in titration and neutralization assays.

8.6 Analysis of response to TENIVAC immunization

Blood samples collected for immunogenicity will also be used for central analysis of tetanus antibodies, at the following time-points:

- All Cycles (pre-dose)
- 30-Day Safety Follow-Up Visit

8.7 Cytokine Analysis

Approximately 2 mL of blood samples will be collected at the following time-points during Day 1 of Cycle 1 and Cycle 2 for determination of serum cytokines (e.g., IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-17, IL-22, TNF-α, TGF-β, IFN-γ and TL1A).

- Day 1: Pre-dose
- Day 1: 2hr ± 5 minutes from end of PTX-35 infusion

For Cycle 3 and beyond cytokine testing will only be performed if the patient is symptomatic at the time of visit (i.e., flu-like symptoms, fever, or allergic reactions).

9.0 STATISTICS

Demographic data and disease-related characteristics will be summarized using descriptive statistics. Categorical variables will be summarized with incidence and percent. Continuous variables will be summarized with n, mean, median, standard deviation and range (minimum and maximum) as appropriate. Data will be presented by each dose cohort. All patient data will be presented in data listings.

9.1 Analysis Populations

Safety Evaluable Population: All patients who received at least 1 dose of PTX-35.

DLT Evaluable Population: All patients who receive at least 1 dose of PTX-35 and experience a DLT and/or complete the DLT evaluation period.

Efficacy Evaluable Population: All patients with pre-treatment measurable disease by RECIST 1.1, who received at least 1 dose of PTX-35 and who had at least one radiological assessment during treatment.

9.2 Endpoints

9.2.1 Primary

- Number of Dose-limiting toxicities (DLTs) per NCI CTCAE v5.0.
- Frequency of treatment-emergent adverse events (TEAEs) and serious adverse events (SAEs) related to PTX-35 during the trial.

9.2.2 Secondary

- Optimal Immunological Dose (OID; dose where T-reg plateau is observed) or Maximum tolerated dose (MTD; highest dose level at which ≤ 1 patient of at least 6 patients experienced a DLT during the first two treatment cycles).
- Pharmacokinetic parameters will include maximum concentration (C_{max}), area under curve up to the last measurable concentration (AUC_{last}), trough observed serum concentration (C_{trough}) and terminal elimination half-life ($T_{1/2\lambda z}$).

9.2.3 Exploratory

Immune phenotype profiling of immune cell subsets will be conducted by flow cytometry in peripheral blood at various time-points to assess change from baseline in:

- Percentage of Treg cells (immunological effect)
- Frequency of anti-therapeutic antibody (ATA) titers to PTX-35 (Immunogenicity)
- General immunologic expansion: The frequency and expansion of NK, CD4 and CD8 T cell subsets
- Specific antigen driven immune expansion: immune responses to a well-characterized first-time exposure or recall antigen based on the patient's HLA type will be measured at baseline and on study using ELISA, ELISPOT and flow cytometric methods.
- Specific cytokine expression in response to treatment with PTX-35.

Clinical benefit will be evaluated where possible for:

- Best Overall Response, Overall Survival and Progression-Free Survival assessed by RECIST 1.1.

9.3 Safety

Safety will be assessed throughout the study by means of physical examination, weight, vital signs, ECOG performance status, laboratory evaluations (hematology, biochemistry and cytokines), electrocardiogram (ECG), and recording of concurrent illness/therapy and treatment-emergent adverse events. CTCAE version 5.0 will be used to grade all toxicities. All related adverse events will be monitored until resolution or permanent outcome. Concomitant medications will be recorded throughout the study.

Cytokines will be monitored during cycle 1 and 2. Patients will also be monitored for any clinical symptoms associated with elevated cytokine levels. Cytokines will only be monitored at cycle 3 and beyond if patient is symptomatic (i.e., flu-like symptoms, fever, or allergic reactions).

Safety data will be summarized for the safety evaluable population. These data will include Treatment emergent adverse events (TEAEs) and laboratory parameters. Adverse event terms will be coded using the most current version of Medical Dictionary for Drug Regulatory Activities (MedDRA).

9.4 Efficacy

Assessment of tumor response will be performed every 8 weeks (± 1 week) from C1D1 until disease progression according to RECIST 1.1 (APPENDIX II). Best overall response (BOR), Overall Survival (OS) and Progression-Free Survival (PFS) will be determined for each patient.

