Title 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)

Protocol Number:	CAEB1102-101B
Amendment Number:	Amendment 1
Investigational Products:	Pegzilarginase/AEB1102 Pembrolizumab
Study Phase:	Phase 1/2
Sponsor Name:	Aeglea Biotherapeutics, Inc.
Legal Registered Address:	Aeglea BioTherapeutics, Inc 901 S. MoPac Expressway, Barton Oaks Plaza One, Suite 250 Austin, TX 78746
Regulatory Agency Identifier Number(s):	IND: 122558
Protocol History:	Original Protocol: 20-SEP-2017 Amendment 1: 08-NOV-2018

Sponsor Approval: [redacted]

1. STUDY SYNOPSIS

Sponsor:	Aeglea Biotherapeutics, Inc.
Name of Finished Product:	Pegzilarginase (AEB1102, Co-ArgI-PEG) solution for intravenous (IV) infusion
Name of Active Ingredient:	Co-ArgI-PEG
Title of Study:	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 No.:	CAEB1102-101B
Phase of Development:	1/2
Indication:	Patients with ED-SCLC that has progressed on or relapsed within 6 months following platinum-based therapy
Number of Subjects Planned:	84 (up to 18 in phase 1 and up to 66 in phase 2)
Study Duration	Overall study duration is expected to be approximately 24 months

Study Objectives:	**Primary Objective:**
	• Phase 1: 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.
	• Phase 2: 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.
	
	Secondary Objectives:
	• To determine the ORR by iRECIST 1.1 of pegzilarginase in combination with pembrolizumab.
	• To determine the following additional efficacy endpoints by both RECIST 1.1 and iRECIST:
	  ○ Clinical benefit rate (CR + PR + SD lasting at least 8 weeks)
	  ○ Time to response
	  ○ Duration of response
	  ○ Progression-free survival
	• To describe overall survival
	• To evaluate the safety profile of the combination of pegzilarginase with pembrolizumab in the study patient population (Phase 2)
	
	Exploratory Objectives:

	• To evaluate the effect of the combination of pegzilarginase with pembrolizumab on blood levels of circulating pegzilarginase, arginine and additional amino acid profiling
	• 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

Study Design:	This 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 trial. Pembrolizumab will be administered every 3 weeks at a fixed dose of 200 mg intravenously on day 1 of each cycle. Pegzilarginase will be administered weekly (i.e., on days 1, 8 and 15 of each cycle) by IV infusion at the study-defined dose. Phase 1: The dose of pegzilarginase will be escalated in two steps from 0.20 mg/kg and then at the single agent MTD for solid tumors, which will be either 0.27 mg/kg or 0.33 mg/kg, as determined in the CAEB1102-100B study. One cycle will be 3 weeks. The dose-limiting toxicity (DLT) window will be 21 days (1 cycle). Any event occurring after the initial 21 days will not be considered a DLT; however, all adverse events will be considered in determining the safety of the combination and selection of the dose of pegzilarginase for Phase 2. Alternatively, the dose level 0.12 mg/kg may be explored in case the combination of pegzilarginase at 0.20 mg/kg with full dose pembrolizumab is declared to be an unacceptably toxic dose. The dose of pegzilarginase will be escalated and/or de-escalated using a mTPI design. If the MTD of pegzilarginase when given in combination with pembrolizumab is less than the MTD of single-agent pegzilarginase in solid tumors, then an additional dose level of pegzilarginase between the combination MTD and single-agent MTD may be explored. At least 6 patients must be enrolled at the recommended phase 2 dose (RP2D) prior to enrollment into the Phase 2 portion of the trial. 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. If a patient misses a pegzilarginase dose during the first cycle due to treatment-related toxicity, this will be considered a DLT. A safety committee comprised of the investigators and the Sponsor's medical monitor will review the safety information for each dose cohort and determine whether dose escalation will occur and the next higher dose level to be studied. Ultimately, the recommended phase 2 dose of pegzilarginase in combination with pembrolizumab 200 mg q 3 weeks will be determined by assessment of the safety of the combination over the DLT period and available safety information in

	subsequent cycles. Following the identification of the RP2D of pegzilarginase in combination with pembrolizumab, the study will continue to the phase 2 portion. Phase 2: In the phase 2 portion of the trial, patients who meet entry criteria will be treated with the RP2D of the combination of pegzilarginase with pembrolizumab in a minimax Simon 2-stage design (Simon, 1989). Fifty-five patients will be enrolled and treated in stage 1. If 23 or more of those subjects achieve a response (CR or PR), an additional 11 subjects will be enrolled. If a total of 30 or more subjects 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%. All patients in both parts of the trial will be monitored for safety by periodic physical exams, vital signs, ECGs, hematology and chemistry laboratory testing, thyroid function testing, urinalysis, and coagulation laboratory testing. Adverse events (AEs) will be recorded at each study visit. The severity of AEs will be graded per the National Cancer Institute Common Terminology Criteria for Adverse Events version 4 (NCI CTCAE v. 4.0). Dose limiting toxicity will be defined as the occurrence of any of the following treatment-emergent events in the first 21 days (one cycle) of treatment if deemed at least possibly related to either of pegzilarginase, pembrolizumab, or their combination: 1) Grade 5 toxicity 2) Non-hematologic AEs a. Gastrointestinal: i. Grade 4 diarrhea or Grade 4 colitis ii. Grade 2 to 3 diarrhea or colitis that does not resolve to at least Grade 1 before the next cycle b. Endocrinology: i. New onset type 1 diabetes mellitus (T1DM) that is not clinically and metabolically stable before next cycle ii. Grade 3 or 4 hyperglycemia that is not clinically and metabolically stable before next cycle iii. Grade 2 to 4 hypophysitis that does not resolve to at least Grade 1 before the next cycle iv. Grade 4 hyperthyroidism v. Grade 3 hyperthyroidism that does not resolve to at least Grade 1 before the next cycle c. Respiratory: i. Grade 4, Grade 3, or recurrent Grade 2 pneumonitis ii. First episode of Grade 2 pneumonitis that does not resolve to at least Grade 1 before the next cycle d. Renal: i. Grade 3 to 4 renal failure or nephritis

ii. Grade 2 nephritis that does not resolve to at least Grade 1 before the next cycle

e. Cardiovascular:

i. Single reading of blood pressure levels ≥ 200 mmHg systolic or ≥ 120 mmHg diastolic

ii. Grade 3 hypertension that does not resolve, with or without intervention, to ≤ Grade 1 in less than 24 hours

f. Infusion reaction:

i. Grade 3 to 4 infusion reaction

ii. Grade 2 infusion reaction that does not resolve to at least Grade 1 before the next cycle

g. Any other Grade 4 non-hematologic, non-laboratory toxicity not described above

h. Any other Grade 3 non-hematologic, non-laboratory toxicity not specifically mentioned in other criteria lasting >3 days despite optimal supportive care

3) Hematologic AEs:

a. Grade 3 or 4 febrile neutropenia regardless of duration

b. Grade 4 thrombocytopenia regardless of duration if associated with:

i. A bleeding event which requires an elective platelet transfusion, or

ii. A life-threatening bleeding event which results in urgent intervention and admission to an Intensive Care Unit

c. Any other treatment-related Grade 4 hematologic toxicities not described above

d. Any other treatment-related Grade 3 hematologic toxicities that do not resolve to at least Grade 1 before the next cycle or requires the use of transfusion of blood products (including platelets or red blood cells) or administration of colony stimulating factors (including G-CSF, GM-CSF or recombinant erythropoietin)

4) Laboratory, other:

a. Grade 3 or higher AST, ALT or bilirubin, except for:

i. Patients with liver metastasis or underlying liver disease with a baseline Grade 2 AST or ALT, AST or ALT will need to increase by greater than or equal to 50% relative to baseline and last for at least 7 days

b. Grade ≥ 2 increase in transaminases (either AST or ALT) associated with either Grade ≥ 2 increase in total bilirubin or Grade ≥ 2 increase in INR, except for:

i. Patients with liver metastasis or underlying liver disease with a baseline Grade 2 AST or ALT, AST or ALT will need to increase by greater than or equal to 50% relative to baseline and last for at least 7 days

c. Any Grade 3 or Grade 4 non-hematologic laboratory value if:

	i. Medical intervention is required to treat the subject, or ii. The abnormality leads to hospitalization, or iii. The abnormality is asymptomatic and persists at Grade 3 or higher for >7 days iv. Exceptions: Grade 3 isolated electrolyte abnormalities (i.e., those occurring without clinical consequence) that resolve, with or without intervention, to Grade ≤ 1 in < 72 hours 5) Other: a. Prolonged delay (>2 weeks) in initiating cycle 2 due to treatment-related toxicity b. Missing two of the three planned pegzilarginase doses during the first cycle because of AE(s) due to treatment-related toxicity
Study Population:	**Number of Subjects:** Approximately 9 to 12 patients will participate in the phase 1 portion of this trial. In phase 2, up to 55 patients will participate in the initial stage 1. In stage 2, an additional 11 patients will be enrolled. **Inclusion Criteria:** A patient must meet all following criteria to be enrolled in the study: 1) Patient is able and willing to provide written informed consent and to comply with all requirements of study participation (including all study procedures) prior to any screening procedures 2) Be ≥ 18 years of age on day of signing informed consent 3) Have histologically or cytologically confirmed small cell lung cancer that meet the following corresponding requirements: a. Extensive disease per criteria of the International Association for the Study of Lung Cancer (IASLC)-American Joint Committee on Cancer (AJCC) TNM staging system b. Have not tolerated or have progressed or relapsed on or within 6 months of platinum-based chemotherapy 4) Have a performance status of ≤ 1 on the ECOG Performance Scale 5) Have measurable disease based on RECIST 1.1 as determined by the site study team. Tumor lesions in a previously irradiated area are considered measurable if progression has been demonstrated in such lesions 6) Willing to undergo core needle or incisional biopsy to obtain fresh tumor tissue specimens. For patients without accessible tumor for biopsy, an archived, formalin-fixed paraffin embedded (FFPE) tumor tissue specimen may be acceptable if it was obtained after the last cancer-directed treatment, tumor is confirmed to be present in the specimen. If an archived FFPE block is provided this needs to be not older than 3 years. In addition, if pre-cut unstained slides are provided these need to be not older than 6 months 7) Demonstrate adequate organ function, with specimens collected within 10 days prior to day 1 of cycle 1, as defined as: a. Absolute neutrophil count (ANC) ≥1,500 /mcL

b. Platelets ≥100,000 / mcL (without need of transfusion for at least 14 days prior to scheduled C1D1)

c. Hemoglobin ≥9 g/dL or ≥5.6 mmol/L (without need of erythropoietin or transfusion for at least 14 days prior to scheduled C1D1)

d. Renal: serum creatinine ≤1.5 X upper limit of normal (ULN)

   i. Subjects with creatinine levels > 1.5x ULN may qualify if Glomerular Filtration Rate (GFR) OR calculated creatinine clearance per institutional standard (CrCL) are ≥ 60 mL/min/1.73m2

e. Hepatic: serum total bilirubin ≤ 1.5 X ULN

   i. Subjects with total bilirubin > 1.5 ULN if direct bilirubin ≤ ULN

f. AST (SGOT) and ALT (SGPT) ≤ 2.5 X ULN or ≤ 5 X ULN for patients with liver metastases

g. Albumin ≥ 2.5 mg/dL

h. International normalized ratio (INR) or prothrombin time (PT) ≤ 1.5 X ULN

   i. Exception: subjects receiving anticoagulant therapy if PT or INR is within therapeutic range of intended use.

i. Activated partial thromboplastin time (aPTT) ≤ 1.5 X ULN

   i. Exception: subjects receiving anticoagulant therapy if PTT is within therapeutic range of intended use

8) Subjects must have recovered from the effects (except alopecia or neuropathy) of any prior systemic therapy, radiotherapy or surgery to Grade 1 or less

9) If subject received major surgery or radiation therapy of > 30 Gy, recovered from the toxicity and/or complications from the intervention is required

10) If female and of child-bearing potential, has a negative urine or serum pregnancy test within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required

11) If a sexually active male or female, must be surgically sterile post-menopausal (defined as female patient with 12 months of spontaneous amenorrhea, or 6 months of spontaneous amenorrhea with serum FSH levels higher than 40 mIU/ml, or 6 weeks after bilateral surgical oophorectomy), or must agree to use a physician-approved method of birth control during the study through a minimum of 120 days after the last study drug administration.

Exclusion Criteria:

A patient is excluded from the study if the patient meets any of the following criteria:

1) Has received more than 2 platinum-based regimens against SCLC

	2) Has received pembrolizumab, or prior therapy with an anti-PD-1, anti-PD-L1, anti-PD-L2, or anti-Cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) antibody as part of any previous therapy, including trials 3) Has participated in Merck MK-3475 (pembrolizumab) clinical trials 4) Has received pegzilarginase as part of any previous therapy 5) Is currently participating in a study of an investigational agent or received the last dose of an investigational agent.within 4 weeks prior to the first dose of treatment in this study (a shorter interval for kinase inhibitors or other short half-life drugs could be considered after approval from the Sponsor). Is currently participating in a study of an investigational device within 4 weeks of the first dose of treatment. 6) Has a diagnosis of an immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment. The use of topical, short courses (3 days or less), or physiologic doses of corticosteroids (doses not higher than 10 mg daily of prednisone equivalent) may be allowed after consultation with the Sponsor. 7) Has had a prior monoclonal antibody (mAb) within 4 weeks prior to first dose or who has not recovered (i.e., ≤ Grade 1) from adverse reactions due to agents administered more than 4 weeks earlier 8) Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to first dose or who has not recovered (i.e., ≤ Grade 1 or at baseline) from treatment-emergent adverse reactions due to a previously administered agent. Note: Patients with ≤ Grade 2 neuropathy or ≤ Grade 2 alopecia are an exception to this criterion and may qualify for the study after consultation with the Sponsor. If the patient has received prior radiation therapy, participant must has recovered from all radiation-related toxicities, not currently taking systemic corticosteroids due to radiation-related toxicities, and not have had radiation pneumonitis. A 1-week washout is permitted for palliative radiation (≤2 weeks of radiotherapy) to non-CNS disease. 9) If the patient has undergone major surgery, he/she must have recovered adequately from the toxicity and/or complications prior to starting therapy 10) Has received transfusion of blood products (including platelets or red blood cells) or administration of colony stimulating factors (including G-CSF, GM-CSF or recombinant erythropoietin) within 14 days prior to study first dose 11) Has a known additional malignancy that is progressing or has required active treatment within the past 3 years. Exceptions include basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or carcinoma in situ (e.g. breast carcinoma, cervical cancer) that has undergone potentially curative therapy 12) Has known central nervous system (CNS) metastases. However, patients with previously treated brain metastases may participate provided neurologic symptoms have stabilized, there is no evidence of new brain metastases or hemorrhage, and they are not using steroids for brain metastases or for complications derived from their treatment for at least 7 days prior to the first dose of trial treatment 13) Has known carcinomatous meningitis

