CLINICAL STUDY PROTOCOL

Lerapolturev (formerly known as PVSRIPO) with and without Immune Checkpoint Blockade in Advanced PD-1 Refractory Melanoma

Protocol Number	LUMINOS-102 (PVSRIPO ICI M201)
Clinical Phase	Phase 2
US IND Number	IND 26877
Protocol Date:	Amendment 4 14JAN2022
Sponsor:	Istari Oncology, Inc. 430 Davis Drive, Suite 560 Morrisville, NC 27560

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Confidentiality Statement
This study will be performed in compliance with Good Clinical Practices (GCP) and applicable regulatory requirements, including the archiving of essential documents. Information contained in this protocol is confidential in nature, and may not be used, divulged, published or otherwise disclosed to others except to the extent necessary to obtain approval of the institutional review board or independent ethics committee, or as required by law. Persons to whom this information is disclosed should be informed that this information is confidential and proprietary and may not be further disclosed without the express written consent of Istari Oncology, Inc.

Sponsor Signatory:

W. Garrett Nichols, MD, MS
Chief Medical Officer
Istari Oncology, Inc.

Date

Investigator Protocol Agreement

I have read this Istari Clinical Study Protocol and agree to conduct the study as outlined herein, in accordance with Good Clinical Practice (GCP) requirements and the International Conference on Harmonization (ICH) guidelines, the Declaration of Helsinki and complying with the obligations and requirements of Clinical Investigators and all other requirements listed in 21 Code of Federal Regulations (CFR) part 312 and with all applicable local regulations. I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

Investigator Name (Print): _____

Date Signed: _____

Investigator Signature: _____

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SYNOPSIS

Protocol Title	Lerapolturev (formerly known as PVSRIPO) with and without Immune Checkpoint Blockade in Advanced PD-1 Refractory Melanoma
Protocol Number	LUMINOS-102 (PVSRIPO ICI M201)
Rationale and Background	Currently approved systemic immunotherapies fail to provide clinical benefit for a significant portion of patients with unresectable melanoma. Less than 40% of patients respond to anti-programmed cell death receptor-1 (PD-1) therapy (ie, have primary PD-1 resistance)[1,2], and of those who do respond, up to 25% may develop an acquired resistance over time[3]. While combining anti-PD-1 with anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) therapy can increase response rates, this combination is associated with significant toxicity[4]. Options for follow-on therapy remain limited, and outcomes are poor for those with advanced disease[5]. Lerapolturev (formerly PVSRIPO) is a recombinant rhinovirus/polio virus (PV) chimera which has demonstrated clinical anti-tumor activity in primary brain tumors and in a Phase 1 study of unresectable, anti-PD-1 refractory (as per investigator determination) melanoma[6] which warrants further investigation. Preliminary data from the current study in unresectable, anti-PD-1/L1 refractory melanoma (as defined by the Society for Immunotherapy in Cancer's [SITC] Immunotherapy Resistance Taskforce Guidance[7]) show that lerapolturev given every (Q) 3 to 4 weeks, alone or in combination with anti-PD-1, at a dose of up to 6×10^8 tissue culture infectious dose 50% ($TCID_{50}$) is very well tolerated (regimen referred to as **Q3/4W** hereafter); all adverse events (AEs) related to lerapolturev and/or anti-PD-1 remain Grade 1 or 2, with no dose-limiting toxicities (DLTs), treatment-related serious AEs (SAEs) or sign/symptoms of cytokine release syndrome reported as of the 10DEC2021 data cutoff (Table 3 and Table 4). However, the anti-tumor activity noted in Phase 1 has not yet been duplicated. In addition, a literature review shows that other innate immune agonists with clinical activity in anti-PD-1/L1 refractory melanoma, including other oncolytic viruses, use an "induction/maintenance" dose schedule, with more frequent (eg, weekly) injections for 1 to 2 months, followed by less frequent (eg, Q3 or 4 week) maintenance thereafter to induce an upfront anti-tumor immune reaction that can be maintained across time. Therefore, LUMINOS-102 will test the safety and efficacy of weekly x7 lerapolturev followed by Q3 to 4-week administration at a dose of up to 1.6×10^9 $TCID_{50}$, as monotherapy and in combination with

<table>
<tr><td></td><td colspan="2">anti-PD-1 therapy, in a more homogeneous unresectable melanoma population that has failed 1 prior line of anti-PD-1/L1-based therapy.</td></tr>
<tr><td>Key Objectives</td><td colspan="2">
<table>
<tr><th>Objectives</th><th>Endpoints/Evaluation Criteria</th></tr>
<tr><td colspan="2">Primary</td></tr>
<tr><td>To evaluate the anti-tumor activity of lerapolturev with and without anti-PD-1, in participants who have failed anti-PD-1/L1-based therapy</td><td>• Overall Response Rate (ORR): the proportion of participants achieving complete (CR) or partial response (PR), per RECIST 1.1</td></tr>
<tr><td>To evaluate the safety/tolerability of lerapolturev with and without anti-PD-1, in participants who have failed anti-PD-1/L1-based therapy</td><td>• The frequency and severity of treatment-emergent adverse events (AE) via Common Terminology Criteria for Adverse Events (CTCAE, v5.0)
• Changes in laboratory (hematology, chemistry) and vital sign parameters
• Lerapolturev AESIs
• Anti-PD-1 immune-related AEs (irAEs)
• Study treatment discontinuation due to AEs</td></tr>
<tr><td>To evaluate the effect of lerapolturev on the tumor microenvironment of injected and non-injected lesions when administered with and without anti-PD-1 therapy</td><td>• Changes from baseline in the number of CD8+ tumor infiltrating lymphocytes (TILs)
• Changes from baseline in PD-L1 expression</td></tr>
<tr><td colspan="2">Secondary</td></tr>
<tr><td>To evaluate survival and disease control outcomes of lerapolturev with and without anti-PD-1, in participants who have failed anti-PD-1/L1-based therapy</td><td>• Overall survival (OS): time from treatment group assignment until death from any cause
• Duration of Response (DOR): time from confirmed objective response (CR or PR per RECIST 1.1) until unequivocal disease progression or death, whichever occurs first
• Disease control rate (DCR): the proportion of participants achieving confirmed CR, confirmed PR, or stable disease</td></tr>
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		(SD) per RECIST 1.1, as best response • Disease control rate-6months (DCR-6mo): the proportion of participants achieving confirmed CR (any duration), confirmed PR (any duration) or SD (≥ 6 months) per RECIST 1.1 as best response • Durable Response Rate (DRR): the proportion of participants with confirmed CR or PR (per RECIST 1.1) lasting at least 6 months • Progression-free survival (PFS): time (number of months) from treatment group assignment until date of documented radiologic disease progression per RECIST 1.1 or death due to any cause, whichever comes first
Study Design	This multi-center, open-label, randomized, Phase 2 study will investigate the efficacy and safety of lerapolturev alone (Arm 1) or in combination with an anti-PD-1 inhibitor (Arm 2). Following a 6-participant safety run-in period, up to approximately 50 participants with unresectable cutaneous melanoma who previously failed an anti-PD-1/L1-based therapy will be randomized 1:1 to receive either lerapolturev or lerapolturev plus an anti-PD-1. Based on updated eligibility criteria, participants will be stratified based on type of anti-PD-1/L1 resistance (primary versus secondary as per Kluger et al[7].) and baseline lactate dehydrogenase (LDH) levels (normal versus >ULN). As of 10DEC2021, the safety run-in has been completed and a total of 17 participants have been enrolled (safety run-in, n=6; Arm 1, n=5; Arm 2, n=6). Upon review of the safety profile for lerapolturev administered as monotherapy or in combination with anti-PD-1 therapy (Section 1.2.2), and in agreement with the data safety monitoring committee (DSMC), the total lerapolturev dose to be administered will be a maximum of 1.6×10^9 $TCID_{50}$/visit, with up to 6 lesions injected/visit. This dose schedule of administration (referred to hereafter as **QW**) will include weekly injections x 7 followed by either Q3W dosing (lerapolturev monotherapy or in combination with pembrolizumab) or Q4W dosing (combination with nivolumab).	

	Participants enrolled in the safety run-in, or those randomized to Arm 1 (lerapolturev only), are allowed to crossover to Arm 2 following disease progression (per RECIST 1.1), once a PR lasts ≥ 6 months, or after 26 weeks on study without RECIST-defined progression (ie, SD or unconfirmed PR or CR), as described in Section 3. In Arm 2, anti-PD-1 will be administered per the manufacturer's prescribing information concurrently with lerapolturev beginning Day 1. After 12 participants have been treated under Study Protocol Amendment 4 and on study approximately 21 days (ie, once n=6 participants per treatment arm receiving lerapolturev **QW** have completed the DLT evaluation period), an additional safety review will be performed by the DSMC to confirm the safety of lerapolturev with or without anti-PD-1 and/or to make recommendations related to lerapolturev dose and/or schedule adjustments as described in Section 3.1.3. Participants currently receiving the **Q3/4W** schedule of injections will be given the option to change to the **QW** schedule (Section 3.1.2). Participants will be evaluated for key primary and secondary anti-tumor response endpoints based on RECIST 1.1. Additional primary endpoints include characterization of the immunologic response (eg, changes in CD8+ TIL levels and PD-L1 expression) to lerapolturev with and without anti-PD-1 and safety. Because lerapolturev is an immunotherapeutic, participant management with respect to treatment decisions (eg, confirmation of PD for study discontinuation) will occur based on iRECIST criteria. However, participants in the safety run-in or Arm 1 are allowed to crossover to Arm 2 upon RECIST-defined PD. ORR, DOR, and DRR based on iRECIST are exploratory endpoints.
Planned Number of Participants	Up to approximately 56 participants
Study Entry Criteria	Inclusion Criteria: 1. ≥ 18 years of age 2. Prior CDC-recommended vaccination series against poliovirus (PV), and has received a boost immunization with trivalent IPOL® (Sanofi-Pasteur SA) at least 1 week, but less than 6 weeks, prior to Day 1 a. NOTE: Patients who are unsure of their vaccination status must provide evidence of anti-PV immunity prior to enrollment, as applicable

