



STATISTICAL ANALYSIS PLAN

A Phase 1/2 study of pegzilarginase (AEB1102, Co-ArgI-PEG) in combination with pembrolizumab in the treatment of patients with extensive disease (ED) small cell lung cancer (SCLC)

Protocol: CAEB1102-101B

Protocol Number: CAEB1102-101B

Protocol Version and Date: Original Protocol, Version 1, 20SEP2017
Amendment 1, 08NOV2018

Name of Test Drug: AEB1102 (Co-ArgI-PEG, pegzilarginase)
Pembrolizumab

Sponsor: Aeglea BioTherapeutics, Inc.
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Analysis Plan Date: 24JUN2020

Analysis Plan Version: 1.0

Version Number	Summary of Changes	Version Date
1.0	Version 1.0	24JUN2020

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APPROVAL SIGNATURE PAGE

Protocol Title: A Phase 1/2 study of pegzilarginase (AEB1102, Co-ArgI-PEG) in combination with pembrolizumab in the treatment of patients with extensive disease (ED) small cell lung cancer (SCLC)

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APPROVAL SIGNATURE PAGE, continued

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Sponsor Approval

By signing this document, I acknowledge that I have read the document and approve of the planned statistical analyses described herein. I agree that the planned statistical analyses are appropriate for this study, are in accordance with the study objectives, and are consistent with the statistical methodology described in the protocol and the clinical development plan.

Aeglea BioTherapeutics, Inc.

Date

Aeglea BioTherapeutics, Inc.

Date



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LIST OF ABBREVIATIONS

Abbreviation	Definition
ADA	Anti-drug antibody
AE	Adverse event
arginine	Refers to L-arginine, unless otherwise noted
ASL	Argininosuccinate lyase
ATC	Anatomical therapeutic chemical
AUC _{0-t}	Area Under the Curve from time 0 to t
AUC _{0-∞}	Area Under the Curve from time 0 to ∞
BOR	Best overall response
CBR	Clinical benefit rate
CL	Clearance
C _{max}	Maximum PK Concentration
CR	Complete remission
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
CV	Coefficient of variation
DES	DLT evaluable analysis set
DLT	Dose-limiting toxicity
DOR	Duration of response
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic Case Report Form



ED	Extensive disease
FAS	Full analysis set
IV	Intravenous
max	maximum
MedDRA	Medical Dictionary for Regulatory Activities
min	minimum
NCI	National Cancer Institute
ORR	Overall remission rate
PD	Pharmacodynamic(s)
PFS	Progression-free survival
PK	Pharmacokinetic(s)
PKC	PK concentration analysis set
PT	Preferred term
PR	Partial remission
RECIST	Response Evaluation Criteria in Solid Tumors
RES	Response-evaluable analysis set
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SCLC	Small cell lung cancer
SD	Standard deviation or Stable disease, per context
SOC	System organ class
T _{1/2}	Half-life
T _{max}	Time at which maximum concentration (C _{max}) occurs



TEAE	Treatment-Emergent Adverse Event
TTR	Time to response
V _{ss}	Volume of distribution at steady state
WHO	World Health Organization
WHODrug	World Health Organization Drug Dictionary



1. PURPOSE

This document is the statistical analysis plan (SAP) that is used for analysis and reporting, including tables, listings, and figures, for CAEB1102-101B (“101B”), the study of AEB1102 (Co-Arg1-PEG, pegzilarginase) in combination with pembrolizumab in patients who have received platinum-based therapy for extensive disease (ED) small cell lung cancer (SCLC) treatment and either have not tolerated the regimen or have relapsed or progressed within 6 months of treatment. The analyses specified in this SAP may be used to support the clinical study report (CSR), publications, and regulatory submissions.

This SAP is based on protocol amendment 1, 08 NOV 2018.

2. STUDY POPULATION

This study of pegzilarginase in combination with pembrolizumab is planned in a population of patients who have received platinum-based therapy for ED-SCLC treatment and either have not tolerated the regimen or have relapsed or progressed within 6 months of treatment.

3. STUDY OBJECTIVES

Study objectives as per the protocol are presented below:

3.1 Study Objectives

3.1.1 Primary Objective (Phase 1)

The primary objective of the Phase 1 is:

- To determine the safety and select a recommended Phase 2 dose of pegzilarginase when combined with pembrolizumab in patients with ED-SCLC that either have not tolerated the regimen or tumor has progressed or relapsed on or within 6 months after platinum-based therapy.

3.1.2 Primary Objective (Phase 2)

The primary objective of Phase 2 is:

- To determine (using a Simon 2-stage minimax design) the objective response rate (ORR: CR+PR) by RECIST 1.1 of pegzilarginase in combination with pembrolizumab.