9.5 Pharmacokinetics

Plasma concentrations of PTX-35 will be measured by ELISA at the following timepoints at Cycle 1 and 2: pre-dose, and 30min (±5min), 2hr (±5min), 4hr (±5min), 24hr (±2hr), 72hr (±2hr), 144hr (±2hr), and 216hr (±2hr) after end of PTX-35 infusion. Plasma concentrations of PTX-35 will also be measured at the following timepoint for Cycle 3: pre-dose.

Non-compartmental or population pharmacokinetic methods will be used to derive PTX-35 PK parameters, which will be summarized using descriptive statistics (mean, standard deviation, median, maximum, minimum).

9.6 Immunogenicity

Blood samples will be drawn for central analysis of anti-therapeutic antibodies (ATA), which will be collected pre-dose on Day 1 of each cycle and at the 30-day follow up visit. Samples testing positive in the screening assay will undergo a confirmatory assay to demonstrate that the ATAs are specific for the therapeutic protein product. Samples identified as positive in the confirmatory assay will be further characterized in titration and neutralization assays.

9.7 General and Specific Antigen Immune Expansion

Blood samples will be drawn to determine each patient's HLA type (collected pre-dose Cycle 1 Day 1) for exclusive use in determining the patients primary or recall response to the TENIVAC vaccine. Blood samples will also be collected pre-dose on Day 1 of each cycle and at the 30-Day Safety Follow-Up visit to measure anti-TENIVAC antibody titer (TDAR). Blood samples will also be collected pre-dose on Day 1 and Day 7 of Cycles 1 and 2 as well as at the 30-Day Safety Follow-Up visit to measure the general and antigen reactive expansion and activity of NK and T cell subsets.

9.8 Sample Size

This is an exploratory trial and therefore no sample size calculations have been performed. The target number of patients (22 - 34 patients) is based on the planned number of dose escalation cohorts required to identify the OID or MTD. Based on response observed in patients at different dose levels and/or in patients with a particular tumor type, the study protocol may be amended to allow for cohort expansion of patients with specific characteristics.

10.0 QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

10.1 Monitoring of the Study and Regulatory Compliance

The Sponsor, or designee, will make an initiation site visit to each institution to review the protocol and its requirements with the Investigator(s), inspect the drug storage area, fully inform the Investigator of his/her responsibilities and the procedures for assuring adequate and correct documentation. During the initiation site visit the case report forms (CRFs) will be reviewed. Other pertinent study materials will also be reviewed with the Investigator's research staff. During the course of the study, the monitor will make regular site visits in order to review protocol compliance, examine CRFs and individual patient's medical records and assure that the study is being conducted according to pertinent regulatory requirements. All CRF entries will be verified with source documentation. The review of medical records will be done in a manner to assure that patient confidentiality is maintained.

10.2 Curricula Vitae and Financial Disclosure of Investigators

All Principal Investigators will be required to provide a current signed and dated curriculum vitae, a completed FDA Form 1572 and a financial disclosure statement to Sponsor or its designee. All Sub-Investigators will be required to provide a current curriculum vitae and a financial disclosure statement to Sponsor or its designee.

10.3 Protocol Modifications

No modification of the protocol should be implemented without the prior written approval of the Sponsor or the Sponsor's representative. Any such changes which may affect a patient's treatment or informed consent, especially those increasing potential risks, must receive prior approval by the IRB/EC. The exception to this is where modifications are necessary to eliminate an immediate hazard to trial subjects, or when the change involves only logistical or administrative aspects of the trial (e.g., change in monitor, change in telephone number). Other administrative revisions which may impact the clinical portion of a study will be duly reported to the IRB/EC by the Principal Investigator.

10.4 Publication Policy

The publication of the results of the study will be subject to the terms and conditions of the clinical trial agreement between the Sponsor and Investigators. Sponsor approval is required for publication of any data from this trial.

11.0 ETHICAL CONSIDERATIONS

11.1 Informed Consent

The Investigator will obtain written informed consent from each patient, or their authorized representative, participating in the study. The form must be signed, witnessed, and dated. The informed consent form will contain all the Essential Elements of Informed Consent set forth in 21 CFR, Part 50, the European Union Directive 2001/20/EC and its associated Detailed Guidances, European Union GCP Directive 2005/28/EC, the ICH Guideline for Good Clinical Practice, Section 4.8, and the terms of the Declaration of Helsinki (2013). Copies of the signed document should be given to the patient and filed in the Investigator's study file, as well as the patient's medical record if in conformance with the institution's Standard Operating Procedures.