	3. Has biopsy proven unresectable cutaneous melanoma and is willing to undergo tumor biopsy prior to the first dose of study drug(s) and at prespecified intervals during the study. Note the following details: a. Patients with ocular, acral or mucosal melanoma are not eligible b. Patients with M1c or M1d disease are NOT eligible c. Submission of an archival biopsy sample is allowed in lieu of the baseline tumor biopsy, provided the tissue is ≤4 months old and the participant received no intervening systemic/intratumoral anti-cancer therapy since the biopsy was acquired. d. Must have at least 1 lesion that is amenable to biopsy. The lesion must be safely accessible as determined by the investigator and should not be located at sites that require significant risk procedures to biopsy. Examples of sites considered to be of significant risk include but are not limited to the following: the brain, lung, mediastinum, pancreas, or endoscopic procedures extending beyond the esophagus, stomach, or bowel wall. 4. Has ≥ 2 melanoma lesions that are accurately measurable by caliper or a radiological method according to RECIST 1.1 criteria a. One lesion must be injectable--defined as a visible or palpable cutaneous, subcutaneous, or nodal melanoma lesion ≥ 10mm in longest diameter or multiple injectable melanoma lesions which in aggregate have a longest diameter of ≥ 10mm and where the minimal lesion size is ≥ 5mm. b. Note that visceral lesions (eg, liver, lung, retroperitoneal, subpleural lesions) are not considered injectable for the purposes of this trial. 5. Has had confirmed progression of disease (PD) while receiving at least 6 weeks (> 1 dose) of an FDA-approved anti-PD-1/L1 therapy (as monotherapy or in combination) for the treatment of melanoma. Note the following details: a. Initial PD as defined by RECIST v1.1 b. Confirmation of PD per iRECIST must occur by repeat assessment ≥ 4 weeks from initial evidence of PD, in the absence of rapid clinical progression.

	c. Those who discontinue anti-PD-1/L1 therapy after at least 6 weeks (> 1 dose) and have confirmed PD per iRECIST within 12 weeks of their last anti-PD-1/L1 dose are also eligible, provided the anti-PD-1/L1 was not stopped due to toxicity requiring permanent discontinuation a. Those treated with anti-PD-1/L1 in the adjuvant setting and who have biopsy-confirmed progression either while receiving anti-PD-1/L1-based therapy or ≤ 12 weeks after their last dose of anti-PD-1/L1 therapy are allowed NOTE: Adjuvant is defined as therapy received after surgical resection of disease such that the patient has no evidence of disease when the anti-PD-1/L1 therapy is initiated. Patients with known BRAF mutation must have also failed or refused to receive BRAF-targeted therapy (alone or in combination with MEK inhibitor) to be eligible. 6. Eastern Cooperative Oncology Group (ECOG) status of 0-1 7. Serum lactate dehydrogenase (LDH) levels ≤3 x upper limit of normal (ULN) 8. Adequate bone marrow, liver and renal function as assessed by the following: a. Hemoglobin ≥9.0 g/dl, patients may be transfused b. Lymphocyte count ≥0.5 x 10^9/L (500 µL) c. Absolute neutrophil count (ANC) ≥1.5 x 10^9/L (1500 µL) d. Platelet count ≥100 x 10^9/L (100,000 µL) without transfusion e. AST, ALT, and alkaline phosphatase (ALP) ≤2.5 x upper limit of normal (ULN), with the following exceptions: i. Patients with documented liver metastases: AST and ALT ≤5 x ULN ii. Patients with documented liver or bone metastases: ALP ≤5 x ULN f. Serum bilirubin ≤1.5 x ULN with the following exception: i. Patients with known Gilbert disease: serum bilirubin level ≤3 x ULN g. Measured or calculated (per institutional standards) creatinine clearance ≥30 ml/min (GFR can also be used in place of creatinine clearance) h. For patients not receiving therapeutic anticoagulation: INR, PT, PTT (or aPTT) ≤1.5 x ULN 9. Life expectancy of >12 weeks

	10. Signed informed consent form (ICF) indicating that participant understands the purpose of, and procedures required for the study, and is willing/able to participate in the study Exclusion Criteria: 1. Has biopsy-proven ocular, acral or mucosal melanoma 2. Has M1c or M1d disease 3. No more than one prior systemic anti-cancer regimen (monotherapy or combination) for management of melanoma. Additional details noted below: a. Adjuvant anti-cancer therapy administered ≥ 6 months prior to the first injection of lerapolturev does NOT count as a line of treatment. b. Patients with BRAF mutant melanoma may enroll if they have received ≤ 2 prior lines of systemic anti-cancer therapy only if one of those lines of therapy was a BRAF-targeted regimen (alone or in combination with MEK inhibitor). c. A line of therapy is defined as a regimen in which at least 2 doses of systemic anti-cancer therapy (monotherapy or combination) was administered, and the regimen was discontinued because of progressive disease 4. Uncontrolled tumor-related pain. Participants requiring pain medication must be on a stable regimen at study entry a. Symptomatic lesions amenable to palliative radiotherapy (eg, bone metastases or metastases causing nerve impingement) should be treated prior to enrollment. Patients should be recovered from the effects of radiation. There is no required minimum recovery period b. Asymptomatic metastatic lesions that would likely cause functional deficits or intractable pain with further growth (eg, epidural metastasis that are not currently associated with spinal cord compression) should be considered for loco-regional therapy if appropriate prior to enrollment 5. Grade ≥2 pleural effusion, pericardial effusion, or ascites 6. Active or history of autoimmune disease or immune deficiency within previous 2 years, with the following exceptions:

	a. History of autoimmune-related endocrinopathy (e.g. adrenal insufficiency, hypothyroidism, Type 1 diabetes mellitus, etc.) that is managed by hormone replacement therapy (e.g. hydrocortisone, thyroid hormone, insulin, etc.) b. Eczema, psoriasis, or lichen simplex chronicus with dermatologic manifestations only (eg, patients with psoriatic arthritis are excluded), provided all of the following conditions are met: i. Rash must cover <10% of body surface area ii. Disease is well-controlled at baseline and requires only low-potency topical corticosteroids iii. No occurrence of acute exacerbations of the underlying condition requiring psoralen plus ultraviolet A radiation, methotrexate, retinoids, biologic agents, oral calcineurin inhibitors, or high potency or oral corticosteroids within 12 months of Day 1 7. History of idiopathic pulmonary fibrosis, organizing pneumonia (eg, bronchiolitis obliterans), drug-induced or idiopathic pneumonitis, or evidence of active pneumonitis on screening chest computed tomography (CT) scan a. History of radiation pneumonitis in the radiation field (fibrosis) is allowed 8. History of a positive HIV RNA test (HIV 1 or 2 RNA by PCR) 9. Known active hepatitis B virus (HBV) infection (chronic or acute) a. NOTE: Participants with a negative HBsAg test and a positive total hepatitis B core antibody (HBcAb) test are allowed 10. Known active hepatitis C virus (HCV) infection a. NOTE: History of a positive HCV antibody test, but negative HCV RNA test, is allowed 11. Active tuberculosis 12. Significant cardiovascular disease, such as New York Heart Association Class II or greater cardiac disease, myocardial infarction, or cerebrovascular accident within 3 months of Day 1, unstable arrhythmia, or unstable angina 13. Has received prior systemic anti-cancer therapy including investigational agents within 4 weeks or 5 elimination half-

	lives—whichever is shorter—prior to treatment, or has not recovered from all AEs due to previous therapies to ≤Grade 1 or baseline. Participants with ≤Grade 2 neuropathy are eligible a. Note: Anti-PD-1/L1 within 4 weeks of Day 1 is allowed b. If participant received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting study treatment 14. History of other malignancy within 2 years prior to Day 1, with the exception of those with a negligible risk of metastasis or death (eg, resected cutaneous basal cell carcinoma, or other cancers with 5-year OS of >90%) 15. Severe infection within 4 weeks prior to Day 1, including, but not limited to, hospitalization for complications of infection, bacteremia, or severe pneumonia a. Prophylactic antibiotics (eg, to prevent a urinary tract infection or chronic obstructive pulmonary disease exacerbation) are allowed 16. Prior allogeneic stem cell or solid organ transplantation 17. Treatment with a live, attenuated vaccine within 4 weeks prior to Day 1 18. Treatment with systemic immunosuppressive medication within 4 weeks prior to Day 1, with the following exceptions: a. Participants who received acute, low-dose systemic immunosuppressant medication or a one-time pulse dose of systemic immunosuppressant medication (eg, 48 hours of corticosteroids for a contrast allergy) are eligible b. Patients receiving mineralocorticoids (eg, fludrocortisone), or systemic prednisone equivalent corticosteroid doses of <10mg per day are eligible for the study 19. Known hypersensitivity to pembrolizumab, nivolumab, or any of the respective excipients 20. Requires therapeutic anticoagulation and cannot discontinue anticoagulation safely during the day prior, day of, and day after each lerapolturev injection a. NOTE: Participants receiving anticoagulation with warfarin at the time of study entry are allowed if they can be transitioned to an alternative anticoagulant (eg, low molecular weight heparin or direct oral anticoagulants) prior to the first dose of lerapolturev.