	14) Has an active autoimmune disease requiring systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs) or a documented history of clinically severe autoimmune disease, or a syndrome that requires systemic steroids or immunosuppressive agents. Patients with vitiligo or resolved childhood asthma/atopy are an exception to this rule. Patients that require intermittent use of bronchodilators or local steroid injections will not be excluded from the study. Patients with hypothyroidism stable on hormone replacement or Sjogren's syndrome will not be excluded from the study. Replacement therapy (e.g. thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc) is not considered a form of systemic treatment. 15) Has evidence of interstitial lung disease, history of non-infectious pneumonitis that required steroids, or current pneumonitis 16) Has an active infection requiring systemic therapy 17) Inadequately controlled hypertension (defined as systolic blood pressure ≥ 200 mmHg and/or diastolic blood pressure ≥ 120 mmHg) on more than one occasion in the month before planned day of infusion. Patients with more than one episode of inadequately controlled hypertension will be allowed to participate under initiation or modification of antihypertensive treatment provided adequate control (defined as systolic blood pressure < 160 mmHg and/or diastolic blood pressure <100 mmHg) is documented on 3 separate days over in the week before starting treatment 18) Currently taking 3 or more anti-hypertensive medications, including diuretics (if combination tablets are part of the treatment, each active ingredient part of them counts as one) 19) Prior history of hypertensive crisis or hypertensive encephalopathy 20) History of myocardial infarction, unstable angina, cardiac or other vascular stenting, angioplasty, or cardiac or vascular surgery within 6 months prior to day 1 of study treatment 21) Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the patient's participation for the full duration of the trial, or is not in the best interest of the patient to participate, in the opinion of the treating investigator 22) Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial 23) Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment 24) Has a known history of Human Immunodeficiency Virus (HIV) (positive for HIV p24 antigen or HIV 1/2 antibodies) 25) Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected) 26) Has received a live-virus vaccination within 30 days prior to the planned first dose of study therapy. Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are

	not allowed. Other seasonal flu vaccines that do not contain live virus are permitted 27) Has a known history of active tuberculosis (Bacillus tuberculosis) 28) Has a history of hypersensitivity to PEG or any component of the pegzilarginase formulation 29) Severe hypersensitivity (≥ Grade 3) to any excipient of pegzilarginase or pembrolizumab 30) Has had an allogenic tissue/solid organ transplant
Protocol Therapy:	**Investigational Product Dose, Route and Regimen:** Pegzilarginase will be administered IV once weekly. Pembrolizumab will be administered IV every 3 weeks. Trial treatment of pegzilarginase may be administered up to 1 day before or after the scheduled day due to administrative reasons. Trial treatment of pembrolizumab may be administered up to 3 days before or after the scheduled Day 1 of each cycle due to administrative reasons. The total dose of pegzilarginase needs not be recalculated at the beginning of each treatment cycle unless there has been a change in the subject's body weight of ≥ 10% from the weight used to calculate the previous total dose. Pembrolizumab will be administered at a fixed dose of 200 mg following standard institutional practice. **Duration of treatment:** Patients may continue treatment for a maximum of 24 months, or until they have confirmed progressive disease per iRECIST criteria, whichever comes first. Even if patients discontinue treatment, follow-up may continue for the patient until progressive disease is confirmed by iRECIST criteria, or 8 weeks have elapsed since all patients have had their last dose of study drug, whichever comes first. All patients are required to complete a follow-up safety visit 30-44 days after their last dose of study drug (pembrolizumab or pegzilarginase), or prior to the initiation of alternative therapy for SCLC, whichever is earlier. **The following medications are prohibited during the study:** • Chronic administration of systemic corticosteroids (except as described in Section 8.8) • Immunosuppressive agents not specified in the protocol • Investigational therapies • Live vaccines • Hematopoietic growth factors except as described in the protocol • Chemotherapy or any other therapy targeted to the patient's underlying SCLC. Focused therapy (e.g. radiation therapy) with the goal of alleviating symptoms may be permitted after consultation with the Sponsor
Outcome Measures:	**Safety:** Safety outcome measures will include the reporting of AEs and serious AEs; findings on physical exams and ECGs; values for vital signs and routine safety labs (hematology, serum chemistries, coagulation parameters, urinalyses, thyroid function testing) **Efficacy:**

	Primary objective of the phase 2 trial will be to assess the objective response rate (ORR:CR+PR) per RECIST 1.1 Additional efficacy endpoints will be evaluated, including: • ORR (iRECIST) • The following endpoints as assessed by both RECIST 1.1 and iRECIST: - Clinical benefit rate (CR + PR + SD lasting at least 8 weeks) - Time to response - Duration of response - Progression free survival • Overall survival **PK/PD/ADA:** • Plasma arginine, additional amino acid profiling, and pegzilarginase levels will be determined at selected timepoints. • Development of antibodies against pegzilarginase and its pegylation will be evaluated and correlated against plasma arginine levels. **Biomarkers:** • Exploratory biomarkers: - Expression of PD-L1 and ASS1 in tumors - Immune composition and mutational burden of tumors - Immuno profiling of circulating immune cells - Cytokine profiling - Other biomarkers potentially related to arginine depletion and/or PD-L1 blockade
Statistical Methods:	**Sample size:** 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 18 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. Details are provided in the efficacy analysis section below. **Safety Analysis**: AEs, laboratory data and vital signs will be listed and summarized for the overall safety set and by phase. The number of subjects reporting AEs and SAEs will be summarized by body system, dose group, and preferred term. Demographics and disposition will be summarized for the overall safety set and by phase. **Efficacy Analysis:** In the phase 2 portion of the trial, patients who meet entry criteria will be treated with the RP2D of pegzilarginase in combination with pembrolizumab in a minimax Simon 2-stage design (Simon, 1989). Up to 55 patients will be treated in stage 1. If 23 or more of those subjects achieve a response (CR or PR), an additional 11 subjects will be enrolled. If a total of 30 or more subjects 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%. For summaries of the data, all subjects (even those who were over-enrolled) will be included, and primary summaries will be computed without adjustment for the fact that the data were derived from a 2-stage design. The impact of such adjustments may be explored as a supplementary analysis. Details of types of response (CR, PR, etc), will be summarized. Logistic regression analyses will be performed to understand the impact of different variables on response. Full details will be provided in the SAP.

Table 1. Schedule of Events

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			

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**
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 subjects 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.

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3. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
AML	acute myelogenous leukemia
ADA	anti-drug antibodies
ADI	arginine deiminase
AE	adverse event
ANC	absolute neutrophil count
ALT	alanine aminotransferase
aPTT	activated partial thromboplastin time
ARG1	Arginase 1
arginine	Refers to L-arginine, unless otherwise noted
ASL	argininosuccinate lyase
ASS1	argininosuccinate synthase 1
AST	aspartate aminotransferase
AUC	area under the curve
BCG	Bacille Calmette-Guerin
BUN	blood urea nitrogen
C	Celsius
Ca^{2+}	calcium
CBC	Complete Blood Count
CD-1	Cluster of Differentiation-1
CFR	Code of Federal Regulations
CI	Confidence Interval
CL	serum clearance of drug per unit time following IV dosing
Cl^{-}	chloride
Cmax	Maximum observed concentration
CNS	Central Nervous System
Co, Co^{2+}	cobalt
Co-ArgI-PEG	AEB1102 drug substance
CR	complete response
CRA	Clinical Research Associate
CrCL	creatinine clearance
CRF	Case Report Form
CRO	Contract Research Organization
CT	Computed tomography scan
CTLA-4	Cytotoxic T-lymphocyte-associated antigen-4
DRF	dose-range-finding
dL	deciliter(s)
DLT	dose-limiting toxicity
DMP	Data Management Plan
DNA	Deoxyribonucleic acid
EC	Ethics committee
ECG	Electrocardiogram

Abbreviation	Definition
ECI	Events of Clinical Interest
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic case report form
ED	Extensive Disease
ED-SCLC	Extensive disease Small Cell Lung Cancer
EDC	Electronic data capture
EPA	Environmental Protection Agency
ER	Emergency Room
FDA	Food and Drug Administration
FDG	fluorodeoxyglucose
FFPE	Formalin-fixed and paraffin embedded
FSH	Follicle Stimulating Hormone
FT3	Free triiodothyronine
FT4	Free thyroxine
GI	Gastrointestinal
GCP	Good Clinical Practice
G-CSF	Granulocyte-colony stimulating factor
GFR	Glomerular Filtration Rate
GI	gastrointestinal
GLP	Good Laboratory Practice
GM-CSF	Granulocyte Macrophage Colony Stimulating Factor
Grade	When written as “Grade 1”, “Grade 2”, etc, the phrase refers to Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0
Gy	Gray
H	Hydrogen
HCO_3-	bicarbonate
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
hr	hour(s)
IB	Investigator’s Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonization
iCR	Complete Response per iRECIST
IM	intramuscular
INR	international normalized ratio
IP	investigational product: “A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use.” (from E6 ICH). The terms “IP” and “study drug” may be used interchangeably in the protocol.
iPR	Partial Response per iRECIST
IRB	Institutional Review Board
iRECIST	RECIST for immunotherapeutics

Abbreviation	Definition
iSD	Stable Disease per iRECIST
ITT	Intent-to-treat
IV	Intravenous
K^+	potassium
k_{cat}	overall catalytic rate of an enzyme in Michaelis–Menten kinetics
kg	kilogram(s)
K_m	substrate concentration at 50% maximum reaction rate in Michaelis–Menten kinetics
L	liter(s)
m	meter(s)
mAB	monoclonal antibody
mcL	microliter(s)
MDS	Myelodysplastic syndromes
MedDRA	Medical Dictionary for Regulatory Activities
Mg, Mg2+	magnesium
mg	milligram(s)
µg	microgram(s)
min	minute(s)
mIU	milli international unit(s)
mL, ml	milliliter(s)
mm	millimeter(s)
mmHg	millimeter(s) of mercury
mM, mmol	millimole(s), millimolar
µM	micromole(s), micromolar
Mn, Mn^{2+}	manganese
MRI	Magnetic Resonance Imaging
mRNA	messenger Ribonucleic Acid
MTD	maximum tolerated dose
mTPI	modified toxicity probability interval
n	number (of patients contributing to a particular result)
Na^+	sodium
N/A	Not applicable
NCA	Noncompartmental analysis
NCI CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NOAEL	No-observable-adverse-effect level
NSAID	Nonsteroidal anti-inflammatory drug
NSCLC	Non-small cell lung cancer
ORR	overall response rate
OS	Overall Survival
OTC	ornithine transcarbamylase or over-the-counter, per context
PD	Pharmacodynamics or Progressive disease, per context
PD1	Programmed cell death 1
PD-L1	Programmed cell death ligand 1

Abbreviation	Definition
PD-L2	Programmed cell death ligand 2
PEG	polyethylene glycol
PET	positron emission tomography
PFS	Progression-free survival
PI	Principal Investigator
PK	Pharmacokinetics
plasma arginine	Used to indicate the biomarker assessed from clinical samples from study patients
PO_4^{3-}	Phosphate
PR	Partial response
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
q	every
Q2W	Every 2 weeks
Q3W	Every 3 weeks
RBC	red blood cell
RP2D	recommended phase two dose
RDW	red blood cell distribution width
RECIST	Response Evaluation Criteria in Solid Tumors
RT-PCR	Reverse transcription polymerase chain reaction
s	second(s)
SAE	serious adverse event
SAP	statistical analysis plan
SCLC	small cell lung cancer
SD	standard deviation or stable disease
$T_{1/2}$	half life
T1DM	Type 1 Diabetes Mellitus
T3	Triiodothyronine
TK	Toxicokinetics
Tmax	Time of the maximum observed concentration
TNM	Tumor-node-metastasis staging system for cancer
TSH	Thyroid stimulating hormone
UA	urinalysis
ULN	upper limit of normal
US	United States
Vss	Volume of distribution following IV dosing
WBC	white blood cell
WHO	World Health Organization

4. ETHICS

4.1 Institutional Review Board or Ethics Committee

Prior to initiating the study, the Investigator will obtain written confirmation that the Institutional Review Board (IRB) or Ethics Committee (EC) is properly constituted and compliant with ICH Guidelines and GCP requirements, applicable laws and local regulations. A copy of the confirmation from the IRB/EC will be provided to the Sponsor or designee. The Principal Investigator (PI) will provide the IRB/EC with all appropriate material, including the protocol, Investigator's Brochure (IB), the Informed Consent Form (ICF) including compensation procedures, and any other written information provided to the subjects, including all consent forms translated to a language other than the native language of the clinical site. The study will not be initiated and Investigational Product (IP) supplies will not be shipped to the site until appropriate documents from the IRB/EC confirming unconditional approval of the protocol, the ICF, and all subject recruitment materials are obtained in writing by the PI and copies are received at the Sponsor or designee. The approval document should refer to the study by protocol title and Sponsor Study Number (if possible), identify the documents reviewed, and include the date of the review and approval. The Sponsor or designee will ensure that the appropriate reports on the progress of the study were made to the IRB/EC and the Sponsor by the PI in accordance with applicable governmental regulations.

4.2 Ethical Conduct of Study

This study will be conducted in accordance with the following:

1. US Code of Federal Regulations (CFR) sections that address clinical research studies, and/or other national and local regulations, as applicable
2. E6 International Conference on Harmonisation (ICH) Guideline for Good Clinical Practice

Specifically, this study is based on adequately performed laboratory and animal experimentation; the study will be conducted under a protocol reviewed and approved by an IRB/EC; the study will be conducted by scientifically and medically qualified persons; the benefits of the study are in proportion to the risks; the rights and welfare of the subjects will be respected; the physicians conducting the study do not find the hazards to outweigh the

potential benefits; and each subject, or his/her legally authorized representative, will provide written, informed consent before any study–related tests or evaluations are performed.

4.3 Subject Information and Informed Consent

A properly written and executed ICF, in compliance with E6 ICH (GCP Guideline, Section 4.8) and 21 CFR §50 and other applicable local regulations, will be obtained for each subject prior to entering the subject into the study. The Investigator will prepare the ICF and provide the documents to the Sponsor for approval prior to submission to the IRB/EC. The Sponsor and the IRB/EC must approve the documents before they are implemented. A copy of the approved ICF, and if applicable, a copy of the approved subject information sheet, and all ICFs translated to a language other than the native language of the clinical site must also be received by the Sponsor prior to the delivery of IP.

The Investigator will provide copies of the signed ICF to each subject and will maintain the original in the subject's file.

5. INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

Prior to beginning the study, the PI at each site must provide to the Sponsor or designee a fully executed and signed Form FDA 1572 and a Financial Disclosure Form. All sub–Investigators must be listed on Form FDA 1572. Financial Disclosure Forms must also be completed for all sub–Investigators listed on the Form FDA 1572 who will be directly involved in the treatment or evaluation of research subjects in this study.

The study will be administered by and monitored by employees or representatives of the Sponsor or designee. Clinical Research Associates (CRAs) or trained designees will monitor each site on a periodic basis and perform verification of source documentation for each subject as well as other required review processes. The Sponsor or designee will be responsible for the timely reporting of serious adverse events (SAEs) to appropriate regulatory authorities as required.

7. STUDY OBJECTIVES

7.1 Primary Objectives

The primary objectives of the study are

- Phase 1: 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.
- Phase 2: 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.

7.2 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 RECIST 1.1 and iRECIST:
  - Clinical benefit rate (CR + PR + SD lasting at least 8 weeks)
  - Time to response
  - Duration of response
  - Progression-free survival
- To describe overall survival
- To evaluate the safety profile of the combination of pegzilarginase with pembrolizumab in the study patient population (Phase 2)

7.3 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

8. INVESTIGATIONAL PLAN

8.1 Overall Study Design and Plan

This 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 SCLC treatment and either have not tolerated the regimen or have relapsed or progressed within 6 months of treatment may participate in the trial.

Pembrolizumab will be administered every 3 weeks at a fixed dose of 200 mg by intravenous (IV) infusion on day 1 of each cycle. Pegzilarginase will be administered weekly (i.e., on days 1, 8 and 15 of each cycle) by IV infusion.

Phase 1: Pegzilarginase will be administered by IV infusion weekly, and one cycle will be 3 weeks. The dose of pegzilarginase will be escalated in two steps from 0.20 mg/kg and then at the single agent MTD for solid tumors, which will be either 0.27 mg/kg or 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. The dose escalation plan is described in Section 13.10.

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

Ultimately, the recommended phase 2 dose (RP2D) of pegzilarginase in combination with pembrolizumab 200 mg q 3 weeks will be determined by assessment of the safety of the combination over the DLT period and available safety information in subsequent cycles.

Phase 2: In the phase 2 part of the trial, patients who meet entry criteria will be treated with the RP2D of pegzilarginase with 200 mg pembrolizumab in a minimax Simon 2-stage design (Simon, 1989). Fifty-five patients will be enrolled and treated in stage 1. If 23 or more of those subjects achieve a response (CR or PR), an additional 11 subjects will be enrolled. If a total of 30 or more subjects 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%.

All patients in both parts of the trial will be monitored for safety by periodic physical exams, vital signs, ECGs, hematology, electrolytes and chemistry laboratory testing, thyroid function testing, urinalysis, and coagulation laboratory testing. AEs will be recorded at each study visit.

DLT will be defined as the occurrence of any of the treatment-emergent events described in Section 8.7.6 in the first 21 days (one cycle) of treatment if deemed at least possibly related to the combination of pegzilarginase with pembrolizumab. The severity of AEs will be graded per the National Cancer Institute Common Terminology Criteria for Adverse Events version 4 (NCI CTCAE v. 4.0).

Combination regimen of pembrolizumab and pegzilarginase must be withheld for drug-related toxicities and severe or life-threatening AEs as described in TablesTable 3 and Table 4.

8.2 Number of Patients

Approximately 9 to 18 patients will participate in the phase 1 trial. For the phase 2 trial, up to 55 patients will participate in the initial stage 1. In the stage 2, additional 11 patients will be enrolled. Patients who have signed informed consent but who have not yet been treated when a particular stage is closed may continue with screening and treatment in the study, with agreement of the sponsor.

8.3 Expected Duration of Patient Participation

Patients may continue treatment for a maximum of 35 cycles (approximately 24 months), or until they have confirmed progressive disease per iRECIST criteria, whichever comes first. Even if patients discontinue treatment, follow-up may continue for the patient until progressive disease is confirmed by iRECIST criteria, or 8 weeks have elapsed since all patients have had their last dose of study drug, whichever comes first. All patients are required to complete a follow-up safety visit 30-44 days after their last dose of study drug (pembrolizumab or pegzilarginase), or prior to the initiation of alternative therapy for SCLC, whichever is earlier.