3.1.3 Secondary Objectives

The secondary objectives of the study are:

- To determine the ORR by iRECIST 1.1 of pegzilarginase in combination with pembrolizumab.
- To determine the following additional efficacy endpoints by both Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 and modified Response Evaluation Criteria in Solid Tumors 1.1 for immune-based therapeutics (iRECIST):
 - Clinical benefit rate (CBR), (i.e., CR + PR + SD lasting at least 8 weeks)
 - Time to response (TTR)
 - Duration of response (DOR)
 - Progression-free survival (PFS)
- To describe overall survival
- To evaluate the safety profile of the combination of pegzilarginase with pembrolizumab in the study patient population (Phase 2)

3.1.4 Exploratory Objectives (Dose Escalation and Expansion)

The exploratory objectives of the study are:

- To evaluate the effect of the combination of pegzilarginase with pembrolizumab on blood levels of circulating pegzilarginase, arginine and additional amino acids
- To evaluate the immunogenicity of pegzilarginase
- To explore the relationship between response to combination therapy with pegzilarginase and pembrolizumab and:
 - PD-L1 expression in tumor
 - ASS1 expression in tumor
 - Immune composition of tumors determined by PanCancer Immune profiling and interferon gamma signature
 - Mutational burden of tumors
 - Immune profiling of circulating immune cells
 - Cytokine profiling
 - Other biomarkers potentially related to arginine depletion and/or PD-L1 blockade

4. STUDY METHODS

This section of the SAP (Section 4) provides background for the reader and is intended to summarize methodology from the protocol. No new methodology or additional detail is



introduced. Any discrepancies between the protocol language and the text in this section are inadvertent and should not be interpreted as a change to the protocol language. This section will not be updated for changes to the protocol in subsequent amendments.

4.1 Overview of Study Design

Study 101B is a Phase 1/2 study of pegzilarginase in combination with pembrolizumab in patients with ED-SCLC. Patients who have received platinum-based therapy for ED-SCLC treatment and either have not tolerated the regimen or have relapsed or progressed within 6 months of treatment may participate in the study. Detailed Inclusion/Exclusion criteria are found in protocol sections 8.5.1 and 8.5.2.

4.1.1 Phase 1

Dose escalation: The dose of pegzilarginase will be escalated in two steps from 0.20 mg/kg and then at the single agent MTD (0.33 mg/kg as determined in the CAEB1102-100B study). If pegzilarginase at the single agent MTD exceeds the MTD when given in combination with pembrolizumab, intermediate dose levels between the prior dose level and the dosed that exceeded the MTD may be explored. One cycle will be 3 weeks.

Patients will be observed for a dose-limiting toxicity (DLT) during the first Cycle (3 weeks) of treatment. Please refer to Section 8.6.1 for more information regarding the dose-escalation plan as it relates to DLT findings.

A safety committee consisting of all participating investigators and the Sponsor's medical monitor will review the safety information for each dose cohort and determine whether dose escalation will occur at the next higher dose level to be studied.

4.1.2 Phase 2

In the Phase 2 part of the study, patients who meet entry criteria will be treated with the recommended Phase 2 dose (RP2D) of pegzilarginase with 200 mg pembrolizumab in a minimax Simon 2-stage design. Fifty-five patients will be enrolled and treated in stage 1. If 23 or more of those patients achieve a response (CR or PR), an additional 11 patients will be enrolled.

4.2 Number of Patients and Method of Assigning Patients to Treatment Groups

Up to 18 (9 minimum) patients may participate in the Phase 1 portion and up to 66 in the Phase 2 portion (i.e., 55 in stage 1 and 11 additional for stage 2), for a total of approximately 84 patients.

4.3 Administration of Study Drugs

Patients will receive pegzilarginase by slow IV infusion once weekly at their assigned dose level. Pembrolizumab will be administered dose of 200 mg on Day 1 of each



3-week treatment cycle after all procedures and assessments have been completed as detailed in the Schedule of Assessments.

4.3.1 Replacement of Patients

If a patient in the dose-escalation phase of the study does not experience a DLT, but also cannot be declared DLT-free due to insufficient follow-up or insufficient dosing, the patient will not be included in determination of RP2D but will be included in other analyses. To be considered DLT-free, a patient must receive at least two of the three planned pegzilarginase doses in the first cycle of treatment without dose reduction and be observed for at least 21 days. An additional patient will be screened and treated to meet the requirements of the design for determining RP2D.