	Anyone transitioned from warfarin to an oral anticoagulant prior to the first dose of lerapolturev should have an INR <1.5x upper limit of normal in order to participate. Antiplatelet agents (eg, aspirin, clopidogrel, etc.) are not considered anticoagulants for the purposes of this study (ie, are allowed) 21. A pregnant or nursing female, or women of child-bearing potential and men who are sexually active and not willing/able to use medically acceptable forms of contraception starting from signed ICF through 150 days after last anti-PD-1 dose 22. History of human serum albumin allergy 23. History of neurological complications due to polio virus infection 24. History of agammaglobulinemia 25. Concurrent participation in a separate interventional clinical trial during this study 26. Any underlying medical condition for which, in the opinion of the investigator, participation would not be in the best interest of the participant (eg, compromises the participant's well-being) or that could prevent, limit, or confound protocol-specified assessments
Concomitant Treatment	Pembrolizumab or nivolumab (Arm 2 only)
Investigational Drugs	Lerapolturev (formerly PVSRIPO): Oncolytic Polio/Rhinovirus Recombinant
Planned Study Sites	Up to approximately 30 sites
Criteria for Assessments	**Efficacy:** Changes in skin lesion and/or radiographic tumor measurements and survival will be assessed per RECIST 1.1 at regular intervals to evaluate efficacy: participants achieving objective response (along with associated durations) or disease control by Week 26 or upon anti-PD-1 re-challenge (Arm 1 and safety run-in participants that crossover to Arm 2, analyzed from time of crossover but separately from Arm 2) through study completion. Assessment of anti-tumor response by iRECIST will be exploratory. **Safety:** Laboratory and spontaneously reported AEs, to evaluate safety and tolerability to determine incidence and severity of treatment-emergent AEs for duration of the study

	Biomarkers: Biological samples will be collected as indicated in the schedule of assessments (SOA) to evaluate immune, genetic, or cytologic factors impacting lerapolturev mechanism of action (MOA), anti-tumor response, and/or to evaluate immune-related AEs
Statistical Methods	The primary endpoint for measuring anti-tumor activity will be ORR, based on RECIST 1.1, which will be summarized as the overall number (%) of participants with CR or PR in each treatment arm, along with 95% confidence interval (CI). Participants will be analyzed based on the treatment arm to which they were randomized, as well as by lerapolturev dose/schedule (ie, **QW** and **Q3/4W**). Other endpoints will be summarized by descriptive statistics, as appropriate. Further details for these and other analyses will be described in the statistical analysis plan (SAP).
Determination of Sample Size	Sample size was determined based on feasibility and logistical considerations. Based on an estimated sample size of 15 participants in Arm 2 receiving lerapolturev QW in combination with anti-PD-1, five objective responses are needed for the 95% confidence interval (CI) to be above 10% (33% ORR; 95% CI: 11.8% to 61.6%), which would indicate the combination has anti-tumor activity in this anti-PD-1 resistant population. See Section 8.3 for additional details regarding sample size determination.
Study Duration	Up to approximately 24 months (104 weeks)

CLINICAL TRIAL SCHEMAT

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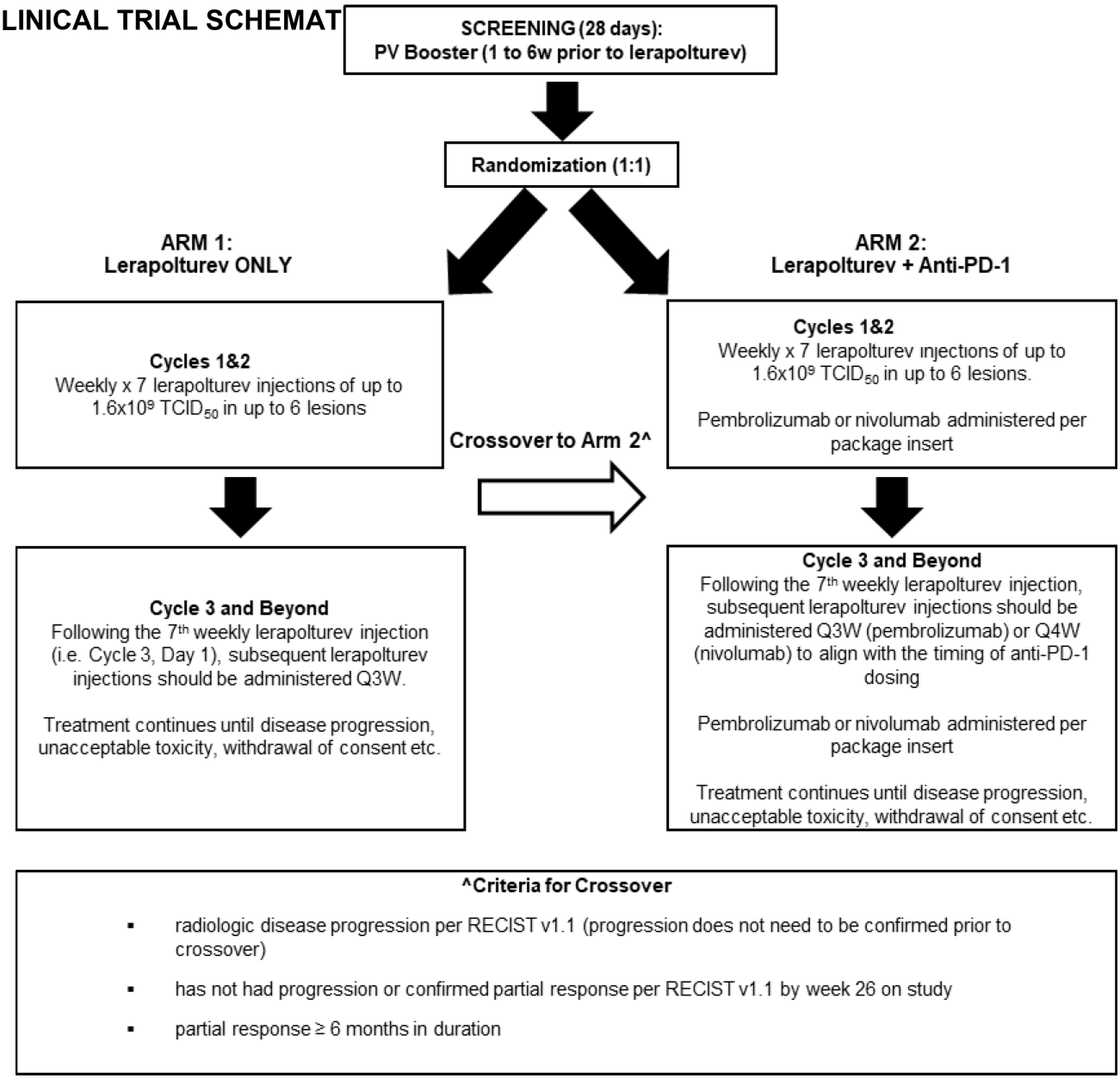

Table 1. Schedule of Assessments for Lerapolturev Monotherapy or Lerapolturev + Pembrolizumab

NOTE: Cycle length for lerapolturev monotherapy or lerapolturev + pembrolizumab is 3 weeks. Lerapolturev is administered weekly during Cycles 1 & 2 and every 3 weeks thereafter on the days indicated in the table below. Refer to the table below for the timing of other study assessments to be completed within each cycle.

Study Procedure[3]	Screening Period	Treatment			EOT Visit[15]	Survival Follow-up[16]
		Cycle 1	Cycle 2	≥ Cycle 3		
	(≤ 28D prior to C1D1)		+3W (+7D)	Q3W (+7D)	≤28D after last treatment	
Informed Consent[1]	X					
Medical History	X					
Physical Exam [2,3]	X	*C1D1*	*C2D1*	*Day 1 of each Cycle*	X	
Vital Signs[3]	X	*C1D1, C1D8, C1D15*	*C2D1, C2D8, C2D15*	*Day 1 of each Cycle*	X	
ECOG Performance Status[3]	X	*C1D1*	*C2D1*	*Day 1 of each Cycle*	X	
PV Immunization Booster[1]	*≥1W, but ≤6W before C1D1*					
Adverse Events	*Continuous from signing ICF until 30 days after last dose of study therapy*					
Concomitant Medications	*Continuous from signing ICF until 30 days after last dose of study therapy*					
Subsequent Anticancer Therapy					X	X
AESI, irAEs and SAEs	*Continuous from signing ICF until 90 days after last dose of study therapy or resolution/stabilization*					
Hematology [3,4]	X	*C1D1*	*C2D1*	*C3D1 then Q6W (ie, at every other visit) and as clinically indicated*	X	
Chemistry[3,4]	X	*C1D1*	*C2D1*	*C3D1 then Q6W (ie, at every other visit) and as clinically indicated*	X	
INR, PT, PTT (or aPTT)	X	*As clinically indicated*				
Thyroid Monitoring [3,5]	X	*Q6W (±7D) and as clinically indicated*				
Pregnancy Test[3,6]	*≤2D before C1D1*	*Q12W (±14D)*				