8.4 Discussion of Study Design, Including Choice of Control Group

This will be a single-arm, open-label trial with a dose-escalation period and a phase 2 period.

8.5 Selection of Study Population

8.5.1 Inclusion Criteria

A patient must meet all the following criteria to be enrolled in this study:

1) Patient is able and willing to provide written informed consent and to comply with all requirements of study participation (including all study procedures) prior to any screening procedures
2) Be > 18 years of age on day of signing informed consent
3) Have histologically or cytologically confirmed SCLC that meet the following corresponding requirements:
    a) ED per criteria of the International Association for the Study of Lung Cancer (IASLC)-American Joint Committee on Cancer (AJCC) TNM staging system
    b) Have not tolerated or have progressed or relapsed on or within 6 months of platinum-based chemotherapy
4) Have a performance status of ≤ 1 on the ECOG Performance Scale
5) Have measurable disease based on RECIST 1.1 as determined by the site study team. Tumor lesions in a previously irradiated area are considered measurable if progression has been demonstrated in such lesions
6) Willing to undergo core needle or incisional biopsy to obtain fresh tumor tissue specimens. For patients without accessible tumor for biopsy, an archived, formalin-fixed paraffin embedded (FFPE) tumor tissue specimen may be acceptable if it was obtained after the last cancer-directed treatment and tumor is confirmed to be present in the specimen. If an archived FFPE block is provided this needs to be not older than 3 years. In addition, if pre-cut unstained slides are provided these need to be not older than 6 months.
7) Demonstrate adequate organ function, with specimens collected within 10 days prior to day 1 of cycle 1, as defined as:
    a) Absolute neutrophil count (ANC) ≥1,500 /mcL
    b) Platelets ≥100,000 / mcL (without need of transfusion for at least 14 days prior to scheduled C1D1)
    c) Hemoglobin ≥9 g/dL or ≥5.6 mmol/L (without need of erythropoietin or transfusion for at least 14 days prior to scheduled C1D1)
    d) Renal: serum creatinine ≤1.5 X upper limit of normal (ULN)
        i) Subjects with creatinine levels > 1.5x ULN may qualify if Glomerular Filtration Rate (GFR) OR calculated creatinine clearance per institutional standard (CrCL) are ≥ 60 mL/min/1.73m2

e) Hepatic: serum total bilirubin ≤ 1.5 X ULN

i) Subjects with total bilirubin > 1.5 ULN if direct bilirubin ≤ ULN

f) AST (SGOT) and ALT (SGPT) ≤ 2.5 X ULN or ≤ 5 X ULN for patients with liver metastases

g) Albumin ≥ 2.5 mg/dL

h) International normalized ratio (INR) or prothrombin time (PT) ≤ 1.5 X ULN

i) Exception: subjects receiving anticoagulant therapy if PT or INR is within therapeutic range of intended use.

i) Activated partial thromboplastin time (aPTT) ≤ 1.5 X ULN

i) Exception: subjects receiving anticoagulant therapy if PTT is within therapeutic range of intended use

8) Subjects must have recovered from the effects (except alopecia or neuropathy) of any prior systemic therapy, radiotherapy or surgery to Grade 1 or less

9) If subject received major surgery or radiation therapy of > 30 Gy, recovered from the toxicity and/or complications from the intervention is required

10) If female and of child-bearing potential, has a negative urine or serum pregnancy test within 72 hours prior to receiving the first dose of study medication. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required

11) If a sexually active male or female, must be surgically sterile post-menopausal (defined as female patient with 12 months of spontaneous amenorrhea, or 6 months of spontaneous amenorrhea with serum FSH levels higher than 40 mIU/ml, or 6 weeks after bilateral surgical oophorectomy), or must agree to use a physician-approved method of birth control during the study through a minimum of 120 days after the last study drug administration

8.5.2 Exclusion Criteria

A patient is excluded from this study if he/she meets any of the following criteria:

1) Has received more than 2 platinum-based regimens against SCLC

2) Has received pembrolizumab, or prior therapy with an anti-PD-1, anti-PD-L1, anti-PD-L2, or anti-Cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) antibody as part of any previous therapy, including trials

3) Has participated in Merck MK-3475 (pembrolizumab) clinical trials

4) Has received pegzilarginase as part of any previous therapy

5) Is currently participating in a study of an investigational agent and received the last dose within 4 weeks of the first dose of treatment (a shorter interval for kinase inhibitors or other short half-life drugs could be considered after approval form the Sponsor). Is currently participating in a study of an investigational device within 4 weeks of the first dose of treatment.

6) Has a diagnosis of an immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 7 days prior to the first dose of trial treatment. The use of topical, short courses (3 days or less), or physiologic doses of corticosteroids (doses not higher than 10 mg daily of prednisone equivalent) may be allowed after consultation with the Sponsor.

7) Has had a prior monoclonal antibody (mAb) within 4 weeks prior to first dose or who has not recovered (i.e., ≤ Grade 1) from adverse reactions due to agents administered more than 4 weeks earlier

8) Has had prior chemotherapy, targeted small molecule therapy, or radiation therapy within 2 weeks prior to first dose or who has not recovered (i.e., ≤ Grade 1 or at baseline) from treatment-emergent adverse reactions due to a previously administered agent. Note: Patients with ≤ Grade 2 neuropathy or ≤ Grade 2 alopecia are an exception to this criterion and may qualify for the study after consultation with the Sponsor. If the patient has received prior radiation therapy, participant must has recovered from all radiation-related toxicities, not currently taking systemic corticosteroids due to radiation-related toxicities, and not have had radiation pneumonitis. A 1-week washout is permitted for palliative radiation (≤2 weeks of radiotherapy) to non-CNS disease.

9) If the patient has undergone major surgery, he/she must have recovered adequately from the toxicity and/or complications prior to starting therapy

10) Has received transfusion of blood products (including platelets or red blood cells) or administration of colony stimulating factors (including G-CSF, GM-CSF or recombinant erythropoietin) within 14 days prior to study first dose

11) Has a known additional malignancy that is progressing or has required active treatment within the past 3 years. Exceptions include basal cell carcinoma of the skin, squamous cell carcinoma of the skin, or carcinoma in situ (e.g. breast carcinoma, cervical cancer) that has undergone potentially curative therapy.

12) Has known central nervous system (CNS) metastases. However, patients with previously treated brain metastases may participate provided neurologic symptoms have stabilized, there is no evidence of new brain metastases or hemorrhage, and they are not using steroids for brain metastases or for complications derived from their treatment for at least 7 days prior to first dose of trial treatment

13) Has known carcinomatous meningitis

14) Has an active autoimmune disease requiring systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs) or a documented history of clinically severe autoimmune disease, or a syndrome that requires systemic steroids or immunosuppressive agents. Patients with vitiligo or resolved childhood asthma/atopy are an exception to this rule. Patients that require intermittent use of bronchodilators or local steroid injections will not be excluded from the study. Patients with hypothyroidism stable on hormone replacement or Sjogren's syndrome will not be excluded from the study. Replacement therapy (e.g. thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc) is not considered a form of systemic treatment.
15) Has evidence of interstitial lung disease, history of non-infectious pneumonitis that required steroids, or current pneumonitis
16) Has an active infection requiring systemic therapy
17) Inadequately controlled hypertension (defined as systolic blood pressure ≥ 200 mmHg and/or diastolic blood pressure ≥ 120 mmHg) on more than one occasion in the month before planned day of infusion. Patients with more than one episode of inadequately controlled hypertension will be allowed to participate under initiation or modification of antihypertensive treatment provided adequate control (defined as systolic blood pressure < 160 mmHg and/or diastolic blood pressure <100 mmHg) is documented on 3 separate days over in the week before starting treatment
18) Currently taking 3 or more anti-hypertensive medications, including diuretics (If combination tablets are part of the treatment, each active ingredient part of them counts as one)
19) Prior history of hypertensive crisis or hypertensive encephalopathy
20) History of myocardial infarction, unstable angina, cardiac or other vascular stenting, angioplasty, or cardiac or vascular surgery within 6 months prior to day 1 of study treatment
21) Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the patient's participation for the full duration of the trial, or is not in the best interest of the patient to participate, in the opinion of the treating investigator
22) Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial
23) Is pregnant or breastfeeding, or expecting to conceive or father children within the projected duration of the trial, starting with the pre-screening or screening visit through 120 days after the last dose of trial treatment
24) Has a known history of Human Immunodeficiency Virus (HIV) (positive for HIV p24 antigen or HIV 1/2 antibodies)

25) Has known active Hepatitis B (e.g., HBsAg reactive) or Hepatitis C (e.g., HCV RNA [qualitative] is detected)
26) Has received a live-virus vaccination within 30 days prior to the planned first dose of study therapy. Note: Seasonal influenza vaccines for injection are generally inactivated flu vaccines and are allowed; however intranasal influenza vaccines (e.g., Flu-Mist®) are live attenuated vaccines, and are not allowed. Other seasonal flu vaccines that do not contain live virus are permitted
27) Has a known history of active tuberculosis (Bacillus tuberculosis)
28) Has a history of hypersensitivity to PEG or any component of the pegzilarginase formulation
29) Severe hypersensitivity (≥ Grade 3) to any excipient of pegzilarginase or pembrolizumab
30) Has had an allogenic tissue/solid organ transplant

8.6 Removal of Patients from Treatment or Assessment

Patients have the right to withdraw from the study at any time for any reason. The investigator may discontinue a patient from the study for any of the following reasons:

- Occurrence of an AE, development of an intercurrent illness/condition, or change in patient's condition that leads the investigator to be concerned about the patient's welfare
- Intolerable toxicity
- Disease progression
- Patient is uncooperative or non-compliant with study procedures/responsibilities
- Pregnancy in a female patient during the study
- Withdrawal of consent
- Patient is lost to follow-up
- Death

The reason for a patient's withdrawal from the study will be recorded in the electronic case report form (eCRF). Patients who withdraw from study participation for any reason may not re-enter the study at any time.

Patients who are discontinued from the study will be asked to complete follow-up visit procedures.

8.6.1 Subject Identification and Replacement of Subjects

All patients who provide informed consent will be assigned a unique patient identifier.

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. An additional patient will be screened and treated to meet the requirements of the design for determining RP2D.

All patients who receive at least one dose of pegzilarginase or pembrolizumab will be included in assessments of safety, regardless of their inclusion in the RP2D determination.

8.7 Treatments

8.7.1 Treatments Administered

Patients will receive pegzilarginase by slow IV infusion once weekly at their assigned dose level. Please refer to the Pharmacy Manual for specific details.

8.7.2 Identity of Investigational Product

Pegzilarginase is a cobalt substituted, pegylated, recombinant human ARG1.

8.7.2.1 Product Characteristics and Labeling

Pegzilarginase drug product will be supplied as a liquid formulation in 10 mL single-use glass vials containing 5 mL of formulated drug product at a concentration of 1 mg/mL or 6 mL of formulated drug product at a concentration of 5 mg/mL. Pegzilarginase is formulated in 50mM NaCl, 1 mM KH_2PO_4, 4 mM K_2HPO_4, and 1.5% w/v glycerol.

8.7.3 Storage

Filled vials of pegzilarginase drug product should be stored frozen at -≤ 65°C.

8.7.4 Dose Selection and Directions for Administration

8.7.4.1 Pembrolizumab Dose Selection and Directions for Administration

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. The rationale for selection of dose of pembrolizumab to be used in this trial

is provided in Section 6.2.2. Details on preparation and administration of pembrolizumab are provided in the Pharmacy Manual.

Pembrolizumab will be administered using a 30-minute IV infusion. Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window between -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes -5 min/+10 min).

8.7.4.2 Pegzilarginase Dose Selection, Directions for Administration, and Escalation

On days where both pembrolizumab and pegzilarginase are dosed, pegzilarginase will be administered 30 minutes after the completion of the pembrolizumab infusion.

The pegzilarginase dose will be calculated based on the patient's weight and the assigned dose level. For a patient whose weight is more than 100 kg, the dosing calculation will be capped at 100 kg body weight. Please refer to the Pharmacy manual for additional details.

Pegzilarginase will be administered in a location at the site equipped with immediately accessible resuscitation equipment. Patients will be observed for a minimum of 1 hour following each infusion during the first two cycles and a minimum of 30 minutes following each infusion in subsequent cycles. A central venous access port may be considered for patients with poor IV access at the Investigator's discretion.

The dose of pegzilarginase will be escalated in two steps from 0.20 mg/kg and then at the single agent MTD for solid tumors, which will be either 0.27 mg/kg or 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. Dose escalation details are provided in Section 13.10.1. The dose of pegzilarginase selected for the phase 2 part of the study will be called the RP2D and will not change during phase 2 of the study.

8.7.5 Method of Assigning Subjects to Treatment Groups

This is an open-label study. Patients are assigned to a fix dose of pembrolizumab during the study; the dose of pegzilarginase is escalated during Phase 1 and fixed during Phase 2 at the RP2D.

8.7.6 Definition of Dose-Limiting Toxicity (DLT)

DLT will be defined as the occurrence of any of the following treatment-emergent events in the first 21 days (one cycle) of treatment if deemed at least possibly related to the combination of pegzilarginase with pembrolizumab. The severity of AEs will be graded per the National Cancer Institute Common Terminology Criteria for Adverse Events version 4 (NCI CTCAE v. 4.0):

1) Grade 5 toxicity
2) Non-hematologic AEs
    a) Gastrointestinal:
        i. Grade 4 diarrhea or Grade 4 colitis
        ii. Grade 2 or 3 diarrhea or colitis that does not resolve to at least Grade 1 before the next cycle
    b) Endocrinology:
        i. New onset type 1 diabetes mellitus (T1DM) that is not clinically and metabolically stable before next cycle
        ii. Grade 3 or 4 hyperglycemia that is not clinically and metabolically stable before next cycle
        iii. Grade 2 to 4 hypophysitis that does not resolve to at least Grade 1 before the next cycle
        iv. Grade 4 hyperthyroidism
        v. Grade 3 hyperthyroidism that does not resolve to at least Grade 1 before the next cycle
    c) Respiratory:
        i. Grade 4, Grade 3, or recurrent Grade 2 pneumonitis
        ii. First episode of Grade 2 pneumonitis that does not resolve to at least Grade 1 before the next cycle
    d) Renal:
        i. Grade 3 or 4 renal failure or nephritis
        ii. Grade 2 nephritis that does not resolve to at least Grade 1 before the next cycle
    e) Cardiovascular:

i. Single reading of blood pressure levels ≥ 200 mmHg systolic or ≥ 120 mmHg diastolic

ii. Grade 3 hypertension that does not resolve, with or without intervention, to ≤ Grade 1 in less than 24 hours

f) Infusion reaction

i. Grade 3 or 4 infusion reaction

ii. Grade 2 infusion reaction that does not resolve to at least grade 1 before the next cycle

g) Any other grade 4 non-hematologic, non-laboratory toxicity not described above

h) Any other grade 3 non-hematologic, non-laboratory toxicity not specifically mentioned in other criteria lasting >3 days despite optimal supportive care

3) Hematologic AEs:

a) Grade 3 or 4 febrile neutropenia regardless of duration

b) Grade 4 thrombocytopenia regardless of duration if associated with:

i. A bleeding event which requires an elective platelet transfusion, or

ii. A life-threatening bleeding event which results in urgent intervention and admission to an Intensive Care Unit

c) Any other treatment-related grade 4 hematologic toxicities not described above

d) Any other treatment-related grade 3 hematologic toxicities that do not resolve to at least grade 1 before the next cycle or requires the use of transfusion of blood products (including platelets or red blood cells) or administration of colony stimulating factors (including G-CSF, GM-CSF or recombinant erythropoietin)

4) Laboratory, other:

a) Grade 3 or higher AST, ALT or bilirubin, except for:

i. patients with liver metastasis or underlying liver disease with a baseline grade 2 AST or ALT, AST or ALT will need to increase by greater than or equal to 50% relative to baseline and last for at least 7 days

b) Grade ≥ 2 increase in transaminases (either AST or ALT) associated with either grade ≥ 2 increase in total bilirubin or Grade ≥ 2 increase in INR, except for:

i. patients with liver metastasis or underlying liver disease with a baseline grade 2 AST or ALT, AST or ALT will need to increase by greater than or equal to 50% relative to baseline and last for at least 7 days

c) Any Grade 3 or Grade 4 non-hematologic laboratory value if:

i. Medical intervention is required to treat the subject, or

ii. The abnormality leads to hospitalization, or

iii. The abnormality is asymptomatic and persists at grade 3 or higher for >7 days

iv. Exception: Grade 3 isolated electrolyte abnormalities (i.e., those occurring without clinical consequence) that resolve, with or without intervention, to grade ≤ 1 in < 72 hours

5) Other:

a) Prolonged delay (>2 weeks) in initiating cycle 2 due to treatment-related toxicity

b) Missing two of the three planned pegzilarginase doses during the first cycle because of AE(s) due to treatment-related toxicity Dose Modification

8.7.7 Dose Modification and Toxicity Management Guidelines for Pembrolizumab

The treatment and dose modifications for pembrolizumab and pegzilarginase are found in Table 3 and Table 4, respectively.