4.3.2 Dose Adjustments and Stopping Rules

No dose modification is permitted during the first treatment cycle in the dose-escalation portion of the study (except after resolution of a DLT). Patients who experience a DLT may be allowed to continue in the study with a pegzilarginase dose modification after consultation with the Sponsor's medical monitor and after resolution of the toxicity to less than Grade 2 or baseline.

After the first cycle of treatment, pegzilarginase dose modification will be allowed in patients who experience toxicity according to Table 4: Pegzilarginase Dose Modification due to Adverse Events in Section 8.7.8 of the protocol. Dose modification and toxicity management guidelines for immune-related AEs (irAEs) associated with pembrolizumab are provided in Table 3 in Section 8.7.8 of the protocol.

5. STUDY ENDPOINTS

5.1.1 Safety Outcome Measures

Safety outcome measures will include adverse events (AEs), serious adverse events (SAEs), changes in physical exam findings, vital signs, electrocardiograms (ECGs), hematology, urinalysis, electrolytes, coagulation parameters, thyroid function, and serum chemistries.

Immunogenicity safety measures will include levels of anti-drug antibodies (ADAs) and anti-PEG antibodies.

5.1.2 Pharmacodynamic Outcome Measures

The primary pharmacodynamic (PD) outcome measure is plasma arginine levels obtained at specific time points according to the Schedule of Assessments (Section 12.1).



Ornithine and other amino acids may be explored as part of secondary outcomes.

5.1.3 Pharmacokinetic Outcome Measures

PK assessments of C_{max} , T_{max} , $T_{1/2}$, AUC_{0-t} , $AUC_{0-\infty}$, V_{ss} and CL will be based on blood samples for pegzilarginase concentrations obtained at various time points according to the Schedule of Assessments (Section 12.1).

5.1.4 Efficacy Outcome Measures

The primary efficacy outcome measure is overall remission rate (ORR) as assessed by investigators. Clinical benefit rate (complete remission [CR] + partial remission [PR] + stable disease [SD] lasting at least 8 weeks or iCR + iPR + iSD lasting 8 weeks) will be assessed according to RECIST v1.1 and iRECIST. Time to response (TTR), Duration of response (DOR), Progression-free survival, Overall survival and CBR comprise the list of secondary efficacy endpoints.

Additional exploratory analyses look to establish relationships between combination therapy and certain baseline characteristics, including PD-L1 expression in tumor, ASS1 expression in tumor, immune composition of tumors determined by PanCancer Immune profiling and interferon gamma signature, mutational burden of tumors, immune profiling of circulating immune cells, and cytokine profiling.

6. DATA CAPTURE AND PROCESSING

Data will be captured on electronic case report forms (eCRFs) and maintained in a Medidata Rave clinical database. All data will undergo a rigorous review process including electronic and manual data checks. Periodic data quality reviews will also be conducted to minimize errors and omissions.

7. ANALYSIS AND REPORTING

7.1 Interim Analyses

There is no formal interim data analysis planned for this study.

7.2 Final Analysis

Final data summaries, figures, and listings will be prepared as identified in this SAP after patients in all cohorts complete the study and following database lock. Interim outputs may be generated for purposes of data review and publication at scientific meetings; such analyses will be considered descriptive rather than confirmatory. Any changes to the analyses described in the SAP will be documented in the CSR.



8. SAMPLE SIZE DETERMINATION

The sample size for the Phase 1 portion of the study is based on the clinical criteria of a modified Toxicity Probability Interval (mTPI) design. At least 9 patients, with a maximum of 12 patients, are anticipated to participate.

Using a standard Simon 2-stage, minimax, design the sample size for the Phase 2 portion will be 55 patients for stage 1 and 11 additional patients for stage 2. If 23 or more of stage 1 patients achieve a response (CR or PR), an additional 11 patients will be enrolled. If a total of 30 or more patients in the two stages achieve a response, then we will conclude that the response rate is greater than 35%. This design has a 1-sided type 1 error rate of 5%, and a power of 80% if the true response rate is at least 50%.

9. ANALYSIS SETS

Data for all enrolled (consented) patients will be listed regardless of whether they received Study Medication or not (e.g., any patient enrolled [consented] but never treated will be included in data listings and listed under “never treated”), unless otherwise noted. Analysis sets for tabulations and figures are defined below for the following analysis sets: consented patient set, full analysis set (FAS), the Phase 1 Set (P1S), the DLT Evaluable Analysis Set (DES), the Phase 2 Set (P2S), the response-evaluable analysis set (RES), the PK concentration analysis set (PKC), and the PD parameter analysis set (PD).

9.1 Consented Patient Set

The Consented Patient Set will consist of all patients who sign informed consent.