Cutaneous, Subcutaneous and Nodal Lesion Measurement and Photographs[3,7]	X	*C1D1, C1D8, C1D15*	*C2D1, C2D8, C2D15*	*Day 1 of each Cycle*	X	*per SOC*
Tumor Imaging[8]	*≤6W before C1D1*	*6 & 12W after C1D1, then Q12W thereafter. Crossover participants must have re-baseline imaging within 28D prior to administration of crossover C1D1 (i.e. first dose of anti-PD-1 therapy)*				per *SOC*
Tissue Biopsy[9] *All samples to be collected prior to dosing at the given timepoint*	*Prior to PV Immunization Booster*	*C1D8*	*C2D1*	*At the time of PD ± 1 week (per RECIST)* ***Crossover Only:** within 1 week prior to crossover C1D1, 1 week after crossover C1D1 (pre-dose, if applicable), 3 weeks (+7d; pre-dose) after crossover C1D1, and at the time of PD ± 1 week (per RECIST) on the crossover regimen*		
Blood Collection[10] *All samples to be collected prior to dosing at the given timepoint*	*Prior to PV Immunization Booster*	*C1D1, C1D8*	*C2D1*	*C3D1 and at the time of PD ± 1 week (per RECIST)* ***Crossover Only:** within 1 week prior to crossover C1D1, 1 week after crossover C1D1 (pre-dose, if applicable), 3 weeks (+7d; pre-dose) after crossover C1D1, and at the time of PD ± 1 week (per RECIST) on the crossover regimen*		
Lerapolturev Administration[12]		*C1D1, C1D8, C1D15*	*C2D1, C2D8, C2D15*	*C3D1 and Q3W thereafter*		
ARM 2: Anti-PD-1 Administration (pembrolizumab) [11]		*C1D1*	*C2D1 (if given Q3W)*	*C3D1 and Q3W or Q6W thereafter per pembrolizumab package insert*		
Crossover from Arm 1 to Arm 2 [13]		*If participant meets protocol-defined criteria*				
Lerapolturev Shedding: Injected Lesion[14]		*C1D1 (post-injection), C1D4, C1D8, C1D15 (post-injection), C1D18*	*C2D1, C2D15 (post-injection), C2D18*	*C3D1*		
Lerapolturev Shedding: Stool			*C2D8 (±3D)*			

AESI = adverse event of special interest; aPTT = activated partial thromboplastin time; C= cycle; CBC = complete blood count; CMP = comprehensive metabolic panel; CT = computed tomography; D = day; ECOG = Eastern Cooperative Oncology Group; EOT = End of Treatment; ICF = Informed consent form; IgG = immunoglobulin G; INR = international normalized ratio; LDH = lactate dehydrogenase; MRI = magnetic resonance imaging; PD-1 = Programmed cell death protein 1; PT = prothrombin time; PTT = partial thromboplastin time; PV = poliovirus; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid stimulating hormone; SAE = serious adverse event; W = weeks

1. **Informed Consent** must be signed prior to initiation of screening activities.

2. **Physical Exam**: The screening physical examination should be a complete physical exam of major body systems and include the general appearance of the participant, height and weight, vital signs (temperature, respiratory rate, blood pressure (systolic and diastolic [mmHg]), and heart rate [bpm]), examination of the skin, ears, nose, throat, lungs, heart, abdomen, extremities, musculoskeletal system, lymphatic system, and nervous system. During treatment and at the end of treatment (EOT) visit, weight assessment and limited symptom-directed physical examination are required. An evaluation of all skin lesions, including those injected with lerapolturev at the previous treatment visit, should be performed prior to subsequent lerapolturev injection.
3. **Procedures and/or Assessments** unless otherwise specified should occur on D1 of each Cycle. Any assessment or procedure scheduled on the same day as lerapolturev and/or anti-PD-1 administration should always precede receipt of study treatments. Note that laboratory assessments (e.g. hematology, chemistry, thyroid monitoring) may be done up to 2 calendar days prior to Day 1 of the cycle.
4. **Hematology and Chemistry:** Blood draws for hematology and chemistry occurring on the same day as study treatment should be collected prior to lerapolturev injection and/or prior to anti-PD-1 infusion (as applicable). **Hematology** should include hemoglobin, white blood cell (WBC) count with differential and platelet count. **Chemistry** measured in serum should include the following: LDH, glucose, calcium, sodium, potassium, bicarbonate, chloride, BUN, creatinine, total protein, albumin, ALP, AST, ALT, total bilirubin, amylase, and lipase.
5. **Thyroid Monitoring:** Should include TSH, free T4, and free T3.
6. **Pregnancy Test:** A pregnancy test (urine-based allowed) must be performed for all female participants of childbearing potential during Screening (≤2 days of lerapolturev injection) and approximately every 12 weeks while on study. If a urine-based test produces an equivocal result, a serum-based test should be performed.
7. **Measurement of Cutaneous, Subcutaneous and Nodal Lesions:** Measurement by ruler or caliper (along with photographs) should occur prior to lerapolturev administration to determine/verify lerapolturev injection volumes for each lesion. For timepoint response assessment of target lesions not visible by radiography, ruler/caliper measurement with photographs should be performed prior to lerapolturev injection.
8. **Tumor Imaging:** Baseline imaging assessments should include CT of the chest, abdomen, and pelvis, a brain MRI, as well as photography of all cutaneous/visible lesions. Participants without evidence of central nervous system (CNS) disease at screening are not required to have brain imaging on study, provided clinical evidence of CNS disease does not emerge. All imaging should be performed with and without IV contrast (oral contrast can be used per institutional guidelines), provided participant can tolerate contrast agent (brain CT—preferably with IV contrast--is acceptable for those who cannot tolerate MRI). Photographic evaluation of visible lesions should precede lerapolturev injection, if occurring in the same visit. All qualifying scans prior to and during screening used to determine enrollment eligibility (ie, baseline scans prior to initiating anti-PD-1, as well as initial and confirmatory scans demonstrating disease progression per iRECIST prior to enrollment) should be provided to the central imaging vendor, where possible. Imaging should be performed using identical techniques and equipment, where possible.
9. **Tissue Biopsy for Biomarker Assessment:** Participants are required to provide a qualifying biopsy. Archival tissue collected ≤4 months prior to Day 1 is allowed in lieu of the qualifying biopsy, provided the participant has not received intervening systemic/intratumoral anti-cancer therapy since the biopsy was performed; if not available, the participant should have a qualifying biopsy taken during the Screening Period prior to polio boost vaccination. Biopsy of a previously irradiated lesion is not allowed unless there is documented disease progression in that lesion.
    - **NOTE:** At all timepoints, biopsies from all three sites (injected lesion, noninjected lesions and draining lymph nodes) should be collected if the biopsy is technically feasible and does not put the participant at significant risk. Examples of sites considered to be of significant risk include, but are not limited to, the following: biopsies of the brain, lung, mediastinum, pancreas, or endoscopic procedures extending beyond the esophagus, stomach, or bowel wall. For situations where the investigator determines biopsy of a lesion would (1) result in a significant decrease in the size of the lesion such that RECIST 1.1 response assessment would be confounded, or (2) increase the risk of lerapolturev leakage from the lesion, the mandatory biopsy for that timepoint can be waived. In addition, complete resection of target lesions is prohibited unless necessary for participant's safety as determined by the investigator.
    - Tissue from additional biopsies taken as part of standard of care may also be collected for analysis.

10. **Blood Collection:** Participants who experience an AE of Special Interest (AESI) of cytokine release syndrome (CRS) or immune effector cell-associated neurotoxicity syndrome (ICANS) should have a blood sample drawn as soon as possible upon learning of the AESI, in order to allow for the profiling of cytokine changes related to the event.
11. **Arm 2 Anti-PD-1 Administration:** Participants randomized to Arm 2 should receive anti-PD-1 concurrently with lerapolturev while on study. Administration of the first anti-PD-1 dose should begin on C1D1 (+2 days). Where possible, participants should receive the same anti-PD-1 therapy that they previously failed prior to enrollment, dosed according to manufacturer's prescribing information.
12. **Lerapolturev Administration:** Up to 6 lesions may be injected with lerapolturev injection volume proportional to lesion size as determined from Table 7. The same or different lesion(s) may be injected at each treatment visit, as described in Section 5.3. The lerapolturev treatment cycle is Q3W for participants on Arm 1 and for Arm 2 or crossover participants receiving pembrolizumab, whether pembrolizumab is given Q3 or Q6W.
    - After Cycle 3, lerapolturev should be administered Q3W. If pembrolizumab is administered Q6W, lerapolturev should be continued Q3W.
    - **NOTE:** Participants previously treated with the **Q3/4W** lerapolturev schedule are allowed to receive the **QW** lerapolturev schedule per the guidance in Section 3.1.2.
    - Where possible, at least one target lesion should remain uninjected for as long as feasible while on study to facilitate the anti-tumor response assessment of non-injected target lesions
13. **Crossover from Arm 1 to Arm 2:** Participants enrolled in the safety run-in or randomized to Arm 1 are allowed to crossover to Arm 2 following radiologic disease progression (per RECIST criteria), once a PR lasts ≥ 6 months, or after 26 weeks on study without progression or confirmed PR (per RECIST criteria). Where possible, participants should receive the same anti-PD-1 therapy on which they had disease progression prior to enrollment, which should be administered according to manufacturer's prescribing information. The below assessments should be completed for participants at the time of crossover:
    - **Tumor Imaging:** All participants crossing over to Arm 2 should have a complete tumor assessment (ie, scans and ruler/caliper measurement of skin lesions with photography) within 28 days prior to the first cycle of the lerapolturev/anti-PD-1 combination. Scans and photographic ruler/caliper assessments performed to document disease progression prior to crossover are acceptable, provided they occur within 28 days prior to the initial treatment with the lerapolturev/anti-PD-1 combination.
    - **Cutaneous and Subcutaneous Measurements and Imaging:** For participants crossing over from Arm 1 to Arm 2, ruler/caliper measurement with photographs should be performed within 28 days prior to their first treatment with the lerapolturev/anti-PD-1 combination (along with scans).
    - **Lerapolturev Administration:** See Section 3.1.2 for guidance.
    - **Tissue Biopsy**: Prior to crossover, a mandatory tissue biopsy (injected lesions, noninjected lesions and draining lymph nodes) should be performed, within 1 week prior to the first cycle of the lerapolturev/anti-PD-1 combination (see Section 7.4); the biopsy taken at the time of PD can be used as the biopsy prior to the first crossover treatment cycle. Additional mandatory biopsies (injected lesions, noninjected lesions, and draining lymph nodes) should be performed 1 week (±2days) and 3 weeks (+7d) after Day 1 of crossover, and within 7 days of confirmed crossover PD. Any biopsy scheduled on same day as lerapolturev injection should be collected prior to treatment. .
14. **Lerapolturev Viral Shedding**:
    - The individual lesion receiving the largest injection volume of lerapolturev at C1D1, C1D15, and C2D15 should be designated for swabbing during that treatment cycle on the days indicated above (ie, the same lesion should be swabbed at each timepoint within a given cycle). Separate swabs should be used to test each area in triplicate (ie, samples are not to be combined or pooled), as described. Following sample collection, the swabbed lesion should be wiped with alcohol, and redressed with an occlusive dressing as required.
    - The lesion injected with lerapolturev at C1D1, C1D15, and C2D15 should be swabbed at least 15 minutes, but less than 4 hours, after the injection site has been cleaned and bandaged post-lerapolturev injection, as described in Section 3.2. All other lesion swabbing timepoints have a ±1D window for collection, but those scheduled on the same day of a given treatment cycle must be collected prior to lerapolturev administration.