Dose modification and toxicity management for immune-related AEs associated with pembrolizumab

AEs associated with pembrolizumab exposure may represent an immunologic etiology. These immune-related AEs (irAEs) may occur shortly after the first dose or several months after the last dose of pembrolizumab treatment and may affect more than on body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical trial data, most irAEs were reversible and could be managed with interruptions of pembrolizumab, administration of corticosteroids and/or other supportive care. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation. Based on the severity of irAEs, withhold or permanently discontinue pembrolizumab and administer corticosteroids. Dose modification and toxicity management guidelines for irAEs associated with pembrolizumab are provided in Table 3.

Table 3. Dose modification and toxicity management guidelines for immune-related AEs associated with pembrolizumab

General instructions:

1. Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks.
2. For situations where pembrolizumab has been withheld, pembrolizumab can be resumed after AE has been reduced to Grade 1 or 0 and corticosteroid has been tapered. Pembrolizumab should be permanently discontinued if AE does not resolve within 12 weeks of last dose or corticosteroids cannot be reduced to ≤ 10 mg prednisone or equivalent per day within 12 weeks.
3. For severe and life-threatening irAEs, IV corticosteroid should be initiated first followed by oral steroid. Other immunosuppressive treatment should be initiated if irAEs cannot be controlled by corticosteroids.

Immune-related AEs	Toxicity grade or conditions (CTCAEv4.0)	Action taken to pembrolizumab	irAE management with corticosteroid and/or other therapies	Monitor and follow-up
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1-2mg/kg prednisone or equivalent) followed by taper	• Monitor subjects for signs and symptoms of pneumonitis • Evaluate subjects with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment • Add prophylactic antibiotics for opportunistic infections
	Grade 3 or 4, or recurrent grade 2	Permanently discontinue		
Diarrhea / colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1-2mg/kg prednisone or equivalent) followed by taper	• Monitor subjects for signs and symptoms of enterocolitis (i.e. diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (i.e. peritoneal signs and ileus). • Subjects with ≥ Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis. • Subjects with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion.
	Grade 4	Permanently discontinue		
AST / ALT elevation or	Grade 2	Withhold	• Administer corticosteroids (initial dose of 0.5-	• Monitor with liver function tests (consider weekly or more frequently until liver

General instructions:

1. Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks.
2. For situations where pembrolizumab has been withheld, pembrolizumab can be resumed after AE has been reduced to Grade 1 or 0 and corticosteroid has been tapered. Pembrolizumab should be permanently discontinued if AE does not resolve within 12 weeks of last dose or corticosteroids cannot be reduced to ≤ 10 mg prednisone or equivalent per day within 12 weeks.
3. For severe and life-threatening irAEs, IV corticosteroid should be initiated first followed by oral steroid. Other immunosuppressive treatment should be initiated if irAEs cannot be controlled by corticosteroids.

Immune-related AEs	**Toxicity grade or conditions (CTCAEv4.0)**	**Action taken to pembrolizumab**	**irAE management with corticosteroid and/or other therapies**	**Monitor and follow-up**
Increased Bilirubin			1mg/kg prednisone or equivalent) followed by taper	enzyme value returned to baseline or is stable
	Grade 3 or 4	Permanently discontinue	• Administer corticosteroids (initial dose of 1-2mg/kg prednisone or equivalent) followed by taper	
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	Newly onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold	• Initiate insulin replacement therapy for subjects with T1DM • Administer anti-hyperglycemic in subjects with hyperglycemia	• Monitor subjects for hyperglycemia or other signs and symptoms of diabetes.
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated.	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue1		
Hyperthyroidism	Grade 2	Continue	• Treat with non-selective beta-blockers (e.g. propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders.
	Grade 3 or 4	Withhold or Permanently discontinue1		
Hypothyroidism	Grade 2-4	Continue	• Initiate thyroid replacement hormones (e.g. levothyroxine or	• Monitor for signs and symptoms of thyroid disorders.

General instructions:

1. Corticosteroid taper should be initiated upon AE improving to Grade 1 or less and continue to taper over at least 4 weeks.
2. For situations where pembrolizumab has been withheld, pembrolizumab can be resumed after AE has been reduced to Grade 1 or 0 and corticosteroid has been tapered. Pembrolizumab should be permanently discontinued if AE does not resolve within 12 weeks of last dose or corticosteroids cannot be reduced to ≤ 10 mg prednisone or equivalent per day within 12 weeks.
3. For severe and life-threatening irAEs, IV corticosteroid should be initiated first followed by oral steroid. Other immunosuppressive treatment should be initiated if irAEs cannot be controlled by corticosteroids.

Immune-related AEs	**Toxicity grade or conditions (CTCAEv4.0)**	**Action taken to pembrolizumab**	**irAE management with corticosteroid and/or other therapies**	**Monitor and follow-up**
			liothyroinine) per standard of care	
Nephritis and renal dysfunction	Grade 2	Withhold	• Administer corticosteroids (prednisone 1-2mg/kg or equivalent) followed by taper.	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1 or 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
All Other immune-related AEs	Intolerable/ persistent Grade 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology or exclude other causes
	Grade 3	Withhold or discontinue based on the type of event. Events that require discontinuation include and not limited to: Guillain-Barre Syndrome, encephalitis		
	Grade 4 or recurrent Grade 3	Permanently discontinue		

NOTES:

1. Withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician.
2. For subjects with Grade 3 or 4 immune-related endocrinopathy where withhold of pembrolizumab is required, pembrolizumab may be resumed when AE resolves to ≤ Grade 2 and is controlled with hormonal replacement therapy or achieved metabolic control (in case of T1DM).

8.7.8 Dose Modification for Pegzilarginase

Table 4 and 5 present the guidelines for dose modification due to adverse events for pegzilarginase.

During Phase 1, dose modifications will not be allowed during the first cycle (3 weeks) of treatment with pegzilarginase (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.

After the first cycle of treatment, pegzilarginase dose modification will be allowed in patients who experience toxicity; the guidelines in Table 4 and 5 will be used to determine the dose modification required. If the investigator believes that the guidelines should not apply, he/she will contact the Sponsor's medical monitor to discuss the circumstances and the appropriate dose modification.

Table 4. Pegzilarginase Dose Modification due to Adverse Events (Other)

Toxicity	Occurrence	Action
Hematological ▪ Grade 3 or 4 neutropenia with fever, or ▪ Grade 4 neutropenia for ≥ 5 days, or ▪ Grade 4 platelets count decrease, or ▪ Grade 3 thrombocytopenia with bleeding	1st	Hold study drug. Once toxicity has resolved to Gr < 2, restart pegzilarginase at dose level -1*.
	2nd	Hold study drug. Once toxicity has resolved to Gr < 2, restart pegzilarginase at dose levels -1 dosing 2 out of the 3 planned doses per cycle*.
	3rd	Discontinue pegzilarginase.
Non-Hematological ▪ Grade 3 nausea, vomiting, or diarrhea if persistent for > 72 hours despite optimal antiemetic or antidiarrheal therapy ▪ Grade 3 increase in ALT or AST if persists for > 72 hours ▪ Any other Grade 3 toxicity considered	1st	Hold study drug. Once toxicity has resolved to Gr < 2, restart pegzilarginase at the next lower dose level -1*.
	2nd	Hold study drug. Once toxicity has resolved to Gr < 2, restart pegzilarginase at dose level -1 dosing 2 out of the 3 planned doses per cycle*.
	3rd	Discontinue pegzilarginase.

Toxicity	Occurrence	Action
clinically significant by the investigator ▪ Any other Grade 4 clinical toxicity ▪ Any Grade 4 laboratory toxicity considered clinically significant by the investigator		

* Starting dose: 0.27 mg/kg
Dose level -1: 0.20 mg
Note: After 4 weeks without a recurrent AE, the dose may be escalated at the Investigator's discretion

Table 5. Guidelines for Pegzilarginase Dose Modification (Events of Tremor, Generalized Muscle Weakness, and Gait Disturbance)

Toxicity	Grade 1	Grade 2 or 3
Tremor	No change	0.27 mg/kg: hold study drug until ≤ Grade 1, then reduce to 0.20 mg/kg
		0.20 mg/kg: hold study drug until ≤ Grade 1, then restart pegzilarginase at dose level -1 dosing 2 out of the 3 planned doses per cycle
		0.20 mg/kg dosing 2 out of the 3 planned doses per cycle: discontinue study
Generalized muscle weakness	No change	0.27 mg/kg: hold study drug until ≤ Grade 1, then reduce to 0.20 mg/kg
		0.20 mg/kg: hold study drug until ≤ Grade 1, then restart pegzilarginase at dose level -1 dosing 2 out of the 3 planned doses per cycle
		0.20 mg/kg dosing 2 out of the 3 planned doses per cycle: discontinue study
Gait disturbance	0.27 mg/kg: reduce to 0.20 mg/kg	0.27 mg/kg: hold study drug until ≤ Grade 1, then reduce to 0.20 mg/kg
	0.20 mg/kg: restart pegzilarginase at dose level -1 dosing 2 out of the 3 planned doses per cycle	0.20 mg/kg: hold study drug until ≤ Grade 1, then restart pegzilarginase at dose level -1 dosing 2 out of the 3 planned doses per cycle
	0.20 mg/kg dosing 2 out of the 3 planned doses per cycle: no change[1]	0.20 mg/kg dosing 2 out of the 3 planned doses per cycle: discontinue study

* Starting dose: 0.27 mg/kg

Dose level -1: 0.20 mg/kg
1 Dose may be omitted at the Investigator's discretion after discussion with the Sponsor.
Note: After 4 weeks without a recurrent AE, the dose may be escalated at the Investigator's discretion.

8.7.9 Infusion Reaction Treatment Guidelines

Pembrolizumab may cause severe or life-threatening infusion-reactions including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Among patients with Arginase I Deficiency participating in pegzilarginase trials, hypersensitivity reactions have been observed that were managed with antihistamines and infusion rate decrease. Please refer to the IB for further details related to the hypersensitivity reaction observed. Dose modification and toxicity management guidelines on pembrolizumab and pegzilarginase associated infusion reaction are provided in Table 6, and additional information is available in the Investigator's Brochures.

Table 6. Infusion Reaction Treatment Guidleines

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	• Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires therapy or infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics, IV fluids); prophylactic medications indicated for < =24 hrs	• **Stop Infusion.** • Additional appropriate medical therapy may include but is not limited to: o IV fluids o Antihistamines o NSAIDS o Acetaminophen o Narcotics • Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator. • If symptoms resolve within one hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g., from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the subject should be premedicated for the next scheduled dose. **Subjects who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further trial treatment administration.**	Subject may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab (MK-3475) or pegzilarginase with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of analgesic).
Grades 3 or 4 **Grade 3:** Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other	• **Stop Infusion.** • Additional appropriate medical therapy may include but is not limited to: o Epinephrine** o IV fluids o Antihistamines o NSAIDS o Acetaminophen o Narcotics o Oxygen o Pressors	No subsequent dosing

NCI CTCAE Grade	Treatment	Premedication at subsequent dosing
clinical sequelae (e.g., renal impairment, pulmonary infiltrates) **Grade 4:** Life-threatening; pressor or ventilatory support indicated	○ Corticosteroids • Increase monitoring of vital signs as medically indicated until the subject is deemed medically stable in the opinion of the investigator • Hospitalization may be indicated. **In cases of anaphylaxis, epinephrine should be used immediately. **Subject is permanently discontinued from further trial treatment administration.**	
Appropriate resuscitation equipment should be available in the room and a physician readily available during the period of drug administration. For further information, please refer to the Common Terminology Criteria for Adverse Events v4.0 (CTCAE) at http://ctep.cancer.gov		

Other allowed dose interruptions for pembrolizumab

Pembrolizumab may be interrupted for situations other than treatment-related AEs such as medical / surgical events or logistical reasons not related to study therapy. Subjects should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor. The reason for interruption should be documented in the patient's study record

8.7.10 Replacement of Patients

Patients are not "replaced" in this study. Patients in the dose escalation portion of the study who do not experience a DLT, but who have either inadequate follow-up or inadequate dosing to be considered DLT-free, will not be included in the assessment of MTD and the determination of the RP2D. For each such patient, an additional patient will be treated at the same dose level in the escalation period, so that the assessment of MTD and determination of RP2D can be made based on the number of patients required by the design. All patients in the dose escalation phase who are treated with one dose of either pembrolizumab or pegzilarginase will be included in safety evaluations, regardless of their inclusion in the MTD and RP2D determinations.

All patients who are treated in the phase 2 part of the study will be included in the analysis of the primary endpoint as described in the statistical analysis section of this protocol and in the statistical analysis plan.

8.8 Prior and Concomitant Medications

8.8.1 Concomitant Medications/Vaccinations (Allowed & Prohibited)

Medications or vaccinations specifically prohibited in the exclusion criteria are not allowed during the ongoing trial. If there is a clinical indication for any medication or vaccination specifically prohibited during the trial, discontinuation from trial therapy or vaccination may be required. The investigator should discuss any questions regarding this with the Sponsor Clinical Director. The final decision on any supportive therapy or vaccination rests with the investigator and/or the subject's primary physician. However, the decision to continue the subject on trial therapy or vaccination schedule requires the mutual agreement of the investigator, the Sponsor and the subject.

8.8.1.1 Acceptable Concomitant Medications

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care, including blood products or transfusions, antibiotics, pain medications, bisphosphonates, and replacement hormonal therapies.

Antiemetic agents may be used for the treatment of nausea and vomiting, including as prophylaxis prior to cycle 1 of treatment with pegzilarginase at the discretion of the investigator. If patients develop nausea or vomiting during study treatment, anti-emetics should be initiated or optimized through dosage increase or combination regimens according to local practices or institutional guidelines. The antiemetics American Society of Clinical Oncology (ASCO) practice guideline is also recommended.

During the first cycle. hematopoietic growth factors should be used only for severe or prolonged neutropenia or for neutropenic sepsis; however, they may be used during subsequent cycles of treatment (adherence to the American Society of Clinical Oncology (ASCO) guidelines is recommended). Erythropoietin should not be administered during the first cycle of pegzilarginase treatment.

Agents for treatment of infusion reactions, including diphenhydramine and acetaminophen, may not be given prophylactically. Patients experiencing an infusion reaction may receive prophylaxis prior to subsequent injection; please refer to Table 6 for specific instructions.

No information is available on potential drug-drug interactions with pegzilarginase. All concomitant medication will be recorded on the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications and fluids. If changes occur during the trial period, documentation of drug dosage, frequency, route, and date may also be included on the CRF.

All concomitant medications received within 30 days before the first dose of trial treatment and 30 days after the last dose of trial treatment should be recorded. Concomitant medications administered after 30 days after the last dose of trial treatment should be recorded for SAEs and ECIs as defined in Section 9.3.

8.8.1.2 Prohibited Concomitant Medications

Subjects are prohibited from receiving the following therapies during the Screening and Treatment Phase (including retreatment for post-complete response relapse) of this trial:

- Antineoplastic systemic chemotherapy or biological therapy
- Immunotherapy not specified in this protocol
- Chemotherapy not specified in this protocol
- Investigational agents other than pegzilarginase and pembrolizumab
- Radiation therapy
    - Note: Radiation therapy to a symptomatic solitary bone lesion may be considered on an exceptional case by case basis after consultation with Sponsor. The subject must have clear measurable disease outside the radiated field. Administration of palliative radiation therapy will be considered clinical progression for the purposes of determining PFS.
- Live vaccines within 30 days prior to the first dose of trial treatment and while participating in the trial. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, chicken pox, yellow fever, rabies, BCG, and typhoid (oral) vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed. However, intranasal influenza vaccines (e.g. Flu - Mist®) are live attenuated vaccines and are not allowed.
- Systemic glucocorticoids for any purpose other than to modulate symptoms from an adverse event or event of clinical interest of suspected immunologic etiology. The use of topical, short courses (3 days or less), or physiologic doses of corticosteroids may be approved after consultation with the Sponsor.
    - Note: Inhaled steroids are allowed for management of asthma.

Subjects who, in the assessment by the investigator, require the use of any of the afore mentioned treatments for clinical management should be removed from the trial. Subjects may receive other medications that the investigator deems to be medically necessary.