9.2 Full Analysis Set (FAS)

The FAS includes all patients who receive at least one dose of pegzilarginase (referred to as the safety analysis set in the Protocol). The FAS is used for evaluating patient characteristics, treatment administration, and safety endpoints.

9.3 Phase 1 Set (P1S)

The Phase 1 Set will consist of all patients in the FAS who participate in the dose escalation portion of the study.

9.4 DLT Evaluable Analysis Set (DES)

All patients in the dose-escalation portion of the study who receive at least 66% of the planned doses in the first cycle or discontinue due to toxicity after receiving any amount of study drug in the first cycle. Patients who withdraw from the study prior to completing 66% of the planned doses in the first cycle for reasons other than toxicity will be considered



unevaluable for DLT assessments and will be replaced. All DLT data will be summarized using the DES.

9.5 Phase 2 Set (P2S)

The Phase 2 Set will consist of all patients in the FAS who participate in the Phase 2 portion of the study and who have baseline radiological assessment.

9.6 Response-Evaluable Analysis Set (RES)

All patients who have received study drug and have baseline and at least one post-baseline on-study assessment of tumor response.

9.7 Pharmacokinetic Concentration Analysis Set (PKC)

The PKC includes all patients in the FAS who are enrolled (consented) in the study and have any measurable pegzilarginase concentration-time data.

9.8 Pharmacodynamic Analysis Set (PD)

The PD includes all patients in the FAS who are enrolled (consented) in the study and have any valid PD data.

10. GENERAL STATISTICAL CONSIDERATIONS

10.1 General Statistical Methodology

Patient data collected on the eCRFs will be listed and summarized. Patient profiles will also be provided. All data (from both planned and unplanned visits) for all enrolled (consented) patients will be listed.

Unless otherwise noted, data will be summarized by dose groups where dose groups are defined as follows:

- Each specific dose investigated in the dose escalation portion of the study;
- Each stage investigated in Phase 2 (i.e., stage 1 and stage 2);
- All Phase 2 patients; and
- All patients treated at the RP2D.

By visit analyses will include data collected at planned, protocol-specified study visits only. Any data from early termination visits, unscheduled visits and follow-up visits will be listed only.

For continuous variables, number of non-missing observations (n), mean, standard deviation (SD), median, minimum (min) and maximum (max) will be presented by visit and time point. Where noted, change from baseline and/or percent change from baseline

will be reported. The number of patients (n), mean, SD, median, min, max, coefficient of variation (CV%), geometric mean, and geometric CV will be presented for PK parameters, as appropriate. Analysis of PK parameters may be provided in a separate report, in which case the analysis methods specified in that document will take precedence over any analysis methods mentioned in this SAP.

For vital signs, ECGs, and laboratory data, baseline is defined as the latest non-missing assessment attained prior to the date/time of first dose of study treatment.

Discrete variables will be summarized as the number (n) and percentage (%) of observations within each category by visit and time point. As needed, a category of “Not Reported” will be included in summaries of categorical variables to capture observations that are unavailable. The number of patients with non-missing data will be used as the denominator for percentage calculations unless otherwise noted.

Due to a lack of efficacy study enrollment was halted early and, therefore, no statistical testing will be conducted nor will the efficacy endpoint be provided in any outputs. .

No formal hypothesis testing is planned.

10.1.1 Handling of Missing Data

All data will be analyzed as they were collected in the database. In general, missing data, unless noted otherwise (see, for example, Section 10.1.2), will not be imputed.

10.1.2 Derived and Computed Variables

The following derived and computed variables have been initially identified as important for pre-specified and exploratory analyses. Additional computed variables may be required to aid in analysis. The SAP will not be amended to describe additional computed variables. Computed variables added after approval of the SAP will be described in the CSR.

Missing Date Components of AEs, Concomitant Medications and Medical History

Every attempt will be made to obtain complete dates for AEs, concomitant medications, and medical history; however, in the case of incomplete dates, the missing Day, Month or Year will be represented in listings as “UN”, “UNK” or “UNKN”, respectively. Missing Hour or Minute in dates will be represented as “UN”.

Missing date components will not be imputed; however, AEs with incomplete start dates will be attributed to treatment according to the following algorithm:

- Only the year is reported: Unless the year of the event precedes the year of the first dose, the event will be considered treatment-emergent.
- Only the month and year are reported: Unless the month (and year) of the event



precedes the month (and year) of the first dose, the event will be considered treatment-emergent.