11.2 Institutional Review Board/Ethics Committee

The study will not be initiated without approval of the appropriate Institutional Review Board/ Ethics Committee (IRB/EC) and compliance with all administrative requirements of the governing body of the institution. This protocol, consent procedures, and any amendments must be approved by the IRB/EC in compliance with current regulations of the FDA and the European Union as applicable and in accordance with ICH/GCPs. A letter of approval will be sent to the Sponsor prior to initiation of the study and when any subsequent modifications are made. The IRB/EC will be kept informed by the Investigator, Sponsor, or its designee, as required by national regulations, as to the progress of the study as well as to any serious and unexpected adverse events.

11.3 Patient Privacy

In order to maintain patient confidentiality, all case report forms, study reports and communications relating to the study will identify patients by initials and assigned patient numbers; patients should not be identified by name. In accordance with local, national, or federal regulations, the Investigator will allow the Sponsor or designee personnel access to all pertinent medical records in order to verify the data gathered on the case report forms and to audit the data collection process. Regulatory agencies such as the US Food and Drug Administration (FDA) may also request access to all study records, including source documentation for inspection. Clinical information will not be released without the written permission of the patient as outlined in the patient consent form.

12.0 DATA HANDLING AND RECORD KEEPING

12.1 Recording of Data

Data collected during the study will be entered in the patient's Case Report Form (CRF) by the investigational site staff. The staff will keep records of the patient's visit in the files considered as source documents for the site, e.g., hospital chart, research chart. The Investigator will be responsible for the recording of all data on the CRF and for submitting the data to the Sponsor or their designee in a timely manner. Should any value be significantly different from normal, the Investigator will comment in the appropriate sections provided in the CRF.

The Investigator will provide access to his/her original records to permit a representative from the Sponsor to verify the proper transcription of data. To facilitate photocopying, entries must be recorded legibly in black ink only. Erroneous entries will be crossed out with a single line, so as to remain legible. The correct value will be entered above the error and then initialed and dated by the person authorized to make the correction.

12.2 Study Records

U.S. Federal laws require that an Investigator maintain all study records for the indication under investigation for two years following the date a Product Licensing Application is approved or, if no application is to be filed or if the application is not approved for such indication, until two years after the investigation is discontinued and the FDA is notified.

13.0 REFERENCES

1. Smith-Garvin JE, Koretzky GA, Jordan MS. T Cell Activation. *Annu Rev Immunol*. 2009;27(1):591-619. doi:10.1146/annurev.immunol.021908.132706

2. Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nat Rev Immunol*. 2013;13(4):227-242. doi:10.1038/nri3405

3. Schreiber TH, Wolf D, Tsai MS, et al. Therapeutic Treg expansion in mice by TNFRSF25 prevents allergic lung inflammation. *J Clin Invest*. 2010;120(10):3629-3640. doi:10.1172/JCI42933

4. Slebioda TJ, Rowley TF, Ferdinand JR, et al. Triggering of TNFRSF25 promotes CD8+ T-cell responses and anti-tumor immunity. *Eur J Immunol*. 2011;41(9):2606-2611. doi:10.1002/eji.201141477

5. Melero I, Hirschhorn-Cymerman D, Morales-Kastresana A, Sanmamed MF, Wolchok JD. Agonist antibodies to TNFR molecules that costimulate T and NK cells. *Clin Cancer Res*. 2013;19(5):1044-1053. doi:10.1158/1078-0432.CCR-12-2065

6. Segal NH, Logan TF, Hodi FS, et al. Results from an integrated safety analysis of urelumab, an agonist anti-CD137 monoclonal antibody. *Clin Cancer Res*. 2017;23(8):1929-1936. doi:10.1158/1078-0432.CCR-16-1272

7. Segal NH, He AR, Doi T, et al. Phase i study of single-agent utomilumab (PF-05082566), a 4-1bb/cd137 agonist, in patients with advanced cancer. *Clin Cancer Res*. 2018;24(8):1816-1823. doi:10.1158/1078-0432.CCR-17-1922

8. Papp KA, Gooderham MJ, Girard G, Raman M, Strout V. Phase I randomized study of KHK4083, an anti-OX40 monoclonal antibody, in patients with mild to moderate plaque psoriasis. *J Eur Acad Dermatology Venereol*. 2017;31(8):1324-1332. doi:10.1111/jdv.14313