8.8.2 Rescue Medications & Supportive Care

Participants should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of AEs with potential immunologic etiology are outlined along with the dose modification guidelines in Sections 8.7.7, 8.7.8 and 8.7.9 [Tables 3, 4, and 5]. Where appropriate, these guidelines include the use of oral or IV treatment with corticosteroids, as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the investigator determines the events to be related to pembrolizumab, pegzilarginase, or their combination.

Note: If after the evaluation of the event, it is determined not to be related to pembrolizumab, pegzilarginase, or their combination, the investigator does not need to follow the treatment guidance. Refer to Tables 3, 4 and 5 for guidelines regarding dose modification and supportive care.

It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event.

8.8.3 Treatment Compliance

Patients who are non-compliant with study treatment for reasons other than AEs will be withdrawn from the study. Consideration will be given to withdrawing from the study any patient who misses ≥2 consecutive study drug administrations. Patients missing ≥2 consecutive study drug administrations should be discussed with the Medical Monitor prior to resuming treatment or withdrawal from the study.

8.9 Investigational Product Accountability

The PI or designee is responsible for maintaining accurate records (including dates and quantities) of IP received, subjects to whom IP is dispensed (subject–by–subject dose specific accounting), and IP lost or accidentally or deliberately destroyed. The PI or designee must retain all unused or expired study supplies until the study monitor has confirmed the accountability data.

8.9.1 Return and Disposition of Clinical Supplies

Unused study drug must be kept in a secure location for accountability and reconciliation by the study monitor. The Investigator or designee must provide an explanation for any destroyed or missing study drug or study materials.

Unused study drug may be destroyed on site, per the site's standard operating procedures, but only after Aeglea has granted approval for drug destruction. The monitor must account for all study drug in a formal reconciliation process prior to study drug destruction. All study drug destroyed on site must be documented. Documentation must be provided to Aeglea and retained in the PIs study files.

If a site is unable to destroy study drug appropriately, the site can return unused study drug to Aeglea or designee upon request. The return of study drug or related materials must be accounted for on a Study Drug Return Form provided by the Sponsor.

All study drug and related materials should be stored, inventoried, reconciled, and destroyed or returned according to applicable state and federal regulations and study procedures.

8.10 Outcome Measures

The Schedule of Events in the Synopsis describes the timing of required evaluations.

8.10.1 Safety Outcome Measures

Safety outcome measures will include SAEs, AEs, changes in physical exam findings, vital signs, ECG's, hematology urinalysis, electrolytes, thyroid function, and serum chemistries.

8.10.2 Efficacy Outcome Measures

8.10.2.1 Primary Efficacy Endpoint

The primary efficacy endpoint is ORR as assessed by RECIST 1.1.

8.10.2.2 Secondary Efficacy Endpoints

The duration of response by RECIST 1.1 criteria as assessed by local investigators at their sites will serve as additional measures of efficacy. Overall survival will be included as it is a standard assessment of clinical benefit in subjects with SCLC. PFS will also be assessed. RECIST 1.1 will be used to determine the dates of progression as this methodology is accepted by regulatory authorities.

Immune-related RECIST

RECIST 1.1 will be used as a basis for all protocol guidelines related to disease status (e.g., tumor response, disease progression, discontinuation of study treatment). Although RECIST 1.1 references a maximum of 5 target lesions in total and 2 per organ, this protocol allows a maximum of 10 target lesions in total and 5 per organ, if clinically relevant to enable a broader sampling of tumor burden.

iRECIST is based on RECIST 1.1 but adapted to account for the unique tumor response characteristics seen with treatment of pembrolizumab. Immunotherapeutic agents such as pembrolizumab may produce antitumor effects by potentiating endogenous cancer-specific immune responses. The response patterns seen with such an approach may extend beyond the typical time course of responses seen with cytotoxic agents and can manifest a clinical response after an initial increase in tumor burden or even the appearance of new lesions. Standard RECIST may not provide an accurate response assessment of immunotherapeutic agents such as pembrolizumab. Therefore, RECIST 1.1 will be used with the following adaptations:

If radiologic imaging by central imaging vendor (if applicable) verifies initial PD, tumor assessment should be repeated ≥4 weeks later in order to confirm PD with the option of continuing treatment per below while awaiting radiologic confirmation of progression.

If repeat imaging shows < 20% tumor burden compared to nadir, stable or improved previous new lesion (if identified as cause for initial PD), and stable/improved non-target disease (if identified as cause for initial PD), treatment may be continued / resumed.

If repeat imaging confirms PD due to any of the scenarios list below, subjects will be discontinued from study therapy.

In determining whether or not the tumor burden has increased or decreased, site study team should consider all target lesions as well as non-target lesions (please refer to the Procedure Manual/TIP Sheet).

Scenarios where PD is confirmed at repeat imaging:

- Tumor burden remains ≥ 20% and at least 5 mm absolute increase compared to nadir
- Non-target disease resulting in initial PD is worse (qualitative)
- New lesion resulting in initial PD is worse (qualitative)
- Additional new lesion(s) since last evaluation

In subjects who have initial evidence of radiological PD, it is at the discretion of the treating physician whether to continue a subject on study treatment until repeat imaging is obtained. This clinical judgment decision should be based on the subject's overall clinical condition, including performance status, clinical symptoms, and laboratory data. Subjects may receive pembrolizumab and pegzilarginase treatment while waiting for confirmation of PD if they are clinically stable as defined by the following criteria:

- Absence of signs and symptoms indicating disease progression
- No decline in ECOG performance status
- Absence of rapid progression of disease
- Absence of progressive tumor at critical anatomical sites (e.g., cord compression) requiring urgent alternative medical intervention
- No requirements for intensified management, including increased analgesia, radiation, or other palliative care

When feasible, subjects should not be discontinued until progression is confirmed. This allowance to continue treatment despite initial radiologic progression takes into account the observation that some subjects can have a transient tumor flare in the first few months after the start of immunotherapy, but with subsequent disease response. Subjects that are deemed clinically unstable are not required to have repeat imaging for confirmation of progressive disease.

iRECIST Assessment of Disease

As noted above, if tumor imaging shows initial disease progression, the study site may elect to continue treatment, repeat imaging ≥ 4 weeks later and assess tumor response or confirmed progression per iRECIST (see Appendix 3).

iRECIST is RECIST 1.1 adapted as described below to account for the unique tumor response seen with immunotherapeutic drugs. iRECIST will be used by site investigator/local radiology review to assess tumor response and progression and make treatment decisions. This data will be collected in the clinical database. Treatment efficacy based on iRECIST as assessed by central imaging vendor review will be evaluated retrospectively, if needed.

Table 7. Imaging and Treatment after First Radiologic Evidence of PD

	Clinically Stable		Clinically Unstable	
	Imaging	**Treatment**	**Imaging**	**Treatment**
1st radiologic evidence of PD by RECIST 1.1	Repeat imaging at 4 to 8 weeks at site to confirm PD	May continue study treatment at the local site Investigator's discretion while awaiting confirmatory tumor imaging by site by iRECIST.	Repeat imaging at 4 to 8 weeks to confirm PD per physician discretion only	Discontinue treatment
Repeat tumor imaging confirms PD (iCPD), by iRECIST at the local site	No additional imaging required	Discontinue treatment (exception is possible upon consultation with sponsor)	No additional imaging required	N/A
Repeat tumor imaging shows iUPD by iRECIST at the local site	Repeat imaging at 4 to 8 weeks to confirm PD. May occur at next regularly scheduled imaging visit	Continue study treatment at the Investigator's discretion	Repeat imaging at 4 to 8 weeks to confirm PD per Investigator's discretion only	Discontinue treatment
Repeat tumor imaging shows iSD, iPR or iCR by iRECIST by the local site	Continue regularly scheduled imaging assessments	Continue study treatment at the Investigator's discretion	Continue regularly scheduled imaging assessments	May restart study treatment if condition has improved and/or clinically stable per Investigator's discretion. Next tumor image should occur according to the regular imaging schedule

iCPD = iRECIST confirmed progressive disease; iCR = iRECIST complete response; iRECIST = modified Response Evaluation Criteria in Solid Tumors 1.1 for immune-based therapeutics; iSD = iRECIST stable disease; iUPD = iRECIST unconfirmed progressive disease; PD = progressive disease; RECIST 1.1 = Response Evaluation Criteria in Solid Tumors 1.1.

- In determining whether or not the tumor burden has increased or decreased, local study site investigators should consider all target lesions as well as non-target lesions (please refer to iRECIST TIP Sheet). Subjects that are deemed clinically unstable are not required to have repeat tumor imaging for confirmation.

- For a **clinically stable** subject with first radiologic evidence of progressive disease by RECIST 1.1 (i.e., **unconfirmed progression of disease**), it is at the discretion of the site investigator to continue treating the subject with the assigned treatment per protocol until progression of disease is confirmed at least 28 days from the date of the scan first suggesting PD. If radiologic progression is confirmed by subsequent scan, then the subject will be discontinued from trial treatment. If radiologic progression is not confirmed by iRECIST per the site, then the subjects may continue on treatment and follow the regular imaging schedule intervals until progression is confirmed at a later timepoint by the site.
    - **NOTE**: If a subject has confirmed radiographic progression (i.e. 2 scans at least 4 weeks apart demonstrating progressive disease) per iRECIST, but the subject is achieving a clinically meaningful benefit, and there is no further increase in the tumor burden at the confirmatory tumor imaging, an exception to continue treatment may be considered following consultation with the Sponsor. In this case, if treatment is continued, tumor imaging should continue to be performed following the intervals as outlined in sections 6.0 Study Flowchart.
- Any subject deemed **clinically unstable** should be discontinued from trial treatment at 1st radiologic evidence of PD and is not required to have repeat imaging for PD confirmation.
- In subjects who discontinue study therapy without documented disease progression, every effort should be made to continue monitoring their disease status by tumor imaging using the same imaging schedule used while on treatment (see Table 1), until (1) the start of new anti-cancer treatment, (2) disease progression (3) death, or (4) the end of the study, whichever occurs first.

iRECIST data will be collected in the clinical database.

8.10.3 Pharmacodynamic Outcome Measures

The primary pharmacodynamic outcome measure is plasma arginine level. Ornithine and other amino acids may be explored as part of secondary outcomes.

8.10.4 Planned Genetic Analysis

Understanding genetic determinants of drug response is an important endeavor during medical research. This research will evaluate whether genetic variation within a clinical trial population correlates with response to the treatment(s) under evaluation. If genetic variation is found to predict efficacy or AEs, the data might inform optimal use of therapies in the patient population. This research contributes to understanding genetic determinants of efficacy and safety associated with the treatments in this study.

Samples will be collected to assess the relationship between high-content immune signatures and response to study therapy.

8.10.5 Exploratory Outcome Measures

Exploratory outcome measures include the relationship ASS1 expression and response to therapy. Other markers relevant to the arginine pathway, such as ASL and OTC enzymes, may also be explored. These will be measured in tumor tissue (both fresh when available and archived).

8.11 Diet/Activity/Other Considerations

8.11.1 Diet

Subjects should maintain a normal diet unless modifications are required to manage an AE such as diarrhea, nausea or vomiting.

8.11.2 Contraception

Pembrolizumab or pegzilarginase may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab or pegzilarginase has transient adverse effects on the composition of sperm.

Subjects should be informed that taking the study medications may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. In order to participate in the study subjects of childbearing potential must adhere to the contraception requirement (described above) from the day of study medication initiation (or 14 days prior to the initiation of study medication for oral contraception) throughout the study period up to 120 days after the last dose of trial therapy. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

8.11.3 Use in Pregnancy

If a subject inadvertently becomes pregnant while on treatment with pembrolizumab or pegzilarginase, the subject will immediately be removed from the study. The site will contact the subject at least monthly and document the subject's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to the Sponsor

without delay and within 24 hours if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn). The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or newborn to the Sponsor.

8.11.4 Use in Nursing Women

It is unknown whether pembrolizumab or pegzilarginase is excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, subjects who are breast-feeding are not eligible for enrollment.

8.12 Subject Withdrawal/Discontinuation Criteria

8.12.1 Discontinuation of Study Therapy

Discontinuation of study treatment does not represent withdrawal from the study. During this time, patients may continue until disease progression, unacceptable toxicity, withdrawal of consent, physician's decision to stop therapy for the patient, or sponsor's decision to terminate the study.

A participant must be discontinued from study treatment but continue to be monitored in the study for any of the following reasons:

- Recurrent Grade 2 pneumonitis
- Completion of 35 treatments (approximately 2 years) of pembrolizumab
- Discontinuation of treatment may be considered for subjects who have attained a confirmed CR that have been treated for at least 8 cycles (24 weeks), receiving at least 2 cycles including 2 doses of pembrolizumab and at least 4 out of 6 of the planned doses of pegzilarginase beyond the date when the initial CR was declared.

8.13 Termination of Study by the Sponsor

The Sponsor may terminate the trial at any time after informing the participating sites/PIs through adequate channels.

9. ADVERSE EVENTS

An AE is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal IP, whether or not considered related to the IP. As patients in this study receive both pegzilarginase and pembrolizumab, "relatedness to IP" means that an AE is deemed related to either pegzilarginase, pembrolizumab, or both.

An AE may include intercurrent illnesses or injuries that represent an exacerbation (increase in frequency, severity, or specificity) of pre-existing conditions (eg, worsening of asthma). Whenever possible, it is preferable to record a diagnosis as the AE term rather than a series of terms relating to a diagnosis. Progression of the underlying SCLC will not be considered an AE.

The reporting period for AEs is the period from the first dose of study drug continuing through the last study follow-up visit or 30 days following last dose of study drug, whichever is the latest. Events occurring after signing the ICF and before the use of IP will be recorded as medical history. If a nonserious AE remains unresolved, it will be followed until the last planned follow-up visit, or the patient receives a new therapy directed against SCLC, including palliative care, whichever occurs first. In other scenarios the PI and Medical Monitor or designee will make a joint clinical assessment as to whether continued follow up of the AE is warranted; the results of this assessment must be documented. Resolution is defined as the return to baseline status or stabilization of the condition, with the expectation that it will remain chronic.

The Investigator record their assessments of AEs in both the source documents and eCRF for:

- severity, using NCI CTCAE v. 4.03. (http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf),
- relationship to IP (see Table), and
- seriousness, i.e., whether the AE meets the definition of SAE in Section 9.1.

Table 8. Adverse Event Relationship to Study Drug

Relationship	Description
Not Related	No relationship between the experience and the administration of study drug; related to other etiologies such as concomitant medications or patient's clinical state. Scenarios: Exposure to the IP has not occurred. OR The administration of the IP and the occurrence of the AE are not reasonably related in time. OR The AE is considered likely to be related to an etiology other than the use of the IP; that is, there are no facts [evidence] or arguments to suggest a causal relationship to the IP.
Unlikely Related	The current state of knowledge indicates that a relationship is unlikely. Timing is poor and there are other causes more likely to explain it. Scenarios The administration of the IP and the occurrence of the AE are related in time but the relationship is improbable based on the current state of knowledge AND The AE could be explained equally well by factors or causes other than exposure to the IP.
Possibly Related	A reaction that follows a plausible temporal sequence from administration of the study drug and follows a known response pattern to the suspected study drug. The reaction might have been produced by the patient's clinical state or other modes of therapy administered to the patient, but this is not known for sure. Therefore, there is good timing, but other causes are possible. Scenarios: The administration of the IP and the occurrence of the AE are reasonably related in time. AND The AE could not be explained equally well by factors or causes other than exposure to the IP.
Probably Related	A reaction that follows a plausible temporal sequence from administration of the study drug and follows a known response pattern to the suspected study drug. The reaction cannot be reasonably explained by the known characteristics of the patient's clinical state or other modes of therapy administered to the patient. There is good timing and other causes are unlikely. The AE follows a clinically reasonable response on withdrawal. Scenarios:

	The administration of IP and the occurrence of the AE are reasonably related in time. AND The AE is more likely explained by exposure to the IP than by other factors or causes.
Definitely Related	A reaction that follows a plausible temporal sequence from administration of the study drug and follows a known response pattern to the suspected study drug and can be confirmed with a positive re-challenge test or supporting laboratory data.

AE = adverse event; IP = investigational products.

In order to classify AEs, preferred terms will be assigned by the Sponsor or Contract Research Organization (CRO) to the original terms entered on the eCRF, using the Medical Dictionary for Regulatory Activities (MedDRA) terminology. The MedDRA version used for classification will be indicated in the clinical study report.