- The injection site, as well as the inside and outside of the occlusive dressing should be swabbed independently (ie, samples are not to be combined or pooled into a single collection tube) in triplicate (ie, 3 swabs collected per area of interest), with the corresponding swab for each tested area labeled and placed into its own container of viral transport medium, according to the laboratory manual. The swabbed lesion should be cleaned and redressed with a new occlusive dressing once lesion swabbing has been completed, as described in Section 5.3.
- Stool samples may be collected at the participant's home up to 3 days prior to or after the C2D8 clinic visit, per the lab manual.

15. **End of Treatment Visit** should be performed within 28 days after the last dose of study drug(s). The visit should include a focused and symptom-directed physical assessment, cutaneous, subcutaneous, and nodal tumor measurements (including photographs), as well as radiographic tumor imaging.
16. **Survival Follow-up:** Scans and cutaneous, subcutaneous and nodal lesion measurements performed per SOC should still be collected for participants in post-EOT survival follow-up who discontinued study treatments for reasons other than disease progression. The collection of lesion measurement (cutaneous, subcutaneous, and nodal, and scans) performed per SOC should continue until confirmed progression or the start of the next anti-cancer therapy. Sites should continue to collect post-study therapies and survival status for follow-up duration

Table 2. Schedule of Assessments for Lerapolturev + Nivolumab

NOTE: Cycle length for lerapolturev + nivolumab is 4 weeks. Lerapolturev is administered weekly during Cycles 1 & 2 and every 4 weeks thereafter on the days indicated in the table below. Refer to the table below for the timing of other study assessments to be completed within each cycle.

Study Procedure[3]	Screening Period	Treatment			EOT Visit[15]	Survival Follow-up[16]
		Cycle 1	Cycle 2	≥ Cycle 3		
	(≤ 28D prior to C1D1)		+4W (+7D)	Q4W (+7D)	≤28D after last treatment	
Informed Consent[1]	X					
Medical History	X					
Physical Exam [2,3]	X	*C1D1*	*C2D1*	*Day 1 of each Cycle*	X	
Vital Signs[3]	X	*C1D1, C1D8, C1D15 C1D22*	*C2D1, C2D8, C2D15*	*Day 1 of each Cycle*	X	
ECOG Performance Status[3]	X	*C1D1*	*C2D1*	*Day 1 of each Cycle*	X	
PV Immunization Booster[1]	*≥1W, but ≤6W before C1D1*					
Adverse Events	*Continuous from signing ICF until 30 days after last dose of study therapy*					
Concomitant Medications	*Continuous from signing ICF until 30 days after last dose of study therapy*					
Subsequent Anticancer Therapy					X	X
AESI, irAEs and SAEs	*Continuous from signing ICF until 90 days after last dose of study therapy or resolution/stabilization*					
Hematology [3,4]	X	*C1D1*	*C2D1*	*C3D1 then Q8W (ie, at every other visit) and as clinically indicated*	X	
Chemistry[3,4]	X	*C1D1*	*C2D1*	*C3D1 then Q8W (ie, at every other visit) and as clinically indicated*	X	
INR, PT, PTT (or aPTT)	X	*As clinically indicated*				
Thyroid Monitoring [3,5]	X		X	*Day 1 of each Cycle*	X	
Pregnancy Test[6]	*≤2D before C1D1*	*Q12W (±14D)*				

Cutaneous, Subcutaneous, and Nodal Lesion Measurement and Photographs[3,7]	X	*C1D1, C1D8, C1D15 C1D22*	*C2D1, C2D8, C2D15*	*Day 1 of each Cycle*	X	*per SOC*
Tumor Imaging[8]	*≤6W before C1D1*	*6 & 12W after C1D1, then Q12W thereafter. Crossover participants must have re-baseline imaging within 28D prior to administration of crossover C1D1 (i.e. first dose of anti-PD-1 therapy)*				*per SOC*
Tissue Biopsy[9] *All samples to be collected prior to dosing at the given timepoint*	*Prior to PV Immunization Booster*	*C1D8, C1D22*		*At the time of PD ± 1 week (per RECIST)* ***Crossover Only:*** *within 1 week prior to crossover C1D1, 1 week after crossover C1D1 (pre-dose, if applicable), 3 weeks (+7d; pre-dose) after crossover C1D1, and at the time of PD ± 1 week (per RECIST) on the crossover regimen*		
Blood Collection[10] *All samples to be collected prior to dosing at the given timepoint*	*Prior to PV Immunization Booster*	*C1D1, C1D8, C1D22*	*C2D15*	*At the time of PD ± 1 week (per RECIST)* ***Crossover Only:*** *within 1 week prior to crossover C1D1, 1 week after crossover C1D1 (pre-dose, if applicable), 3 weeks (+7d; pre-dose) after crossover C1D1, and at the time of PD ± 1 week (per RECIST) on the crossover regimen*		
Lerapolturev Administration[12]		*C1D1, C1D8, C1D15 C1D22*	*C2D1, C2D8, C2D15*	*C3D1 and Q4W thereafter*		
ARM 2: Anti-PD-1 Administration (nivolumab) [11]		*C1D1 (C1D15 if nivolumab Q2W)*	*C2D1 (C2D15 if nivolumab Q2W)*	*C3D1 and Q2W or Q4W thereafter per nivolumab package insert*		
Crossover from Arm 1 to Arm 2 [13]		*If participant meets protocol-defined criteria*				
Lerapolturev Shedding: Injected Lesion[14]		*C1D1 (post-injection), C1D4, C1D8, C1D15 (post-injection), C1D18 C1D22*	*C2D8 (post-injection), C2D11, C2D15*			
Lerapolturev Shedding: Stool			*C2D1 (±3D)*			

AESI = adverse event of special interest; aPTT = activated partial thromboplastin time; C= cycle; CBC = complete blood count; CMP = comprehensive metabolic panel; CT = computed tomography; D= day; ECOG = Eastern Cooperative Oncology Group; EOT = End of Treatment; ICF = Informed consent

form; IgG = immunoglobulin G; INR = international normalized ratio; LDH = lactate dehydrogenase; MRI = magnetic resonance imaging; PD-1 = Programmed cell death protein 1; PT = prothrombin time; PTT = partial thromboplastin time; PV = poliovirus; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid stimulating hormone; SAE = serious adverse event; W = weeks

1. **Informed Consent** must be signed prior to initiation of screening activities.
2. **Physical Exam**: The screening physical examination should be a complete physical exam of major body systems and include, the general appearance of the participant, height and weight, vital signs (temperature, respiratory rate, blood pressure (systolic and diastolic [mmHg]), and heart rate [bpm]), examination of the skin, ears, nose, throat, lungs, heart, abdomen, extremities, musculoskeletal system, lymphatic system, and nervous system. During treatment and at the end of treatment (EOT) visit, weight assessment and limited symptom-directed physical examination are required. An evaluation of all skin lesions, including those injected with lerapolturev at the previous treatment visit, should be performed prior to subsequent lerapolturev injection.
3. **Procedures and/or Assessments** unless otherwise specified should occur on D1 of each Cycle. Any assessment or procedure scheduled on the same day as lerapolturev and/or anti-PD-1 administration should always precede receipt of study treatments. Note that laboratory assessments (e.g. hematology, chemistry, thyroid monitoring) may be done up to 2 calendar days prior to Day 1 of the cycle.
4. **Hematology and Chemistry:** Blood draws for hematology and chemistry occurring on the same day as study treatment should be collected prior to lerapolturev injection and/or prior to anti-PD-1 infusion (as applicable). **Hematology** should include hemoglobin, white blood cell (WBC) count with differential and platelet count. **Chemistry** measured in serum should include the following: LDH, glucose, calcium, sodium, potassium, bicarbonate, chloride, BUN, creatinine, total protein, albumin, ALP, AST, ALT, total bilirubin, amylase, and lipase.
5. **Thyroid Monitoring:** Should include TSH, free T4, and free T3.
6. **Pregnancy Test:** A pregnancy test (urine-based allowed) must be performed for all female participants of childbearing potential during Screening (≤2 days of lerapolturev injection) and approximately every 12 weeks while on study. If a urine-based test produces an equivocal result, a serum-based test should be performed.
7. **Measurement of Cutaneous, Subcutaneous, and Nodal Lesions**: Measurement by ruler or caliper (along with photographs) should occur prior to lerapolturev administration to determine/verify lerapolturev injection volumes for each lesion. For timepoint response assessment of target lesions not visible by radiography, ruler/caliper measurement with photographs should be performed prior to lerapolturev injection.
8. **Tumor Imaging:** Baseline imaging assessments should include CT of the chest, abdomen, and pelvis, a brain MRI, as well as photography of all cutaneous/visible lesions. Participants without evidence of central nervous system (CNS) disease at screening are not required to have brain imaging on study, provided clinical evidence of CNS disease does not emerge. All imaging should be performed with and without IV contrast (oral contrast can be used per institutional guidelines), provided participant can tolerate contrast agent (brain CT—preferably with IV contrast--is acceptable for those who cannot tolerate MRI). Photographic evaluation of visible lesions should precede lerapolturev injection, if occurring in the same visit. All qualifying scans prior to and during screening used to determine enrollment eligibility (ie, baseline scans prior to initiating anti-PD-1, as well as initial and confirmatory scans demonstrating disease progression per iRECIST prior to enrollment) should be provided to the central imaging vendor, where possible. Imaging should be performed using identical techniques and equipment, where possible.
9. **Tissue Biopsy for Biomarker Assessment:** Participants are required to provide a qualifying biopsy. Archival tissue collected ≤4 months prior to Day 1 is allowed in lieu of the qualifying biopsy, provided the participant has not received intervening systemic/intratumoral anti-cancer therapy since the biopsy was performed; if not available, the participant should have a qualifying biopsy taken during the Screening Period prior to polio boost vaccination. Biopsy of a previously irradiated lesion is not allowed unless there is documented disease progression in that lesion.
    - **NOTE:** At all timepoints, biopsies from all three sites (injected lesion, noninjected lesions and draining lymph nodes) should be collected if the biopsy is technically feasible and does not put the participant at significant risk. Examples of sites considered to be of significant risk include, but are not limited to, the following: biopsies of the brain, lung, mediastinum, pancreas, or endoscopic procedures extending beyond the esophagus, stomach, or bowel wall. For situations where the investigator determines biopsy of a lesion would (1) result in a significant decrease in the size of