For the “time since” calculations, the following equation will be used:

$$\text{Time (months)} = (\text{Date of the first dose} - \text{date of diagnosis} + 1) / 30.42.$$

If the month and year of the diagnosis are provided but the day is missing, the missing day is imputed as 15. If only the year is provided, then the missing month and day are imputed as July 1 for the calculation. If the year is missing, the patient will be excluded from the analysis. If the imputed diagnosis date is after the first dose date, the time will be imputed as 1 day.

Study Days Relative to First Administration of Study Medication

Study Day 1 is the day of the first administration of study medication; negative Study Days occur prior to first administration of study medication; and positive Study Days are those after the first administration of study medication – e.g., Study Day -1 is the day immediately preceding the first administration of study medication, Study Day 2 is the day immediately following the first administration of study medication.

AEs with Missing Severity, CTCAE Grade and/or Relationship

Every attempt will be made to obtain complete information for AEs regarding severity Common Terminology Criteria for Adverse Events (CTCAE) Grade and relationship(s); however, in the case of missing data, the AE severity and/or relationship will be classified as “Not Reported”.

Cumulative Actual Dose

The cumulative actual dose in milligrams per kilograms (mg/kg) is defined as the sum of the actual dose administered in mg/kg over all doses administered.

Laboratory Values with < or > Inequality Symbols

Some continuous laboratory data include “<” or “>” symbols. Data will be presented in listings with their inequality symbol; however, for tabulation of summary statistics of safety laboratory results, the number associated with the inequality sign will be used for statistical calculations.

11. PATIENT CHARACTERISTICS AND DEMOGRAPHICS

11.1 Enrollment and Disposition of Patients

Summary statistics including numbers and percentages will be presented for the following by dose group (see Section 10.1) in the FAS:



- Treated patients
- Patients ongoing at time of data cut
- Patients who discontinued the study along with the reasons for discontinuation as follows:
 - AE
 - Death
 - Lost to Follow-up
 - Non-Compliance with Study Drug
 - Pregnancy
 - Withdrawal of Consent
 - Disease Progression
 - Other

The number of patients in the FAS, P1S, DES, P2S, RES, PKC, and PD analysis sets will be summarized by dose group based on the Consented Patient Set.

A patient disposition flowchart, with information similar to that contained in a CONSORT diagram, will be created for this study.

11.2 Demographics and Baseline Characteristics

Demographics – age, sex, child-bearing potential (female patients only [listed only]), race, and ethnicity - and baseline patient characteristics – height (cm), weight (kg), and Eastern Cooperative Oncology Group (ECOG) performance status - will be tabulated by dose group, using all patients in the FAS to descriptively assess the comparability of the groups.

The following baseline characteristics will be summarized as continuous or categorical variables, where appropriate:

- SCLC History
 - Stage of disease at initial diagnosis
 - Time from initial diagnosis to first dose day (in months)
 - Time since ED diagnosis (in months)
 - Treatment type for ED diagnosis
- Other Cancer History
 - Tumor type at initial diagnosis
 - Stage of disease at initial diagnosis
 - Time from initial diagnosis to first dose day (in months)
 - Time from final treatment



- Stage at final treatment
- Treatment response
- Tumor Tissue Sampling at Screening
 - Time from biopsy to first dose day (in months)
 - Tumor tissue type
 - Anatomical location
 - Localized disease stage
 - Extensive disease stage
- Patient prior cancer therapy (by dose group) for each type (i.e., adjuvant, maintenance, neoadjuvant, therapeutic): Total number of prior treatments per patient; the best response of the most recent prior treatment; prior cancer surgery; radiation treatment. Prior cancer treatments will be listed and are coded according to the World Health Organization (WHO) Drug Dictionary (WHODrug) February 2016.

Medical history will be coded using Medical Dictionary for Regulatory Activities (MedDRA®) Version 19.0. Medical history will be listed for all enrolled (consented) patients (system organ class, preferred term, and verbatim term) by dose group in the FAS.

11.3 Drug Exposure and Compliance

The number of cycles initiated, number of doses administered per patient, and the cumulative actual dose in mg/kg will be summarized by dose group for the FAS for both pegzilarginase and pembrolizumab separately.

11.4 Pharmacodynamic Analyses

Arginine (and ornithine) concentration data will be summarized with descriptive statistics (N, mean, SD, median, min, max, CV%) by nominal time point, dose and cycle/day. Individual and mean arginine and ornithine levels will be graphically displayed over time and by dose group.

A listing of arginine and ornithine levels will be provided.

If a particular assessment of a PD concentration is below the limit of quantitation, the result will be included in summaries by imputing half the value of the quantitation limit.

11.5 Pharmacokinetic Analyses

Pegzilarginase concentration data will be summarized with nominal times and descriptive statistics (N, mean, SD, median, min, max, CV%) by time point, dose and cycle/day. Individual and mean pegzilarginase levels will be graphically displayed over time and by



dose group.