9.1 Serious Adverse Events

An SAE is defined as any AE that:

- Results in death
- Is life threatening, that is, places the subject at immediate risk of death from the event as it occurred. This definition does not include a reaction that, had it occurred in a more severe form, might have caused death.
- Requires in-patient hospitalization or prolongation of an existing in-patient hospitalization.
- Results in persistent or significant disability or incapacity. An event qualifies as resulting in a persistent or significant disability or incapacity if it involves a substantial disruption of the subject's ability to carry out usual life functions. This definition is not intended to include experiences of relatively minor or temporary medical significance.
- Is a congenital anomaly or birth defect, ie, an AE that occurs in the child or fetus of a subject exposed to IP, prior to conception or during pregnancy.
- Is an important medical event or serious medical condition that does not meet any of the above criteria but may jeopardize the subject or require medical or surgical intervention to prevent 1 of the outcomes listed above. Examples of such events are intensive treatment in the emergency room, allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

More than one of the above criteria may apply to any specific event.

The reporting period for SAEs is the period from the time of the first dose of study drug(s) continuing through the end of treatment. Any SAEs of which the investigator becomes aware within 90 days following cessation of study treatment or within 30 days following cessation of study treatment if the participant initiates new anticancer therapy, whichever is earlier will be reported to the Sponsor . Events occurring after signing the ICF and before the use of IP will be recorded as medical history. SAEs reported to the Investigator outside of this reporting period will be reported to Aeglea and the Medical Monitor if, in good medical judgment, the event has any bearing on the study data. SAEs must be followed by the Investigator until resolution, stabilization or until the subject is lost to follow up. Resolution of an SAE is defined as the return to baseline status or stabilization of the condition, with the expectation that it will remain chronic. SAE follow-up will stop if the patient initiates alternative anti-cancer therapy.

Any SAE, whether or not considered related to study drug(s), will be reported immediately (within 24 hours) to the Sponsor or its Safety designee by fax or scanned and emailed using the study-specific SAE Report Form. In addition to the outcome of the SAE, any medication or other therapeutic measures used to treat the event will be recorded on the appropriate eCRF page(s). Investigators should not wait to collect additional information that fully documents the event before notifying the Sponsor or its Safety designee of an SAE. The Sponsor may be required to report certain SAEs to regulatory authorities within 7 calendar days of being notified about the event; therefore, it is important that Investigators submit additional information requested by the Sponsor, the Medical Monitor, and/or the Sponsor or its Safety designee as soon as it becomes available.

Reporting of SAEs to the IRB/EC will be done in compliance with the standard operating procedures and policies of the IRB/EC and with applicable regulatory requirements. Adequate documentation must be obtained by the Sponsor showing that the IRB/EC was properly and promptly notified as required. Contact information for the Sponsor safety representative will be provided in a separate document.

A completed SAE Report form signed by the Investigator must be transmitted to the Sponsor’s designated safety representative.

The Investigator is encouraged to discuss with the Medical Monitor any AEs for which the issue of seriousness is unclear or questioned. Contact information for the Medical Monitor will be provided in a separate document.

9.2 Dose Modification Guidelines for Adverse Events

The dose modification guidelines for AEs are presented in Section 1)

9.3 Assessing and Recording Adverse Events and Events of Clinical Interest

Progression of the cancer under study is not considered an AE.

All medical events that occur after the consent form is signed but before treatment allocation must be reported by the investigator if they cause the subject to be excluded from the trial or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, or a procedure.

From the time of first dose through 30 days following cessation of treatment, all AEs must be reported by the investigator. Such events will be recorded at each examination on the Adverse Event case report forms/worksheets. The reporting timeframe for AE meeting any serious criteria is described in section Immediate Reporting of Adverse Events to the Sponsor. The investigator will make every attempt to follow all subjects with non-serious AEs for outcome.

9.3.1 Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor

For this trial, an overdose for pembrolizumab will be defined as ≥1000 mg (5 times the dose) of pembrolizumab. No specific information is available on the treatment of overdose of pembrolizumab. In the event of overdose, the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

An overdose for pegzilarginase will be defined as ≥ 1.25 times the intended dose of pegzilarginase. No specific information is available on the treatment of overdose of pegzilarginase. In the event of overdose, the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

9.3.2 Reporting of Pregnancy and Lactation to the Sponsor

Although pregnancy and lactation are not considered AEs, it is the responsibility of investigators or their designees to report any pregnancy or lactation in a subject (spontaneously reported to them) that occurs during the trial.

Pregnancies and lactations that occur after the consent form is signed but before first dose must be reported by the investigator if they cause the subject to be excluded from the trial, or are the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, or a procedure.

Pregnancies and lactations that occur from the time of first dose through 120 days following cessation of Sponsor's product, or 30 days following cessation of treatment if the subject initiates new anticancer therapy, whichever is earlier, must be reported by the investigator. All reported pregnancies must be followed to the completion/termination of the pregnancy. Pregnancy outcomes of spontaneous abortion, missed abortion, benign hydatidiform mole, blighted ovum, fetal death, intrauterine death, miscarriage and stillbirth must be reported as serious events (Important Medical Events). If the pregnancy continues to term, the outcome (health of infant) must also be reported.

Such events must be reported within 24 hours to the Sponsor either by electronic media or paper. Electronic reporting procedures can be found in the EDC data entry guidelines. Paper reporting procedures can be found in the Investigator Trial File Binder (or equivalent).

9.3.3 Immediate Reporting of Adverse Events and Events of Clinical Interest to the Sponsor

9.3.3.1 Serious Adverse Events

In addition to the SAE criteria defined in Section 9.1, AEs meeting either of the below criteria, although not serious per ICH definition, are reportable to the Sponsor in the same timeframe as SAEs to meet certain local requirements.

Therefore, these events are considered serious by the Sponsor for collection purposes:

- Is a new cancer (that is not a condition of the study);
- Is associated with an overdose.

For the time period beginning when the consent form is signed until first dose, any SAE, or follow up to a SAE, including death due to any cause other than progression of the cancer under study, that occurs to any subject must be reported within 24 hours to the Sponsor if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of 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, whether or not related to the Sponsor's product, must be reported within 24 hours to the Sponsor either by electronic media or paper. Electronic reporting procedures can be found in the EDC data entry guidelines. Paper reporting procedures can be found in the Investigator Trial File Binder (or equivalent)

Additionally, any SAE, considered by an investigator who is a qualified physician to be related to the Sponsor's product that is brought to the attention of the investigator at any time following consent through the end of the specified safety follow-up period specified in the paragraph above, or at any time outside of the time period specified in the previous paragraph also must be reported immediately to the Sponsor.

All subjects with SAEs must be followed up for outcome.

9.3.3.2 Events of Clinical Interest

Selected non-serious and serious AEs are also known as Events of Clinical Interest (ECI) and must be reported to the Sponsor.

For the time period beginning when the consent form is signed until first dose, any ECI, or follow up to an ECI, that occurs to any subject must be reported within 24 hours to the Sponsor if it causes the subject to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, or a procedure.

For the time period beginning at first dose through 30 days following cessation of treatment, any ECI, or follow up to an ECI, whether or not related to the Sponsor's product, must be reported within 24 hours to the Sponsor, either by electronic media or paper. Electronic reporting procedures can be found in the EDC data entry guidelines. Paper reporting procedures can be found in the Investigator Trial File Binder (or equivalent).

Events of clinical interest for this trial include:

1) an overdose of Sponsor's product, as defined in Section 2.7.1 - Definition of an Overdose for This Protocol and Reporting of Overdose to the Sponsor, that is not associated with clinical symptoms or abnormal laboratory results.

2) an elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

   *Note that these criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology. The trial site guidance for assessment and follow up of these criteria can be found in the Investigator Trial File Binder (or equivalent). It may also be appropriate to conduct additional evaluation for an underlying etiology in the setting of abnormalities of liver blood tests including AST, ALT, bilirubin, and alkaline phosphatase that do not meet the criteria noted above. In these cases, the decision to proceed with additional evaluation will be made through consultation between the study investigators and the sponsor Medical Monitor. However, abnormalities of liver blood tests that do not meet the criteria noted above are not ECIs for this trial.

10. APPROPRIATENESS OF MEASUREMENTS

10.1 Safety

The safety measures included in this study are intended to assure the detection of both known and unknown risks related to pegzilarginase and pembrolizumab administration.

10.2 Efficacy

RECIST and iRECIST are standard efficacy measures in oncology clinical studies of solid tumors.

10.3 Pharmacodynamics/Efficacy

The primary pharmacodynamic measure for this study is plasma arginine level.

10.4 Pharmacokinetics

Pegzilarginase (Co-ArgI-PEG) concentration data will be analyzed by noncompartmental analysis (NCA) with Phoenix™ WinNonlin® Version 6.3 or higher, using an intravascular administration model and actual sampling times (relative to actual dose times). The following PK parameters will be evaluated:

T_{max}: time of the maximum observed concentration;

C_{max}: maximum observed concentration;

AUC_{0-t}: area under the curve through the time of the last measurable concentration;

$AUC_{0-\infty}$: area under the curve extrapolated to infinity;

$T_{1/2}$: apparent terminal elimination half-life;

CL: serum clearance of drug per unit time following IV dosing;

V_{ss}: volume of distribution following IV dosing.

11. DEFINITION OF STUDY PROCEDURES

Study procedures should be performed according to the Schedule of Assessments

11.1 Informed Consent

Written informed consent must be obtained from the patient before any study-related procedures are performed and whenever the study procedures change or new safety information becomes available, that may affect the patient's willingness to participate.

11.2 Demographics, Baseline Disease Characteristics, and Medical History

Demographic data will include gender, age, race, and ethnicity, and will be collected during the Screening period. Medical and surgical history will be obtained and will include documentation of the date/year of malignancy diagnosis, as well as documentation of all currently active and relevant medical and surgical conditions. Baseline disease characteristics will be collected in order to confirm eligibility as well as perform exploratory subset analyses.

11.3 Physical Examination

The physical examination will include evaluation of the head, eyes, ears, nose, throat, cardiovascular, dermatological, musculoskeletal, respiratory, gastrointestinal, and neurological systems. ECOG performance status will be assessed at the time of each physical exam. Physical exams should be performed according to the Schedule of Assessments. Before administration of the first dose of study drug, any new or worsened abnormalities (other than those due to protocol-required procedures) should be recorded as medical history. No rectal or pelvic examinations are required.

11.4 Vital Signs

Vital signs (including blood pressure, pulse, respirations, and temperature) will be assessed at each study visit, prior to and following each study drug infusion. Weight should be assessed at least once per cycle.

11.5 Electrocardiogram

A 12-lead ECG will be obtained after at least 5 minutes of rest in the supine or semi-recumbent position using equipment at the site at the visits noted on the Schedule of Assessments

The same position should be used for all assessments. The investigator or designee will evaluate the ECG for abnormalities.

11.6 Laboratory Tests

Clinically significant abnormal findings identified during the Screening period should be recorded on the medical history eCRF as a diagnosis.

Laboratory samples will be obtained according to the Schedule of Assessments. The following tests will be obtained for this study:

- Hematology: RBC; WBC, hemoglobin, hematocrit, WBC differential count (neutrophils, lymphocytes, eosinophils and basophils), platelet count, reticulocyte count, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin, mean corpuscular volume, mean platelet volume, and red blood cell distribution width (RDW).
- Serum Chemistry: includes sodium (Na+), potassium (K+), chloride (Cl-), bicarbonate (HCO3-), magnesium (Mg2+), calcium (Ca2+), phosphate (PO43-), blood urea nitrogen (BUN), creatinine, glucose, lactate dehydrogenase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, total bilirubin, total protein, and albumin.
- Urinalysis (UA) includes appearance, glucose, ketones, blood, protein, nitrate, bilirubin, specific gravity, pH, urobilinogen, and leukocytes. If positive for blood or protein trace, then include microscopy.
- Coagulation: PT / international normalized ratio (INR) and activated partial thromboplastin time (aPTT) tests will be performed.
- Thyroid function testing: includes triiodothyronine (T3) or free triiodothyronine (FT3), free thyroxine (FT4), and thyroid stimulating hormone (TSH).
- Serum pregnancy tests will be performed for all female patients of childbearing potential at the Screening visit and Week 1, Day 1 visit. Urine or serum pregnancy tests (performed at the site) will be obtained as noted in the Schedule of Assessments Patients who have a confirmed positive pregnancy test at any time during the study will be discontinued from further study drug administration and will be followed for pregnancy outcome.

Clinically significant abnormal laboratory test results should be followed until a return to normal or baseline values. Details for the collection, processing, storage, and shipment of samples can be found in the study laboratory manual.

11.7 Pharmacokinetic Samples

Blood samples for PK analysis will be drawn as described in the Schedule of Assessments Time of PK sampling and date as well as start and stop times of each IV infusion will be

recorded. Samples will be shipped to and stored at the bioanalytical laboratory at ≤ -70°C during the course of the study.

11.8 Anti-Drug Antibody Testing

Blood samples for anti-drug antibody (ADA) testing will be drawn as described in the Schedule of Assessments (Table 1). Samples will be shipped to the bioanalytical laboratory at ≤ -70°C during the course of the study.

If the blood sample drawn during the follow up visit indicates that the patient has developed antibodies to the study drug, then whenever possible, the site will collect blood samples for antibody determination every 4-6 weeks until the antibody is no longer present.

11.9 Pharmacodynamic Samples

Blood samples for amino acid analysis including plasma arginine and ornithine, will be drawn at time points designated in the Schedule of Assessments. The time of PD sampling and date and time of each pegzilarginase dose will be recorded. Samples will be shipped to the central laboratory during the course of the study.

11.10 Tumor/Response Assessment

Confirmation of measurable disease based on RECIST 1.1 at screening will be used to determine participant eligibility. Initial tumor imaging at screening must be performed within 21 days prior to the date of first dose. The site study team must review screening images to confirm the participant has measurable disease per RECIST 1.1.

Tumor imaging should be performed by computed tomography (CT). MRI or magnetic resonance imaging should only be used when CT is contraindicated or for imaging in the brain, but the same imaging technique regarding modality, ideally the same scanner, and the use of contrast, should be used in a subject throughout the trial. CT scan is the more commonly used modality and is preferred for the majority of patients. An MRI can be utilized if clinically appropriate. Caution is urged when using or interpreting FDG-PET scans in the context of treatment with pegzilarginase due to the effects that arginine deprivation has on glucose metabolism (Stelter et. al. 2012) Tumor response assessment is made by RECIST 1.1 and iRECIST (Appendix 2).

Per RECIST 1.1, partial or complete response should be confirmed by a repeat tumor imaging assessment not less than 4 weeks from the date the response was first documented. \ Participants will then return to regular scheduled imaging, starting with the next scheduled imaging time point. Participants who receive additional imaging for confirmation do not need to undergo the next scheduled tumor imaging if it is less than 4 weeks later; tumor imaging may resume at the subsequent scheduled imaging time point.

Continue to perform imaging until whichever of the following occurs first:

- Initial site-assessed disease progression is verified by the central imaging vendor (if applicable)
- The start of new anti-cancer treatment
- Withdrawal of consent
- Death
- The end of the study

Per iRECIST (Table 8), disease progression should be confirmed by the site 4 to 8 weeks after site-assessed first radiologic evidence of PD in clinically stable participants. Participants, who have unconfirmed disease progression may continue on treatment at the discretion of the investigator until progression is confirmed by the site, provided they have met the conditions detailed in Table 8. Participants who receive confirmatory imaging do not need to undergo the next scheduled tumor imaging if it is less than 4 weeks later; tumor imaging may resume at the subsequent scheduled imaging time point, if clinically stable. Participants, who have confirmed disease progression by iRECIST, as assessed by the site, will discontinue study treatment. Exceptions are detailed in Table 8. Please refer to Appendix 4 for a description of the iRECIST process for assessment of disease progression.

For participants who discontinue study treatment, tumor imaging should be performed at the time of treatment discontinuation (±4 weeks window). If previous imaging was obtained within 4 weeks prior to the date of discontinuation, then imaging at treatment discontinuation is not mandatory. For participants who discontinue study treatment due to documented disease progression, this is the final required tumor imaging if the investigator elects not to implement iRECIST.

For participants who discontinue study treatment without documented disease progression, every effort should be made to continue monitoring disease status by tumor imaging using the same imaging schedule used while on treatment until the start of a new anticancer treatment, disease progression, pregnancy, death, withdrawal of consent, or the end of the study, whichever occurs first.

11.11 Tissue Samples

Patients with accessible tumor tissue will be asked to consent to a biopsy to obtain a fresh tumor tissue sample for analysis and/or storage. Tumor biopsies will be performed following confirmation of eligibility for the study and prior to first study drug treatment; a second optional tumor biopsy will be obtained before start of the fourth cycle. Tumor tissue samples will be assayed for the expression of PD-L1, and ASS1. ASL, OTC; and other potential biomarkers related to the arginine pathway. If possible, infiltrating immune cells will be characterized and high content gene signatures will be explored to assess for determinants of response. Archival tumor tissue samples will be requested if a fresh biopsy specimen cannot be obtained. Blood samples will be collected and analyzed to determine the immune profiling of circulating immune cells during the trials as well as changes in cytokines under treatment. Similarly, blood samples may be utilized to determine germline genetic variations. Both tumor tissue and bloods samples, or its components, will be banked for future analysis.