the lesion such that RECIST 1.1 response assessment would be confounded, or (2) increase the risk of lerapolturev leakage from the lesion, the mandatory biopsy for that timepoint can be waived. In addition, complete resection of target lesions is prohibited unless necessary for participant's safety as determined by the investigator.

- Tissue from additional biopsies taken as part of standard of care may also be collected for analysis.

10. **Blood Collection:** Participants who experience an AE of Special Interest (AESI) of cytokine release syndrome (CRS) or immune effector cell-associated neurotoxicity syndrome (ICANS) should have a blood sample drawn as soon as possible upon learning of the AESI, in order to allow for the profiling of cytokine changes related to the event.
11. **Arm 2 Anti-PD-1 Administration:** Participants randomized to Arm 2 should receive anti-PD-1 concurrently with lerapolturev while on study. Administration of the first anti-PD-1 dose should begin on C1D1 (+2 days). Where possible, participants should receive the same anti-PD-1 therapy that they previously failed prior to enrollment, dosed according to manufacturer's prescribing information. The lerapolturev treatment cycle is Q4W for participants receiving nivolumab, whether nivolumab is given Q2 or Q4W.
12. **Lerapolturev Administration:** Up to 6 lesions may be injected with lerapolturev injection volume proportional to lesion size as determined from Table 7. The same or different lesion(s) may be injected at each treatment visit, as described in Section 5.3.
    - After Cycle 3, lerapolturev should be administered Q4W in Arm 2 participants receiving nivolumab. If nivolumab is administered Q2W, lerapolturev should be continued Q4W.
    - **NOTE:** Participants previously treated with the **Q3/4W** lerapolturev schedule are allowed to receive the **QW** lerapolturev schedule per the guidance in Section 3.1.2.
    - Where possible, at least one target lesion should remain uninjected for as long as feasible while on study to facilitate the anti-tumor response assessment of non-injected target lesions
13. **Crossover from Arm 1 to Arm 2:** Participants enrolled in the safety run-in or randomized to Arm 1 are allowed to crossover to Arm 2 following radiologic disease progression (per RECIST criteria), once a PR lasts ≥ 6 months, or after 26 weeks on study without progression or confirmed PR (per RECIST criteria). Where possible, participants should receive the same anti-PD-1 therapy on which they had disease progression prior to enrollment, which should be administered according to manufacturer's prescribing information. The below assessments should be completed for participants at the time of crossover:
    - **Tumor Imaging:** All participants crossing over to Arm 2 should have a complete tumor assessment (ie, scans and ruler/caliper measurement of skin lesions with photography) within 28 days prior to the first cycle of the lerapolturev/anti-PD-1 combination. Scans and photographic ruler/caliper assessments performed to document disease progression prior to crossover are acceptable, provided they occur within 28 days prior to the initial treatment with the lerapolturev/anti-PD-1 combination.
    - **Cutaneous and Subcutaneous Measurements and Imaging:** For participants crossing over from Arm 1 to Arm 2, ruler/caliper measurement with photographs should be performed within 28 days prior to their first treatment with the lerapolturev/anti-PD-1 combination (along with scans).
    - **Lerapolturev Administration:** See Section 3.1.1 for guidance.
    - **Tissue Biopsy**: Prior to crossover, a mandatory tissue biopsy (injected lesions, noninjected lesions and draining lymph nodes) should be performed, within 1 week prior to the first cycle of the lerapolturev/anti-PD-1 combination (see Section 7.4); the biopsy taken at the time of PD can be used as the biopsy prior to the first crossover treatment cycle. Additional mandatory biopsies (injected lesions, noninjected lesions, and draining lymph nodes) should be performed 1 week (±2days) and 3 weeks (+7d) after Day 1 of crossover, and within 7 days of confirmed crossover PD. Any biopsy scheduled on same day as lerapolturev injection should be collected prior to treatment.
14. **Lerapolturev Viral Shedding:**
    - The individual lesion receiving the largest injection volume of lerapolturev at C1D1, C1D15, and C2D8 should be designated for swabbing during that treatment cycle on the days indicated above (ie, the same lesion should be swabbed at each timepoint within a given cycle). Separate swabs

should be used to test each area in triplicate (ie, samples are not to be combined or pooled), as described. Following sample collection, the swabbed lesion should be wiped with alcohol, and redressed with an occlusive dressing as required.

- The lesion injected with lerapolturev at C1D1, C1D15, and C2D8 should be swabbed at least 15 minutes, but less than 4 hours, after the injection site has been cleaned and bandaged post-lerapolturev injection, as described in Section 3.2. All other lesion swabbing timepoints have a ±1D window for collection, but those scheduled on the same day of a given treatment cycle must be collected prior to lerapolturev administration.
- The injection site, as well as the inside and outside of the occlusive dressing should be swabbed independently (ie, samples are not to be combined or pooled into a single collection tube) in triplicate (ie, 3 swabs collected per area of interest), with the corresponding swab for each tested area labeled and placed into its own container of viral transport medium, according to the laboratory manual. The swabbed lesion should be cleaned and redressed with a new occlusive dressing once lesion swabbing has been completed, as described in Section 5.3.
- Stool samples may be collected at the participant's home up to 3 days prior to or after the C2D1 clinic visit, per the lab manual.

15. **End of Treatment Visit** should be performed within 28 days after the last dose of study drug(s). The visit should include a focused and symptom-directed physical assessment, cutaneous, subcutaneous, and nodal tumor measurements (including photographs), as well as radiographic tumor imaging.
16. **Survival Follow-up:** Scans and cutaneous, subcutaneous, and nodal lesion measurements performed per SOC should still be collected for participants in post-EOT survival follow-up who discontinued study treatments for reasons other than disease progression. The collection of lesion measurement (cutaneous, subcutaneous and nodal and scans) performed per SOC should continue until confirmed progression or the start of the next anti-cancer therapy. Sites should continue to collect post-study therapies and survival status for follow-up duration.

1 INTRODUCTION

1.1 Background

Metastatic melanoma is often a fatal disease, with less than 28% of patients surviving 5 years[5]. However, many patients are diagnosed with early-stage disease, where if well managed, the chance of cure can be quite high. Unfortunately, patients who recur or are diagnosed with advanced disease suffer a more aggressive disease course, making the likelihood of cure much less. For patients with Stage III disease, over 70% of patients' first recurrence was regional/nodal or systemic[8], meaning localized therapies (eg, surgery or isolated limb perfusion) are likely to have limited effect. Therefore, systemic therapies are often indicated, to mitigate further systemic spread and extend life.

The past 10 years has seen an increase in the number of systemic therapies available to melanoma patients able to improve outcomes. Most notable among these were the FDA approval of immune therapies able to specifically target PD-1/L1 and CTLA-4, which can be used alone or in combination in the front-line setting. When used as a single-agent, less than 40% of patients respond to anti-PD-1 therapy (ie, more than 60% have primary PD-1 resistance)[1,2]. Of those who do respond, 25% or more go on to develop an acquired resistance[3]. While combining PD-1 and CTLA-4 blockade can increase the response rate to around 50%, this combination is associated with significant toxicity, with clinical studies demonstrating ~55% of patients experience Gr 3/4 AEs[4]. Beyond immunotherapies, treatment options are limited. Patients whose tumors harbor BRAF mutations have the option to receive targeted therapies against BRAF and the mitogen-activated protein kinase /extracellular signal-regulated kinase (MEK) pathway. Although BRAF/MEK based targeted therapy has been shown to improve survival, progression free survival is often less than 1 year and systemic toxicities are common[9–11].