Listings of pegzilarginase concentration data and all PK parameters will be provided.

11.6 Efficacy

11.6.1 Primary Efficacy Analysis

As per Section 10.1, no analysis of the primary efficacy endpoint will be provided.

11.6.2 Secondary Efficacy Analyses

As per Section 10.1, no analysis of the secondary efficacy endpoints will be provided.

11.6.3 Exploratory Efficacy Analyses

As per Section 10.1, no exploratory efficacy analyses will be conducted.

11.7 Safety

11.7.1 Adverse Events

All patients will be assessed regularly for the potential occurrence of AEs from the date of first visit until 30 days after the last dose of study treatment. AEs will be coded using the MedDRA terminology. The version of MedDRA that is used will be noted in the outputs. The incidence of AEs will be summarized and tabulated by System Organ Class (SOC) and Preferred Term (PT).

All patients will be assessed regularly for the potential occurrence of serious AEs from the date of first visit until 90 days after the last dose of study treatment, or 30 days after the last dose of study treatment if the subject initiates new anticancer therapy, whichever is earlier; any SAE, or follow up to a SAE, including death due to any cause other than progression of the cancer under study. The National Cancer Institute (NCI) CTCAE (version 4.03) will be used to grade both clinical and laboratory AEs. A patient with several occurrences of the same AE will be counted once and classified by the most severe occurrence.

Any AE that is classified as definitely related, probably related or possibly related to study drug will be considered related. A patient with several occurrences of the same AE will be counted once and classified by the event with the closest relationship to the study drug. An AE with a missing relationship to the medication will be classified as “Not Reported” for the purpose of analysis and summarization.

Treatment-emergent AEs (TEAEs) are defined as the AEs that start or worsen after the first dose of study drug.

An overview of the incidence of TEAEs will be provided including the numbers and percentages of patients with at least 1 DLT, with any TEAE, with any Grade ≥ 3 TEAE,



with any study drug related TEAE, with any Grade ≥ 3 related TEAE, with any TEAE requiring dose reduction, with any TEAE leading to discontinuation of study drug, with any related TEAE leading to discontinuation of study drug, any TEAE with a fatal outcome, with any SAE, and with any study drug related serious TEAE.

TEAEs will be summarized with patient incidence as follows:

- AEs by SOC and PT for patients experiencing a DLT;
- AEs by SOC and PT, and by PT in descending frequency of all doses combined;
- Grade ≥ 3 AEs by PT in descending frequency;
- Grade ≥ 3 AEs related to study drug by PT in descending frequency;
- AEs related to study drug by SOC and PT, and by PT in descending frequency of all doses combined;
- AEs by SOC, PT and maximum CTCAE Grade;
- AEs related to study drug by SOC, PT and maximum CTCAE Grade;
- AEs requiring reduction in dose of study drug by SOC and PT
- AEs requiring delaying or skipping study drug administration by SOC and PT
- AEs leading to discontinuation of study drug by SOC and PT;
- AEs related to study drug and leading to discontinuation of study drug by PT in descending frequency;
- SAEs by SOC and PT;
- SAEs related to study drug by SOC and PT;
- SAEs with a fatal outcome that occurred within 30 days of the last study drug administration by SOC and PT;
- AEs starting or worsening during the first cycle (start date or date of worsening between Day 1 and Day 21) by SOC and PT

Summaries will be provided by dose group for the FAS. Listings will be presented for all AEs with details including dates of onset, severity grade, drug-relationship, action taken, and outcome. Listings will be repeated for SAEs, AEs leading to discontinuation of study drug, AEs requiring reduction in dose, and AEs with a fatal outcome.

11.7.2 Vital Signs

Vital signs including weight (kg), temperature ($^{\circ}\text{C}$), pulse rate (beats/min), respiratory rate (breaths/min), systolic blood pressure (mmHg), and diastolic blood pressure (mmHg) will be presented in a listing.

The primary means of assessing vital sign changes is through assessing AEs related to



vital signs. Summaries of vital signs will also be created by cycle, showing the maximum deviation (increase and decrease) from the pre-infusion vital sign assessment for that cycle. After medical review of the listings and planned summaries, additional summaries and graphs may be created to further explore vital sign results.

11.7.3 Performance Status

ECOG performance status (0, 1, 2, 3, 4, and 5) will be listed.

11.7.4 Electrocardiograms

The primary means of assessing ECG results is through assessing AEs related to ECGs.

ECG results will be listed. The description of all ECG abnormalities will be listed separately. After medical review of the listings, additional summaries and graphs may be created to further explore the ECG results.