11.12 Follow Up

All patients, including patients who withdraw from the study prematurely, will return to the study site for a follow-up visit between 30 to 44 days following the last dose of study drug. Follow-up visit procedures are noted in the Schedule of Assessments.

If a patient fails to return for scheduled visits, a documented effort must be made to determine the reason. If the patient cannot be reached by telephone after two attempts, a certified letter should be sent to the patient (or the patient's legally authorized representative, if appropriate) requesting contact with the Investigator. This information should be recorded in the study records.

12. DATA QUALITY ASSURANCE

Sponsor personnel (and/or designees) will visit the study site prior to initiation of the study to review with the site personnel information about the IP, protocol, and other regulatory document requirements, any applicable randomization procedures, source document requirements, eCRFs, monitoring requirements, and procedures for reporting AEs, including SAEs.

At visits during and after the study, the Sponsor and/or designee will monitor the site for compliance with regulatory documentation, with a focus on accurate and complete recording of data on eCRFs from source documents, adherence to protocol, SAE reporting, and drug accountability records.

A Sponsor-designated clinical data management group will design and program a study database and corresponding eCRFs and provide training for sites and CRAs on data entry and cleaning procedures. Data quality control and analysis will be performed by the Sponsor (or designee), including study monitors and the clinical data management group based on a predefined data management plan (DMP) and statistical analysis plan (SAP).

13. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

13.1 Statistical Analysis Plan (SAP)

The SAP will provide a detailed description of the planned statistical analyses. The SAP will be finalized prior to database lock. Any changes to the methods described in the plan will be described and justified in the final clinical study report.

13.2 General Analysis Conventions

The following general analysis conventions will be used:

1) All data collected in the CRFs will be reported in at least one data listing.
2) Unless otherwise indicated, analyses of efficacy and safety data will be consistent with the Intention-To-Treat (ITT) principle, in that all treated patients will be included in the analysis.
3) All Type I error is spent in this study on the primary analysis of ORR according to the Simon 2 Stage Design. The phrases “statistically significant” or “nominally statistically significant” may be used to describe analyses, whether planned or unplanned, where the p-value is less than 0.05. In these cases, the phrase is descriptive and does not imply that the analysis was confirmatory in the statistical sense.
4) Unplanned exploratory and sensitivity analyses may be performed as deemed appropriate; such analyses will be indicated in the clinical study report. Changes from the planned analyses, if any, will also be indicated.
5) “Baseline” will be defined as the last measurement of a particular endpoint prior to first study treatment, unless otherwise indicated.
6) Summaries and analyses will be provided by dose of pegzilarginase for the dose escalation portion of the study.
7) Summary statistics for continuous endpoints will include the number of subjects with non-missing data, mean, median, minimum, and maximum values.
8) Summary displays for percentages will indicate the number of patients in the numerator and denominator, as well as the computed percentage. Unless otherwise indicated, patients with missing data will be considered as a separate category that will be included in the denominator.

9) In general, percentages will be reported to the nearest whole percent, since there are fewer than 100 patients planned for this study. Clinical data will be reported using precision with which the particular endpoint was measured. Summary statistics such as mean and standard deviation may be reported with more precision than the original measurements. P-values will be reported in the clinical study report with at least 3 decimal places, although the number of decimal places may vary depending on the statistical software used.

13.3 Analysis Sets

13.3.1 Consented Patient Set

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

13.3.2 Full Analysis Set (FAS)

The Full Analysis Set (FAS) will consist of all patients in the Consented Patient Set who receive at least one dose of either investigational product.

13.3.3 Phase 1 Set

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

13.3.4 DLT Evaluable Set

The DLT Evaluable Set will consist of all patients in the Phase 1 Set who either experience a DLT, or who do not experience a DLT and have sufficient follow-up and dosing to be declared DLT-free.

13.3.5 Phase 2 Set

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.

13.3.6 PK/PD Set

The PK/PD Set will consist of all patients in the FAS who have sufficient follow-up data to contribute to the analysis of PK or PD data.

13.4 Missing Data Handling

Although imputation of missing data is not planned, should an imputation method be used for a particular analysis, it will be indicated in the clinical study report.

13.5 Disposition of Subjects

A figure similar to a CONSORT diagram will be created to describe patient flow in the study for all patients in the Consented Patient Set.

The number and percentage of patients entering and completing the clinical study will be presented, along with primary reason for discontinuation for any patients who do not complete the study.

13.6 Demographic and Baseline Characteristics

Demographic information and baseline disease characteristics will be summarized according to the standard analysis conventions.

13.7 Deviation Reporting

Protocol deviations, whether collected on the eCRF or deviation logs at the clinical site, will be listed by subject.

13.8 Efficacy Analyses

Efficacy analyses will be performed on the Phase 2 Analysis Set. Additional descriptive analyses may be performed on the Full Analysis Set.

13.8.1 Primary Analysis

The primary analysis will be performed on the Phase 2 Analysis Set. The primary analysis of the primary endpoint, ORR as assessed by investigators, is the only statistical analysis where Type I error is spent. The formal analysis declares the study statistically significant at one-sided α=0.05 if at least 30 of 66 total patients in the Phase 2 Set achieve an overall response per RECIST 1.1. In this analysis, all treated patients are included and patients who, for any reason, do not achieve ORR are considered "failures". The formal statistical conclusion if the study is positive is that the ORR is statistically significantly greater than 35%.

If either stage is over-enrolled, the 55 (or 66 after stage 2) patients to be included in the analysis will be determined based on order of treatment - date and time. This distinction of the exact 55 (or 66) patients is only relevant for the decision to open Stage 2, and for the statistical inference regarding the primary analysis. Confidence intervals and summary statistics will be computed using all patients treated in the phase 2 part of the study, even

those over-enrolled. Confidence intervals will not be adjusted for the 2-stage design; they will be computed as if the design was a single stage.

13.8.2 Secondary Efficacy Analyses

The secondary efficacy endpoints are, as assessed by both RECIST and iRECIST

- Clinical benefit rate (CR + PR + SD lasting at least 8 weeks, or iCR+iPR+iSD lasting 8 weeks)
- Time to response
- Duration of response
- Progression-free survival

Overall survival will also be assessed.

These endpoints will be summarized for the Phase 2 Set according to the general analysis conventions. 95% confidence intervals will also be created.

13.8.3 Exploratory Efficacy Analyses

Exploratory efficacy analyses describe the impact of pembrolizumab with pegzilarginase on arginine plus additional acids (PD endpoints), as well as levels of circulating pegzilarginase (PK endpoint). These descriptive analyses will be performed using standard analytic techniques, which will be described in detail in a specific analytic plan for these variables. Immunogenicity response, if any, will be provided in listings, and may be modeled with safety data if at least 5 patients exhibit an immune response to either Co-Arg 1 or to PEG.

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. These relationships will be explored primarily through logistic regression or cox proportional hazards regression, depending on the outcome of interest. Further details will be provided in the statistical analysis plan.

13.9 Safety Analysis

Safety Analyses will be performed for the Full Analysis Set and will be performed by dose. AEs will be listed and summarized. The number of patients reporting SAEs, related SAEs,

treatment-emergent SAEs, and related treatment-emergent AEs will be summarized by body system, dose group, and preferred term. In addition, the number of subjects discontinuing from treatment will be tabulated.

Laboratory data will be listed and summarized across time; values outside the normal range will be flagged. Laboratory studies will include hematology, chemistry, urinalysis, and coagulation. Immunogenicity safety measures will include levels of ADA and anti-PEG antibodies.

Vital signs will be listed and summarized across time. Abnormal changes in ECGs and physical examinations will be listed.

13.10 Determination of Sample Size

13.10.1 Sample Size for the Phase 1 Portion of the Study

In the dose escalation part of the study, an mTPI design with a target DLT rate of approximately 30% will be applied for dose escalation and confirmation to determine a RP2D for pegzilarginase in combination with pembrolizumab.

Pre-determined dose levels of pegzilarginase (in combination with a fixed dose of pembrolizumab) will be explored independently. A de-escalation dose of 0.12 mg/kg pegzilarginase is available if the starting dose of 0.20 mg/kg is deemed not tolerable. All dose escalation and de-escalation decisions will be based on the occurrence of DLTs at a given dose during the first 21-day period (Cycle 1) and will be made jointly by the investigators and the sponsor.

The dose of pembrolizumab will remain constant at 200 mg Q3W for each dose level for pegzilarginase.

In Table , the number of patients treated is indicated in the columns and the number of patients who experienced a DLT is indicated in the rows. Dosing decisions include escalate to the next higher dose (E), stay at the current dose (S), de-escalate to the next lower dose (D), and de-escalate to a lower dose and never test this dose again (i.e., unacceptably toxic dose; DU).

During dose escalation, a minimum of 3 patients are required at each dose. Depending on accrual rate, 3, 4, 5 or 6 patients may be enrolled at each new dose until the last of those

patients completes the 21-day DLT assessment period. For example, the dose escalation rules will proceed as follows if 3 patients are enrolled: if 0 out of the first 3 patients at a given dose level develops a DLT, then the dose can be escalated to the next level without further expansion. If 1 out of the first 3 patients at a given dose level develops a DLT, no more than an additional 3 patients should be enrolled at this dose level until additional DLT data are available since this dose would be considered unacceptably toxic if all 3 of the additional patients experience a DLT (i.e., 4 out of 6 patients). If 2 out of the first 3 patients at a given dose level develop a DLT, the dose will be de-escalated to the next lower level. If 3 out of the first 3 patients at a given dose level develop a DLT, this dose will be considered unacceptably toxic (i.e., the dose will be de-escalated and never re-escalated to that dose again). The same principle will be applied whether 3, 4, 5 or 6 patients are enrolled in the same dose cohort according to Table .

Based on the mTPI design, the number of patients who are enrolled at a dose, but are not yet fully evaluable for DLT assessment, may not exceed the number of remaining patients who are at risk of developing a DLT before the dose would be considered unacceptably toxic (denoted as DU in Table). To determine how many more patients can be enrolled at a dose level, one can count steps in a diagonal direction (down and to the right) from the current cell to the first cell marked DU. In total, 3 to 14 patients may be enrolled at a given dose level.

For example, if 1/3 patients have experienced a DLT at a given dose level, no more than an additional 3 patients should be enrolled at this dose level until additional DLT data are available. This is because this dose level would be considered unacceptably toxic if all 3 of the additional patients experience a DLT (i.e., 4/6 patients with DLTs as in Table).

Dose escalation and confirmation will end after a minimum of 6 patients and a maximum of 14 patients have been treated at any of the selected doses. The totality of the data will be considered before a dose is selected (RP2D) to carry forward to Phase 2 and the escalation schedule may be adjusted based on PK, PD, and safety data emerging throughout the study to determine the RP2D.

Note that while 30% was the target toxicity rate used to generate the guidelines in Table the observed rates of patients with DLTs at the MTD may be slightly above or below 30%.

Table 9. Dose Escalation and Confirmation Rules Based on the Modified Toxicity Probability Interval Design

	Number of DLT-Evaluable Patients Treated at Current Dose											
Number of toxicities	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
0	E	E	E	E	E	E	E	E	E	E	E	E
1	S	S	S	E	E	E	E	E	E	E	E	E
2	D	S	S	S	S	S	S	S	E	E	E	E
3	DU	DU	D	S	S	S	S	S	S	S	S	S
4		DU	DU	DU	D	D	S	S	S	S	S	S
5			DU	DU	DU	DU	DU	D	S	S	S	S
6				DU	DU	DU	DU	DU	DU	D	S	S
7					DU	DU	DU	DU	DU	DU	DU	D
8						DU	DU	DU	DU	DU	DU	DU
9							DU	DU	DU	DU	DU	DU
10								DU	DU	DU	DU	DU
11									DU	DU	DU	DU
12										DU	DU	DU
13											DU	DU
14												DU

Target toxicity rate = 30%
Flat non-informative prior Beta (1,1) is used as a prior and ε1=ε2=0.05 (Ji and Wang, 2013).
To be DLT-Evaluable, a patient must either 1) experience a DLT during the DLT-window, or 2) have sufficient dosing and follow-up to be "at risk" for a DLT, but not experience one.
Abbreviations: D = de-escalate to the next lower dose, DU = the current dose is unacceptably toxic, E = escalate to the next higher dose, S = stay at the current dose

13.10.2 Sample Size for the Phase 2 Portion of the Study

In the phase 2 portion of the trial, fifty-five patients will be enrolled and treated in stage 1. If 23 or more of those subjects achieve a response (CR or PR), an additional 11 subjects will be enrolled. If a total of 30 or more subjects 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%.

If a potential patient has provided informed consent and is in the screening process when a specific portion of the trial is closed, that patient may continue on with treatment under the protocol. For this reason, the trial may be over-enrolled by a small number of patients.

13.11 Multiplicity Considerations

The only analysis where Type I error is spent is the primary analysis. There are no multiplicity adjustments for other analyses.

13.12 Changes in the Conduct of the Study or Planned Analyses

Only the Sponsor may modify the protocol. Any change in study conduct considered necessary by the PI will be made only after consultation with the Sponsor, who will then issue a formal protocol amendment to implement the change. The only exception is when an Investigator considers that a subject's safety is compromised without immediate action. In these circumstances, immediate approval of the Chairman of the IRB/EC must be sought, and the Investigator should inform the Sponsor and the full IRB/EC within 2 working days after the emergency occurs. The Sponsor may terminate the trial at any time after informing the participating sites/PIs through adequate channels.

With the exception of minor administrative or typographical changes, the IRB/EC must review and approve all protocol amendments. Protocol amendments that have an impact on subject risk or the study objectives, or require revision of the ICF, must receive approval from the IRB/EC prior to their implementation.

When a protocol amendment substantially alters the study design or the potential risks or burden to subjects, the ICF will be amended and approved by the Sponsor and the IRB/EC, and all active subjects must again provide informed consent.

Note that if discrepancies exist between minor features of the statistical analysis as planned in the protocol, and the final SAP, a protocol amendment will not be issued, and the SAP will prevail.

14. DATA COLLECTION AND MANAGEMENT

This study will be using Electronic Data Capture (EDC) system and the designated investigator staff will enter the data required by the protocol into the Electronic Case Report Forms (eCRF). The eCRFs have been built using fully validated, secure, web-enabled software that conforms to 21 CFR Part 11 requirements. Investigator site staff will not be given access to the EDC system until they have been trained. Automatic validation programs check for data discrepancies in the eCRFs and allow modification or verification of the entered data by the investigator staff. The Principal Investigator is responsible for assuring that the data entered into eCRF is complete and accurate, and that entry and updates are performed in a timely manner.

Sponsor personnel (or designated CRO) will review the data entered by investigational staff for completeness and accuracy. Electronic data queries stating the nature of the problem and requesting clarification will be created for discrepancies and missing values and sent to the investigational site via the EDC system. Designated investigator site staff are required to respond promptly to queries and to make any necessary changes to the data. Concomitant treatments and prior medications entered into the database will be coded using the WHO Drug Reference List, which employs the Anatomical Therapeutic Chemical classification system. AEs will be coded using the Medical dictionary for regulatory activities (MedDRA) terminology.

After database lock, the investigator will receive electronic or paper copies of the patient data for archiving at the investigational site.

15. STUDY MONITORING AND AUDITING

Before study initiation, at a site initiation visit or at an investigator's meeting, Sponsor personnel (or designated CRO) will review the protocol and eCRFs with the investigators and their staff. During the study, a CRA designated by the Sponsor will visit the site regularly to check the completeness of patient records, the accuracy of entries on the eCRFs, the adherence to the protocol and to Good Clinical Practice, the progress of enrollment, and to ensure that study medication is being stored, dispensed, and accounted for according to specifications. Key study personnel must be available to assist the field monitor during these visits.

The investigator must maintain source documents for each patient in the study, consisting of case and visit notes (hospital or clinic medical records) containing demographic and medical information, laboratory data, electrocardiograms, and the results of any other tests or assessments. All information recorded on eCRFs must be traceable to source documents in the patient's file. The investigator must also keep the original signed informed consent form (a signed copy is given to the patient).

The investigator must give the CRA access to all relevant source documents to confirm their consistency with the eCRF entries. Sponsor monitoring standards require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria and documentation of SAEs. Additional checks of the consistency of the source data with the eCRFs are performed according to the study-specific monitoring plan.

Sponsor personnel or designees may conduct an audit of a clinical site at any time during or after completion of the study. The PI will be informed if an audit is to take place and advised as to the scope of the audit. Representatives of the FDA or other Regulatory Agencies may also conduct an audit of the study. If informed of such an inspection, the PI should notify the Sponsor immediately. The PI will ensure that the auditors have access to the clinical supplies, study site facilities, original source documentation, and all study files. All inspections and audits will be carried out giving consideration to data protection as well as subject confidentiality to the extent that local, state, and federal laws apply.