9. Burris HA, Infante JR, Ansell SM, et al. Safety and Activity of Varlilumab, a Novel and First-in-Class Agonist Anti-CD27 Antibody, in Patients With Advanced Solid Tumors. *J Clin Oncol*. 2017;35(18):2028-2036. doi:10.1200/JCO.2016.70.1508

10. Brahmer JR, Lacchetti C, Schneider BJ, et al. irAE_guideline_JCliOnc2018. *J Clin Oncol*. 2018;36(17):JCO.2017.77.638. doi:10.1200/JCO.2017.77.6385

11. Lee DW, Gardner R, Porter DL, et al. Current concepts in the diagnosis and management of cytokine release syndrome. *Blood*. 2014;124(2):188-195. doi:10.1182/blood-2014-05-552729

12. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009;45(2):228-247. doi:10.1016/j.ejca.2008.10.026

APPENDIX I - ECOG Performance Status

Grade	
0	Fully active, able to carry on all pre-disease performance without restriction.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work.
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

APPENDIX II - Tumor Measurement Based on RECIST 1.1 Criteria

See the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee, version 1.1[12] for additional details on RECIST 1.1.

Measurable disease will be defined as the presence of at least one measurable lesion that can be accurately measured in at least one dimension with the longest diameter a minimum size of:

- >10mm by CT scan (CT scan slice thickness no greater than 5 mm)
- 10mm caliper measurement by clinical exam (lesions which cannot be accurately measured with calipers should be recorded as non-measurable).
- 20 mm by chest X-ray.

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

All other lesions, including small lesions (longest diameter <10mm or pathological lymph nodes with ≥10 to <15 mm short axis) as well as truly non-measurable lesions, will be considered non-measurable. Lesions considered truly non-measurable include: leptomeningeal disease; ascites; pleural/pericardial effusion; inflammatory breast disease; lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

All measurements should be recorded in metric notation, using calipers if clinically assessed. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment. The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging based evaluation should always be done rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam. Clinical lesions will only be considered measurable when they are superficial and ≥10 mm diameter as assessed using calipers (e.g., skin nodules). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesions is recommended.

Baseline Documentation of Target and Non-Target Lesions
All measurable lesions up to a maximum of 5 lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as target lesions and will be recorded and measured at baseline.

Target lesions should be selected on the basis of their size (lesions with the longer diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements.

A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize the objective tumor response of the measurable dimension of the disease.

All other lesions (or sites of disease) including pathological lymph nodes should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required, and these lesions should be followed as "present" or "absent", or in rare cases "unequivocal progression".

Evaluation of Target Lesions using RECIST 1.1 Criteria
NOTE: In addition to the information below, also see section 4.3.2 in the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee, version 1.1[12] for special notes on the assessment of target lesions.

Complete response (CR) − Disappearance of all target lesions. Any pathological lymph node (LN) (whether target or non-target) must have decreased in short axis to <10mm.

Partial response (PR) − At least a 30% decrease in the sum of the LD of the target lesions taking as reference the baseline sum LD.

Progressive Disease (PD) − At least a 20% increase in the sum of the LD of the target lesions taking as reference the smallest sum LD recorded since the treatment started including baseline if that is the smallest on study. In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5mm. The appearance of one or more new lesions also constitutes PD.

Stable disease (SD) − Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD taking as references the smallest sum LD since the treatment started.

Evaluation of Non-Target Lesions using RECIST 1.1 Criteria
Complete response (CR) − Disappearance of all non-target lesions and normalization of tumor marker levels. All LN must be non-pathological in size (< 10mm short axis).

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

Progressive disease (PD) − Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions.

Evaluation of Best Overall Response using RECIST 1.1 Criteria

The best overall response is the best response recorded from the start of the study treatment until the end of treatment provided the confirmation criteria are met. To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat studies that should be performed > 4 weeks after the criteria for response are first met. If a CR/PR cannot be confirmed the original "response" should be considered stable disease. The best overall response will be defined according to the following table:

Overall Response First Time Point	Overall Response Subsequent Time Point	BEST Overall Response
CR	CR	CR
CR	PR	SD, PD, or PR[1]
CR	SD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	NE[2]	SD provided minimum criteria for SD duration met, otherwise, NE[2]
PR	CR	PR
PR	PR	PR
PR	SD	SD
PR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
PR	NE[2]	SD provided minimum criteria for SD duration met, otherwise, NE[2]
NE	NE[2]	NE[2]

[1] If a CR is truly met at first time point, then any disease seen at a subsequent time point, even disease meeting PR criteria relative to baseline, makes the disease PD at that point (since disease must have reappeared after CR). Best response would depend on whether minimum duration for SD was met. However, sometimes 'CR' may be claimed when subsequent scans suggest small lesions were likely still present and in fact the patient had PR, not CR at the first time point. Under these circumstances, the original CR should be changed to PR and the best response is PR.