11.7.5 Laboratory Results

Laboratory studies will include hematology, chemistry, urinalysis, coagulation, and amino acid levels (Arg, ornithine). Please refer to section 11.6 of the protocol for the list of laboratory tests conducted.

For quantitative chemistry, hematology, coagulation, and urinalysis parameters, descriptive statistics will be used to summarize laboratory measurements based on the actual value and the change from baseline value at each time point of assessment. In addition, the maximum, minimum, and last observed value during the treatment period will be presented.

Shift tables from baseline to the worst post-baseline values during the treatment period will be provided for the chemistry, hematology, and coagulation parameters that have CTCAE v4.03 toxicity grades.

Laboratory data will be listed with values outside the normal range flagged. Thyroid function test results will be listed.

11.7.6 Immunogenicity

Immunogenicity results including ADA and anti-PEG ADA will be listed.

11.7.7 Concomitant Medications and Procedures

Prior and concomitant medications will be coded using the WHODrug version dated February 2016. Prior medications include medications that were taken prior to the first dose of any study treatment. Concomitant medications during the treatment period include medications that started at any time and were taken at any time after the start of



study treatment until 30 days after the end of study treatment.

The number and percentage of patients taking concomitant medications during the treatment period will be summarized by anatomical therapeutic chemical (ATC) and PT for the FAS. Although a patient may have taken the same medication multiple times, the patient is counted only once within an ATC classification. The same patient may contribute to two or more PTs in the same classification.

Listings of prior medications (used within 30 days prior to the start of the study) and concomitant medications (used during the course of the study) will be provided.

A listing of prior procedures (done within 30 days prior to the start of the study) and concomitant procedures (done during the course of the study) will be provided.

11.7.8 Additional Exploratory Analyses

No additional exploratory analyses will be performed (see section 10.1).



12. APPENDICES

12.1 Schedule of Assessments for Dose Escalation (Protocol Table 1)

Assessment	Screening/Baseline	Treatment Period												Follow-up
		Cycle 1			Cycle 2			Cycle 3			Cycle 4 to 35			
Day of Cycle ^a	Day -21 to -3	1	8	15	1	8	15	1	8	15	1	8	15	Last Dose + 30 to 44 days
Informed consent	X													
Verify inclusion/ exclusion criteria	X													
Medical history	X													
Tumor tissue sample ^b	X									X				
Blood sampling for germline DNA ^p	X													
Contrast-enhanced CT or MRI ^c	X									X			X	
Height	X													
Weight	X	X ^d			X ^d			X ^d			X ^d			X
Targeted physical exam	X	X ^d	X ^d		X ^d			X ^d			X ^d			X
ECOG performance score	X	X ^d			X ^d			X ^d			X ^d			X
Pregnancy test ^e	X	X ^d			X ^d			X ^d			X ^d			X
Coagulation	X ^o	X ^d			X ^d									
Vital signs	X	X ^f	X ^f	X ^f	X ^f	X ^{f,g}	X ^{f,g}	X ^g	X ^g	X ^g	X ^g	X ^g	X ^g	X
PK/PD sampling, Phase 1		X ^h	X ⁱ	X ⁱ	X ^h	X ⁱ	X ⁱ	X ^j	X ^j	X ^j	X ^j			X
PK/PD sampling, Phase 2		X ^j	X ^j	X ^j	X ^j	X ^j	X ^j	X ^j	X ^j	X ^j	X ^j			X
CBC, Chemistry	X ^o	X ^d	X ^d	X ^d	X ^d	X ^d	X ^d	X ^d			X ^d			X
Urinalysis	X	X ^d			X ^d			X ^d			X ^d			X
Thyroid function Testing ^k	X ^o	X						X			X			
ECG	X	X ^l			X ^l			X ^l						
Anti-pegzilarginase and anti-PEG antibodies		X ^d			X ^d			X ^d			X ^d			X
Adverse events ^m		X	X	X	X	X	X	X	X	X	X	X	X	X
Blood sampling for immunoprofiling		X ^d	X ^d			X ^d								
Blood sampling for cytokine analysis		X ^h												
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pembrolizumab administration ⁿ		X			X			X			X			
Pegzilarginase administration ^a		X	X	X	X	X	X	X	X	X	X	X	X	

ECG = electrocardiogram; PK = pharmacokinetic; PD=pharmacodynamic.