Recently, there has been interest in determining whether there is any potential for clinical benefit from anti-PD-1 re-challenge in patients who previously progressed on anti-PD-1/L1 or from treating beyond progression (TBP) in patients that are currently failing an anti-PD-1/L1. To the Sponsor's knowledge, no study has formally addressed whether anti-PD-1 rechallenge following discontinuation is beneficial[12], which may suggest this approach is not viable. For TBP, a pooled FDA analysis of melanoma clinical trials that allowed anti-PD-1 TBP has shown that continued anti-PD-1 beyond RECIST-defined progression can result in an ORR of up to 19%[13]. However, 66% of those treated beyond progression in this analysis were deemed to have progressed at their first post-treatment visit, and the median duration of TBP was only 1.41 months (IQR: 0.69–4.86 months). Of these patients with RECIST-defined PD at their first scan, approximately 36% discontinued due to the appearance of a new lesion without target lesion progression, which can be an immunoprogressive phenomenon that results in subsequent lesion contraction. In addition, the protocols allowing TBP within this pooled analysis had specific criteria to identify which patients were allowed to continue anti-PD-1, including

absence of clinical progression (including laboratory values), no decline in performance status, and absence of disease progression in areas requiring immediate medical treatment[13]. Given median time to response to anti-PD-1 is 2 to 3 months[14,15], it is possible that TBP in this select, well-performing population allowed more patients time for treatment response to occur. In contrast, had the definition of anti-PD-1/L1 resistance from the Society for Immunotherapy of Cancer (SITC) Immunotherapy Resistance Task force been applied to the population of this study (ie, ≥6 weeks of therapy with confirmation of PD by repeat imaging at least 4 weeks later), many patients would not have been included[7]. Thus, one can estimate that the true ORR for anti-PD-1 treatment beyond progression in patients treated per the SITC Immunotherapy Resistance Task Force definition of anti-PD-1 resistance is no greater than 6%, as nearly two-thirds of patients included within the TBP cohort would be excluded because of discontinuation without confirmation of progression 4 weeks later.

In addition, a recent study investigated ipilimumab alone or in combination with anti-PD-1 in an anti-PD-1 refractory population. ORR in the combination arm was 31% vs 12% for ipilimumab alone, and OS at 1-year for the combination was also superior versus ipilimumab alone, suggesting that the addition of new immunotherapies to failing anti-PD-1 therapy improves outcomes when compared to a switch strategy[16]. However, Grade 3 or greater toxicities occurred in over 30% of the patients receiving ipilimumab, whether alone or in combination, meaning risk to patient safety remains a concern. Taken together, there is significant interest in the development of novel immunotherapeutic agents that can increase the anti-tumor response for the broad proportion of melanoma patients unable to benefit from currently approved options.

Lerapolturev (formerly known as PVSRIPO) is a recombinant rhinovirus/polio virus chimera that may affect anti-tumor activity through two mechanisms: direct tumor cell killing and induction of a secondary anti-tumor immune response[17]. Lerapolturev has demonstrated clinical activity in patients with primary brain tumors[18]. Preliminary data suggest lerapolturev has activity, either alone or in combination with an anti-PD-1 inhibitor, in PD-1 refractory melanoma when injected intratumorally (summarized below). Therefore, this study is designed to further characterize lerapolturev activity in advanced/metastatic anti-PD-1/L1 refractory melanoma.

1.2 Rationale

Even when accepting the risk for significant toxicity, most melanoma patients are unable to benefit from currently approved immunotherapy combinations, which suggests alterative mechanisms for immune activation are required to safely trigger an anti-tumor immune response. One alternative approach is the use of oncolytic viruses, which depending on the virus, can cause direct tumor cell killing and secondary anti-tumor immune response. Talimogene laherparepvec (T-VEC, Imlygic), a modified herpes simplex type 1 virus expressing human granulocyte macrophage colony-stimulating factor (GM-CSF), has been approved to treat melanoma based on increased DRR (defined as

the percent of patients with complete response (CR) or partial response (PR) maintained continuously for a minimum of 6 months) compared to GM-CSF alone: The DRR was 16.3% in the IMLYGIC arm and 2.1% in the GM-CSF arm in the overall study population. There was no statistically significant difference in overall survival (OS) between the IMLYGIC and the GM-CSF arms; the median OS in the overall study population was 22.9 months in the IMLYGIC arm and 19.0 months in the GM-CSF arm (p = 0.116)[19].

However, data from a Phase 1b trial investigating T-VEC (administered by intratumoral injection every 2 weeks) in combination with pembrolizumab in advanced anti-PD-1 naïve melanoma showed >60% of participants achieved an objective response, with multiple responses ongoing at the time of publication (median OS and progression-free survival not reached)[20]. Response to the combination was associated with increased CD8+ T-cell infiltration and increased expression of programmed death-ligand 1 (PD-L1; the immunosuppressive ligand of PD-1) following treatment, suggesting an oncolytic virus in combination with anti-PD-1 could potentially overcome mechanisms of anti-PD-1 resistance.

Although these data are encouraging, the follow-on randomized, placebo-controlled Phase 3 study failed to show a survival benefit and was halted for futility[21], suggesting alternative approaches (such as optimized dosing schedules) may be required to translate apparent immune activation into clinical benefit – particularly in populations known to be refractory to anti-PD-1/L1-based therapies. In support of this hypothesis, preliminary data from an ongoing Phase 2 study investigating intratumoral injection of BO-112 (a non-coding double-stranded RNA formulated with the cationic carrier polyethylenimine and administered weekly x 7 followed by every 3-week maintenance) in combination with pembrolizumab has shown activity in anti-PD-1 refractory melanoma. BO-112 induces innate immunity through activation of RNA viral response sensors (eg, RIG-I and MDA5), which resulted in a 27% ORR when combined with pembrolizumab, suggesting a more persistent stimulation of innate immunity may be required to improve immune-mediated anti-tumor response.

Given these data, it is hypothesized that weekly administration of lerapolturev in the setting of a vaccine-generated immune recall response will more effectively activate innate RNA viral response pathways when compared to oncolytic viruses like T-VEC or non-coding double-stranded RNA like BO-112. Lerapolturev is a live-attenuated, serotype 1 poliovirus vaccine (Sabin) that was genetically modified by exchange of the cognate internal ribosomal entry site (IRES) with that of human rhinovirus type 2, thus eliminating its neurovirulence potential[22]. Infection of cells by lerapolturev is mediated via the poliovirus receptor (PVR), CD155--a cell adhesion molecule of the Ig-like superfamily that is broadly expressed in solid tumors, including melanoma[23]. Upon infection, lerapolturev is directly cytotoxic to neoplastic cells, which offer ideal conditions for viral IRES-mediated ribosome recruitment due to the unhinged protein synthesis required in cancer cells[24]. In contrast, lerapolturev infection of antigen-presenting macrophages and dendritic cells

(DC) results in a non-lethal infection, and activation of innate interferon gamma (IFNγ) pathways to increase immune effector responses directed against tumor neoantigens[17], thus driving a secondary immune response. As shown in Figure 1, lerapolturev infection resulted in death of breast and melanoma tumor cells without affecting DC viability. The principal elements determining lerapolturev tumor tropism, tumor-specific cell killing (Figure 1A & B), neuronal incompetence/safety, and immunogenicity are well established empirically[17,25]. Notably, lerapolturev infection in DCs significantly increased PD-L1 expression (Figure 1C), suggesting lerapolturev in combination with anti-PD-1 blockade in the tumor is rational, as the combination could further enhance the immune system's ability to specifically recognize and destroy tumor.

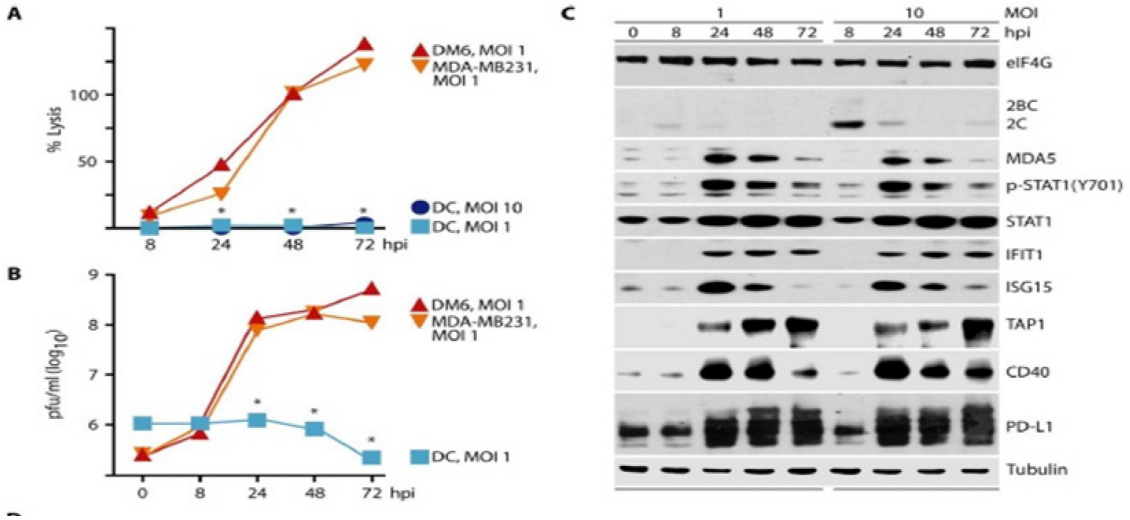

Figure 1. Lerapolturev is directly lethal to breast and melanoma cell lines, yet lerapolturev infection of DCs is sublethal and induces sustained proinflammatory cytokine production.