[2] NE=unevaluable

APPENDIX III - Summary of Changes from Previous Versions

Changes from Protocol v2.1

Section # and Title	Page(s) #	Description
5.1 Inclusion Criteria; Synopsis	8, 27	• Continuous abstinence included in addition to contraception for participants of child-bearing potential
6.1.1.1 PTX-35	30	• Clarification that patient weight from screening should be used for all visit dose calculations (unless there is a 10% weight change from screening requiring recalculation), or unless institutional policy dictates otherwise.
6.1.3 Dose-Limiting Toxicity; Synopsis	9, 33	• Updated DLT definition to exclude Grade 3 lymphopenia
7.0 Study Evaluations	37-41	• Corrections made to Table 2 (Schedule of Assessments) and list of study visit procedures to address inconsistencies and clarify required evaluations.
Table 2, Schedule of Assessments Footnote 'R'; 7.6 Survival Follow-Up	38, 41	• Added electronic communications as a method of contact for survival follow up.
8.4 Analysis of Immunological Effect; 8.5 Analysis of Immunogenicity; 8.6 Analysis of Response to TENIVAC immunization; 8.7 Cytokine Analysis	45-46	• Minor clarification of blood sample collection requirements. • Revised cytokine 2hr post-dose collection timepoint to 2hr ±5 minutes from end of PTX-35 infusion, for consistency and clarity.
Various throughout	Various	• Revised all timepoint references of PTX-35 "post-dose" to "after end of PTX-35 infusion" for clarity.
Various throughout	Various	• Minor formatting changes

Changes from Protocol v2.0

Section # and Title	Page(s) #	Description
Synopsis	7 – 9	• Verbiage updated to match protocol body; the synopsis was erroneously not updated in Protocol v2.0. • Exclusion criteria renumbered to start with #1.
Title Page and 1.3 Medical Monitor	1, 15	• Added name and contact information of Medical Monitor.
4.2 Trial Design and 6.1.2 Dose Escalation Scheme	26, 33	• 28 days changed to 4 weeks. • 2 weeks changed to 14 days.

6.1.1.1 PTX-35	32	• Clarification added to avoid confusion between the 0.7 mL priming volume associated with the infusion set, and the 1.0 mL priming volume required in the Investigational Product Manual.
7.2 Pre-Treatment	40	• Added detail regarding collection of medical history and prior medications.
7.3.4 Day 1 of Cycle 3 and Beyond	41	• Removed the minus two (-2) day option for visit window schedule to ensure that a cycle will not be shorter than 14 days.
Appendix III	58	• Added Summary of Changes from previous version.
Throughout- Various	Throughout	• Removal of the word 'chemokine' and associated list of cytokines for consistency throughout document.

Changes from Protocol v3.0

Section # and Title	Page(s) #	Description
Title Page and 1.3 Medical Monitor	1, 17	• Medical Monitor changed from Vance Sohn, MD to Alan Epstein, MD, JD, MBA, SM
Investigators and Study Centers	6	• Added Rachel Sanborn, MD and study centers in Austin and Portland
Study Overview	7	• Updated to reflect total of 7 dose levels and added cohorts 6 and 7 to table
Target Number of Patients	8	• Updated number of patients from 15 to 30 to 22 to 34
4.2 Overview of Trial Design	29	• Updated to reflect total of 7 dose levels
6.1.1.1 PTX-35	35	• Updated from dose levels 4 and 5 to dose levels 4 through 7 and clarified to permit Investigator to shorten observation period after first treatment cycle
6.1.2 Dose Escalation Scheme	36, 37	• Updated to reflect total of 7 dose levels and added cohorts 6 and 7 to table and clarified dose stagger after first patient only
7.1 Table 2	44	• Footnote l 30-minute pk time point corrected time window from ± 2 minutes to ± 5 minutes
8.3 Pharmacokinetics	52	• Corrected 30-minute pk time point window from ± 2 minutes to ± 5 minutes
9.5 Pharmacokinetics	55	• Corrected 30-minute pk time point window from ± 2 minutes to ± 5 minutes
9.8 Sample Size	56	• Updated sample size from 15 to 30 to 22 to 34