- a. Visit windows will be ± 1 day.
- b. Collection of tumor tissue sample before first dose is mandatory for all patients; a second biopsy obtained between 4 days after cycle 3 day 15 up to a day before cycle 4 day 1 is optional. Tumor tissue samples will need to be freshly obtained via core needle or incisional biopsy and processed following instructions described in the Laboratory Manual; fine needle aspirations are not accepted. For patients without accessible tumor for biopsy, an archived, formalin-fixed and paraffin embedded (FFPE) tumor tissue specimen may be acceptable if it was obtained after the last cancer-directed treatment, and cancer cells are confirmed to be present in the specimen. If an archived FFPE block is provided this needs to be not older than 3 years. If pre-cut unstained slides are provided these need to be from a FFPE block not older than 3 years and have been cut less than 6 months ago. For more details please refer to the Laboratory Manual.
- c. Contrast-enhanced CT or MRI will be obtained during the screening period, and at 9, 18 and 27 weeks (up to -3 days) from cycle 1 day 1. Subsequent tumor imaging should be performed every 12 weeks (up to -3 days). At screening/baseline, imaging of the head, chest, abdomen and pelvis are mandatory. On follow-up assessments, imaging of chest, abdomen and pelvis are mandatory. On follow-up assessments, imaging of the head and other clinically relevant areas will be obtained as deemed clinically appropriate by the treating physician for patients with brain metastases or suspicious symptoms. Imaging obtained during the screening period will be utilized to confirm that patient complies with inclusion/exclusion criteria. MRI can be used in lieu of contrast-enhanced CT for patients who are allergic to contrast. The same methodology should be used throughout the study to follow each lesion. When possible, imaging will be obtained as early as possible for patients that ended their participation for reasons other than documented disease progression.
- d. To be performed on the same day of but prior to the study drug administration.
- e. For female patients of childbearing potential, the screening serum pregnancy test must be negative within 7 days of study start. Urine or serum pregnancy tests will be obtained at all other time points including day 1 of cycles 1 to 3 and every other cycle thereafter (cycle 5, 7, 9, etc).
- f. Vital signs will be obtained 15 min (± 5 min) prior to the start of the first infused agent, and at 15 min (± 5 min), 30 min (± 5 min), 60 min (± 10 min), 90 min (± 10 min), then at 2 hour (± 10 min), 3 hours (± 10 min), and 4 hours (± 10 min) after the start of the first infused agent. Vitals signs at 5 hours (± 10 min) after the start of the first infused agent will be also obtained on day 1 of cycle 1 and 2. From Cycle 1 Day 1 through Cycle 2 Day 1, if there is no evidence of a clinically significant adverse event considered at least possibly/probably related to study drugs that occurred during a day of infusion, then vital signs may be collected as per footnote g.
- g. Vital signs will be obtained 15 min (± 5 min) prior to the start of the first infused agent and at 15 min (± 5 min), 30 min (± 5 min), 60 min (± 10 min), 90 min (± 10 min) and at 2 hours (± 10 min) after the start of the first infused agent.
- h. Samples will be obtained 15 min (± 5 min) prior to and at 1 hour (± 10 min), 4 hour (± 15 min), and 72 hours (± 24 hours) after the start of pegzilarginase infusion.
- i. Samples will be obtained 15 min (± 5 min) prior to and at 1 hour (± 10 min) and 4 hour (± 15 min) after the start of pegzilarginase infusion.
- j. Samples will be obtained 15 min (± 5 min) prior to the start of pegzilarginase infusion during days 1, 8, and 15 of cycle 1-3 and on day 1 of each cycle thereafter through cycle 6.
- k. Thyroid function testing will include triiodothyronine (T3) or free triiodothyronine (FT3), free thyroxine (FT4), and thyroid stimulating hormone (TSH). Samples will be obtained at screening, at on the same day but before the study drug administration on day 1 of cycle 1 and every two cycles thereafter (cycles 3, 5, 7, etc).
- l. A 12-lead ECG will be obtained predose and at 60 min (± 10 min) after the start of pegzilarginase administration on days 1 of cycles 1, 2 and 3.
- m. Determination of ammonia levels: measure ammonia levels per standard local institutional practice in case of occurrence of a Grade 3 or higher adverse event considered at least possibly/probably related to pegzilarginase. In case of hyperammonemia, proceed to treat per standard local institutional practice.
- n. May be administered up to 3 days before or after the scheduled day 1 of each cycle due to administrative reasons.
- o. With specimens collected within 10 days prior to day 1 of cycle 1.
- p. The germline DNA can be collected anytime between screening and prior to dosing on day 1 cycle 1.



13. REFERENCES

US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER). *Guidance for Industry: E9 Statistical Principles for Clinical Trials*. September 1998.

14. TABLES, LISTINGS AND FIGURES

The planned tables, listings, and figures to accompany this SAP will be contained in a separate document.