16. PRIVACY AND CONFIDENTIALITY

All US-based sites and laboratories or entities providing support for this study must, where applicable, comply with Health Insurance Portability and Accountability Act (HIPAA) of 1996. A site that is not a covered entity as defined by HIPAA must provide documentation of this fact to the Sponsor/CRO.

The confidentiality of records that may be able to identify subjects will be protected in accordance with applicable laws, regulations, and guidelines.

After subjects have consented to take part in the study, the Sponsor and/or its representatives review their medical records and data collected during the study. These records and data may, in addition, be reviewed by others including the following: independent auditors who validate the data on behalf of the Sponsor; third parties with whom the Sponsor may develop, register, or market pegzilarginase (Co-ArgI-PEG); national or local regulatory authorities; and the IRB(s)/EC(s) that gave approval for the study to proceed. The Sponsor and/or its representatives accessing the records and data will take all reasonable precautions in accordance with applicable laws, regulations, and guidelines to maintain the confidentiality of subjects' identities.

Subjects are assigned a unique identifying number; however, their initials and date of birth may also be collected and used to assist the Sponsor to verify the accuracy of the data (eg, to confirm that laboratory results have been assigned to the correct subject).

The results of studies containing subjects' unique identifying number, relevant medical records, and possibly initials and dates of birth will be recorded. They may be transferred to, and used in, other countries that may not afford the same level of protection that applies within the countries where this study is conducted. The purpose of any such transfer would include supporting regulatory submissions, conducting new data analyses to publish or present the study results, or answering questions asked by regulatory or health authorities.

17. RETENTION OF RECORDS

The PI must retain all study records required by the Sponsor and by the applicable regulations in a secure and safe facility. The PI must consult a Sponsor representative before disposal of any study records and must notify the Sponsor of any change in the location, disposition, or custody of the study files. The PI/institution must take measures to prevent accidental or premature destruction of essential documents (ie, documents that individually and collectively permit evaluation of the conduct of a study and the quality of the data produced), including paper copies of study records (eg, subject charts), as well as any original source documents that are electronic as required by applicable regulatory requirements.

All study records must be retained for at least 2 years after the last approval of a marketing application in the US or an ICH region and until (1) there are no pending or contemplated marketing applications in the US or an ICH region or (2) ≥2 years have elapsed since the formal discontinuation of clinical development of the IP. The PI/institution should retain subject identifiers for ≥15 years after the completion or discontinuation of the study. Subject files and other source data must be kept for the maximum period of time permitted by the hospital, institution, or private practice, but not less than 15 years. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements or by a Sponsor agreement. The Sponsor must be notified and will assist with retention should PI/institution be unable to continue maintenance of subject files for the full 15 years. It is the responsibility of the Sponsor to inform the PI/institution as to when these documents no longer need to be retained.

18. USE OF INFORMATION AND PUBLICATION

The Sponsor recognizes the importance of communicating medical study data and therefore encourages their publication in reputable scientific journals and at seminars or conferences. The details of the processes of producing and reviewing reports, manuscripts, and presentations based on the data from this study will be described in the Clinical Study Agreement between the Sponsor, CRO's, and the PI.

Due to the confidential nature of this development program, the results of the study may not be published or publicly presented without the prior approval of the Sponsor. Any Investigator wishing to publish or present any study finding must present a manuscript or abstract to the Sponsor one hundred and twenty (120) days prior to submission for publication or presentation to provide the Sponsor an opportunity for review and comment.

19. REFERENCES

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20. INVESTIGATOR RESPONSIBILITIES

20.1 Conduct of Study and Protection of Human Subjects

In accordance with FDA Form 1572, the Investigator will ensure that:

- He/she will conduct the study in accordance with the relevant, current protocol and will only make changes in a protocol after notifying the Sponsor, except when necessary to protect the safety, rights, or welfare of subjects.
- He/she will personally conduct or supervise the study.
- He/she will inform any potential subjects, or any persons used as controls, that the drugs are being used for investigational purposes and he or she will ensure that the requirements relating to obtaining informed consent in 21 Part 50 and IRB review and approval in 21 CFR Part 56 are met.
- He/she will report to the Sponsor adverse experiences that occur in the course of the investigation in accordance with 21 CFR 312.64.
- He/she has read and understands the information in the IB, including potential risks and side effects of the drug.
- His/her staff and all persons who assist in the conduct of the study are informed about their obligations in meeting the above commitments
- He/she will ensure adequate and accurate records in accordance with 21 CFR 312.62 and make those records available for inspection in accordance with 21 CFR 312.68.
- He/she will ensure that the IRB/EC complies with the requirements of 21 CFR Part 56, and other applicable regulations, and conducts initial and ongoing reviews and approvals of the study. He/she will also ensure that any change in research activity and all problems involving risks to human subjects or others are reported to the IRB/EC. Additionally, he/she will not make any changes in the research without IRB/EC approval, except where necessary to eliminate apparent immediate hazards to human subjects.
- He/she agrees to comply with all other requirements regarding the obligations of clinical Investigators and all other pertinent requirements in 21 CFR Part 312.

21. INVESTIGATOR AND SPONSOR SIGNATURE PAGE

Protocol Title: A Phase 1 Multiple Dose, Dose Escalation Trial of pegzilarginase (Co-ArgI-PEG) in Patients with Advanced Solid Tumors

Aeglea Biotherapeutics, Inc. Protocol Number: *CAEB1102-101B, Amendment 1 Dated: 08 Nov 2018*

I have read the forgoing protocol and agree to conduct this study as outlined. I agree to conduct the study in compliance with all applicable regulations and guidelines, including E6 ICH, as stated in the protocol, and other information supplied to me.

Investigator's Signature	Date
Printed Name	

Accepted for the Sponsor:

On behalf of Aeglea Biotherapeutics, Inc., I confirm that Aeglea Biotherapeutics, Inc., as a Sponsor will comply with all obligations as detailed in all applicable regulations and guidelines. I will ensure that the PI is informed of all relevant information that becomes available during the conduct of this study.

Chief Medical Officer/Medical Monitor's Signature	Date
Printed name	

22. APPENDICES

Appendix 1. ECOG Performance Status (Oken, 1982)

Grade	ECOG
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 house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
5	Dead

Appendix 2. RECIST v1.1 Summary

Table 1. Time Point Response: Patients with Target (± Non-target) Disease

Target lesions	Non-target lesions	New lesions	Overall response
CR	CR	No	CR
CR	Non-CR/non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, and NE = inevaluable

Abstracted from Eisenhauer EA, et al. 2009

Table 2. Time Point Response: Patients with Non-target Disease Only

Non-target lesions	New lesions	Overall response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD
Not all evaluated	No	NE
Unequivocal PD	Yes or No	PD
Any	Yes	PD

CR = complete response, PD = progressive disease and NE = inevaluable

A 'Non-CR/non-PD is preferred over 'stable disease' for non-target disease since SD is increasingly used as endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised.

Appendix 3. iRECIST Summary

	Timepoint response with no previous iUPD in any category	**Timepoint response with previous iUPD in any category***
Target lesions: iCR; non-target lesions: iCR; new lesions: no	iCR	iCR
Target lesions: iCR; non-target lesions: non-iCR/non-iUPD; new lesions: no	iPR	iPR
Target lesions: iPR; non-target lesions: non-iCR/non-iUPD; new lesions: no	iPR	iPR
Target lesions: iSD; non-target lesions: non-iCR/non-iUPD; new lesions: no	iSD	iSD
Target lesions: iUPD with no change, or with a decrease from last timepoint; non-target lesions: iUPD with no change, or decrease from last timepoint; new lesions: yes	Not applicable	New lesions confirm iCPD if new lesions were previously identified and they have increased in size (≥5 mm in sum of measures for new lesion target or any increase for new lesion non-target) or number; if no change is seen in new lesions (size or number) from last timepoint, assignment remains iUPD
Target lesions: iSD, iPR, iCR; non-target lesions: iUPD; new lesions: no	iUPD	Remains iUPD unless iCPD is confirmed on the basis of a further increase in the size of non-target disease (does not need to meet RECIST 1.1 criteria for unequivocal progression)
Target lesions: iUPD; non-target lesions: non-iCR/non-iUPD, or iCR; new lesions: no	iUPD	Remains iUPD unless iCPD is confirmed on the basis of a further increase in sum of measures ≥5 mm; otherwise, assignment remains iUPD
Target lesions: iUPD; non-target lesions: iUPD; new lesions: no	iUPD	Remains iUPD unless iCPD is confirmed based on a further increase in previously identified target lesion iUPD in sum of measures ≥5 mm or non-target lesion iUPD (previous assessment need not have shown unequivocal progression)
Target lesions: iUPD; non-target lesions: iUPD; new lesions: yes	iUPD	Remains iUPD unless iCPD is confirmed on the basis of a further increase in previously identified target lesion iUPD sum of measures ≥5 mm, previously identified non-target lesion iUPD (does not need to be unequivocal), or an increase in the size or number of new lesions previously identified
Target lesions: non-iUPD or progression; non-target lesions: non-iUPD or progression; new lesions: yes	iUPD	Remains iUPD unless iCPD is confirmed on the basis of an increase in the size or number of new lesions previously identified
Target lesions, non-target lesions, and new lesions defined according to RECIST 1.1 principles; if no pseudoprogression occurs, RECIST 1.1 and iRECIST categories for complete response, partial response, and stable disease would be the same. *Previously identified in assessment immediately before this timepoint. "i" indicates immune responses assigned using iRECIST. iCR=complete response. iPR=partial response. iSD=stable disease. iUPD=unconfirmed progression. non-iCR/non-iUPD=criteria for neither CR nor PD have been met. iCPD=confirmed progression. RECIST=Response Evaluation Criteria in Solid Tumours.		

This table is taken directly from Table 2 of Seymour and Bogaerts (2017).

Appendix 4: Description of the iRECIST Process for Assessment of Disease Progression

Assessment at Screening and Prior to RECIST 1.1 Progression

Until radiographic disease progression based on RECIST 1.1, there is no distinct iRECIST assessment.

Assessment and Decision at RECIST 1.1 Progression

For participants who show evidence of radiological PD by RECIST 1.1 as determined by the Investigator, the Investigator will decide whether to continue a participant on study treatment until repeat imaging is obtained (using iRECIST for participant management (see Table [X] and Figure [X]). This decision by the Investigator should be based on the participant's overall clinical condition.

Clinical stability is defined as the following:

- Absence of symptoms and signs indicating clinically significant progression of disease
- No decline in ECOG performance status
- No requirements for intensified management, including increased analgesia, radiation, or other palliative care

Any participant deemed clinically unstable should be discontinued from study treatment at [For those studies in which PFS is the primary endpoint, add the following: central verification of] site-assessed first radiologic evidence of PD, and is not required to have repeat tumor imaging for confirmation of PD by iRECIST.

If the Investigator decides to continue treatment, the participant may continue to receive study treatment and the tumor assessment should be repeated 4 to 8 weeks later to confirm PD by iRECIST, per Investigator assessment. Images should continue to be sent in to the central imaging vendor for potential retrospective BICR.

Tumor flare may manifest as any factor causing radiographic progression per RECIST 1.1, including:

- Increase in the sum of diameters of target lesion(s) identified at baseline to ≥20% and ≥5 mm from nadir

o Note: the iRECIST publication uses the terminology “sum of measurements”, but “sum of diameters” will be used in this protocol, consistent with the original RECIST 1.1 terminology.

- Unequivocal progression of non-target lesion(s) identified at baseline
- Development of new lesion(s)

iRECIST defines new response categories, including iUPD (unconfirmed progressive disease) and iCPD (confirmed progressive disease). For purposes of iRECIST assessment, the first visit showing progression according to RECIST 1.1 will be assigned a visit (overall) response of iUPD, regardless of which factors caused the progression.

At this visit, target and non-target lesions identified at baseline by RECIST 1.1 will be assessed as usual.

New lesions will be classified as measurable or non-measurable, using the same size thresholds and rules as for baseline lesion assessment in RECIST 1.1. From measurable new lesions, up to 5 lesions total (up to 2 per organ), may be selected as New Lesions – Target. The sum of diameters of these lesions will be calculated and kept distinct from the sum of diameters for target lesions at baseline. All other new lesions will be followed qualitatively as New Lesions – Non-target.

Assessment at the Confirmatory Imaging

On the confirmatory imaging, the participant will be classified as progression confirmed (with an overall response of iCPD), or as showing persistent unconfirmed progression (with an overall response of iUPD), or as showing disease stability or response (iSD/iPR/iCR).

Confirmation of Progression

Progression is considered confirmed, and the overall response will be iCPD, if ANY of the following occurs:

- Any of the factors that were the basis for the initial iUPD show worsening

o For target lesions, worsening is a further increase in the sum of diameters of ≥5 mm, compared to any prior iUPD time point

o For non-target lesions, worsening is any significant growth in lesions overall, compared to a prior iUPD time point; this does not have to meet the "unequivocal" standard of RECIST 1.1

o For new lesions, worsening is any of these:

□ An increase in the new lesion sum of diameters by ≥5 mm from a prior iUPD time point

□ Visible growth of new non-target lesions

□ The appearance of additional new lesions

- Any new factor appears that would have triggered PD by RECIST 1.1

Persistent iUPD

Progression is considered not confirmed, and the overall response remains iUPD, if:

- None of the progression-confirming factors identified above occurs AND
- The target lesion sum of diameters (initial target lesions) remains above the initial PD threshold (by RECIST 1.1)

Additional imaging for confirmation should be scheduled 4 to 8 weeks from the imaging on which iUPD is seen. This may correspond to the next visit in the original visit schedule. The assessment of the subsequent confirmation imaging proceeds in an identical manner, with possible outcomes of iCPD, iUPD, and iSD/iPR/iCR.

Resolution of iUPD

Progression is considered not confirmed, and the overall response becomes iSD/iPR/iCR, if:

- None of the progression-confirming factors identified above occurs, AND
- The target lesion sum of diameters (initial target lesions) is not above the initial PD threshold.

The response is classified as iSD or iPR (depending on the sum of diameters of the target lesions), or iCR if all lesions resolve.

In this case, the initial iUPD is considered to be pseudo-progression, and the level of suspicion for progression is "reset". This means that the next visit that shows radiographic

progression, whenever it occurs, is again classified as iUPD by iRECIST, and the confirmation process is repeated before a response of iCPD can be assigned.

Management Following the Confirmatory Imaging

If repeat imaging does not confirm PD per iRECIST, as assessed by the Investigator, and the participant continues to be clinically stable, study treatment may continue and follow the regular imaging schedule. If PD is confirmed, participants will be discontinued from study treatment.

NOTE: If a participant has confirmed radiographic progression (iCPD) as defined above, but the participant is achieving a clinically meaningful benefit [For those studies in which PFS is the primary endpoint, add the following: or if RECIST 1.1 PD has not been verified centrally], an exception to continue study treatment may be considered following consultation with the Sponsor. In this case, if study treatment is continued, tumor imaging should continue to be performed following the intervals as outlined in Section 13 and submitted to the central imaging vendor.

Detection of Progression at Visits After Pseudo-progression Resolves

After resolution of pseudo-progression (i.e., achievement of iSD/iPR/iCR), iUPD is indicated by any of the following events:

- Target lesions

o Sum of diameters reaches the PD threshold (≥20% and ≥5 mm increase from nadir) either for the first time, or after resolution of previous pseudo-progression. The nadir is always the smallest sum of diameters seen during the entire trial, either before or after an instance of pseudo-progression.

- Non-target lesions

o If non-target lesions have never shown unequivocal progression, their doing so for the first time results in iUPD.

o If non-target lesions have shown previous unequivocal progression, and this progression has not resolved, iUPD results from any significant further growth of non-target lesions, taken as a whole.

- New lesions
  - o New lesions appear for the first time
  - o Additional new lesions appear
  - o Previously identified new target lesions show an increase of ≥ 5 mm in the new lesion sum of diameters, from the nadir value of that sum
  - o Previously identified non-target lesions show any significant growth

If any of the events above occur, the overall response for that visit is iUPD, and the iUPD evaluation process (see Assessment at the Confirmatory Imaging above) is repeated. Progression must be confirmed before iCPD can occur.

The decision process is identical to the iUPD confirmation process for the initial PD, with one exception: if new lesions occurred at a prior instance of iUPD, and at the confirmatory imaging the burden of new lesions has increased from its smallest value (for new target lesions, the sum of diameters is ≥5 mm increased from its nadir), then iUPD cannot resolve to iSD or iPR. It will remain iUPD until either a decrease in the new lesion burden allows resolution to iSD or iPR, or until a confirmatory factor causes iCPD.

Additional details about iRECIST are provided in the iRECIST publication.