In further support of this rationale, data from an ongoing Phase 1 dose-finding study investigating lerapolturev in anti-PD-1 refractory melanoma (NCT03712358) suggests that lerapolturev is well-tolerated and has anti-tumor activity in this difficult to treat population. As of 23 March 2021, 12 participants were enrolled in the study, each receiving a single lerapolturev injection into a single lesion in up to 3 separate treatments. There were no SAEs noted; all AEs were Grade 1 or 2, with all but two lerapolturev-related AEs (n=1 hot flash and n=1 fatigue; both Grade 1) localized to the injected cutaneous, subcutaneous or nodal lesion. Despite the limited number of treatments administered, four of the 12 participants achieved an objective response, including 2 PRs which were determined to be pathologic complete responses (pCR) upon biopsy (remaining lesions contained melanophages only, without evidence of melanoma). Among participants who received 3 lerapolturev injections (maximum number administered), objective responses were observed in 4/6 (67%) participants. In addition, most participants were subsequently re-challenged with anti-PD-1 after lerapolturev,

which resulted in durable disease control in 3 additional participants, including a PR and a pCR[6]. Taken together, these data suggest lerapolturev is not only safe and well-tolerated, but that lerapolturev could have direct anti-tumor activity in an anti-PD-1 resistant melanoma population and could act synergistically in combination with anti-PD-1.

Given the safety and anti-tumor activity noted in Phase 1 following limited lerapolturev treatment, the purpose of LUMINOS-102 is to characterize the safety and efficacy of lerapolturev when injected into multiple tumors over time, as well as to assess the antitumor activity of lerapolturev in combination with anti-PD-1. This study was originally designed to test lerapolturev injection in up to 6 lesions (or max dose of 6 x 10^8 $TCID_{50}$) given every 3 to 4 weeks, concurrent with or without anti-PD-1 antibody therapy, until progression. Despite a 6-fold increase in lerapolturev dose relative to the dose evaluated in the Phase 1 study, all AEs considered related to lerapolturev, or anti-PD-1 remain Grade 1 or 2; no dose-limiting toxicities (DLTs), treatment-related serious AEs (SAEs) or signs and symptoms of cytokine release syndrome were reported in the 17 participants treated as of the 10 December 2021 data cutoff (see Table 3 and Table 4). Therefore, based on the encouraging safety data generated with lerapolturev to date, together with the observation that more frequent administration of intratumoral therapies with similar mechanisms of action is tolerable and able to induce anti-tumor responses in patients with anti-PD-1 resistant melanoma, the safety and efficacy of lerapolturev given weekly for 7 weeks followed by administration every 3 or 4-weeks (maintenance) at a dose of up to 1.6 x 10^9 $TCID_{50}$, as monotherapy and in combination with anti-PD-1 therapy will be tested.

1.2.1 Nonclinical Studies

Refer to the lerapolturev Investigator's Brochure (IB) for details on nonclinical and preclinical studies. In animal tumor models, oncolytic PVs elicit efficient anti-neoplastic effects resulting in tumor regression and, eventually, destruction[17]. This includes histologic evidence for direct, virus-mediated tumor cell killing and indirect, host-mediated inflammatory responses directed against tumor.

As described in the IB, a murine-specific version of lerapolturev called mRIPO was highly active in a melanoma mouse model[26]. B16-F10.9-OVA-CD155 tumor cells were implanted in the flank of mice and treated with a single intratumoral injection of DMEM (control) or mRIPO ($5x10^7$ $TCID_{50}$). mRIPO therapy significantly delayed tumor growth as measured by tumor volume (Figure 2B) and increased overall survival (Figure 2C). mRIPO therapy, but not the DMEM control, produced anti-tumor cytotoxic T lymphocytes (CTLs): cells harvested from draining inguinal lymph nodes exhibited cytotoxicity against EL4 thymoma cells expressing TRP-2 (B16-F10 melanoma antigen), OVA (B16-F10-OVA antigen), or B16-F10.9-OVA-CD155 cells themselves (Figure 2D). Effector T cells from control treated mice exhibited minimal cytotoxicity against any of the tested target cells

(Figure 2D). Analysis of cytokines in supernatant revealed increased granzyme B, IFN-γ, and TNF-α secretion only in the mRIPO-treated group (Figure 2E), which corroborates CTL activity observed in Figure 2D.

Systemic T cell activation also occurred in spleens harvested 14 days after mRIPO treatment. Splenocytes were co-cultured with B16-F10.9-OVA-CD155 cells for 2 days and supernatant was assessed for T cell effector cytokines and granzyme B. Splenocytes from mRIPO-treated tumor-bearing mice exhibited T cell effector function against B16-F10.9-OVA-CD155 cells (Figure 2F).

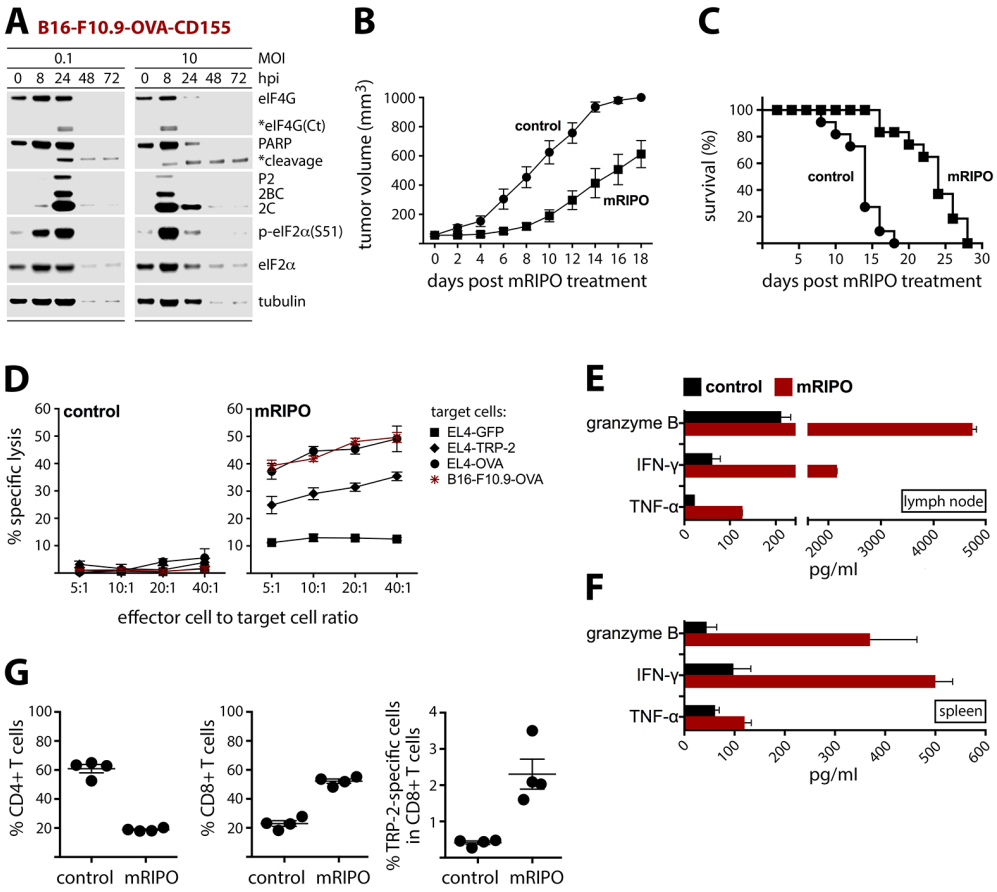

Figure 2. Lerapolturev restricts tumor growth and produces antigen-specific anti-tumor immunity[25]

A. Cytotoxicity characteristics of mRIPO in murine melanoma B16-F10.9-OVA-CD155 cells. **B**., **C**. Subcutaneous B16-F10.9-OVA-CD155 tumors were implanted in C57BL/6-CD155 mice and treated when the volume was ~50-100 mm^3 (~15-18 days post tumor implant). Tumors were injected with either control (DMEM) or mRIPO in a volume of 20 µl. Tumor volume was measured (n = 11 per group) on the days indicated. Mice were sacrificed when the tumor volume reached 1000 mm^3, data from two pooled experiments are shown. **D**. Tumor draining inguinal lymph nodes were harvested from mice (n=4) 7 days post treatment with control DMEM/mRIPO and re-stimulated with antigen expressing cells for 5 days. Re-stimulated cells were harvested, washed, and tested for lytic activity against B16-F10.9-OVA cells or EL4

cells electroporated with RNA encoding: GFP (control), TRP-2 (melanoma antigen), or OVA. **E**. Supernatant from the CTL assay in **D**. was tested for markers of T cell activation and lytic activity by ELISA. **F**. B16-F10.9-OVR-CD155 tumor bearing mice were treated with control DMEM/mRIPO and spleens were harvested 14 days after treatment (n=4). Splenocytes from individual mice were co-cultured with B16-F10.9-OVA-CD155 cells (5:1 ratio) (48h). Supernatant was harvested and tested for markers of T cell activation and lytic activity by ELISA. **G**. Tumor draining inguinal lymph nodes were harvested following treatment with control DMEM/mRIPO and individually re-stimulated in vitro. After 5 days, re-stimulated cells were harvested and analyzed for CD4 and CD8 T cells by flow cytometry. Melanoma antigen TRP-2 specific response was assessed using a H-2Kb TRP-2 tetramer (right panel). TRP-2 specific responses were compared using student t test and $p<0.05$.

Istari and collaborators have conducted preclinical studies to support lerapolturev plus anti-PD1 therapy in this setting, which in summary have produced the following results:

- Up-regulation of PD-L1 expression in lerapolturev infected macrophages and dendritic cells, suggesting lerapolturev in combination with anti-PD-1 blockade in the tumor is rational[17,26].

- Lerapolturev infection of tumor-associated macrophages (TAMs), and type-I/III interferon dominant antiviral inflammation of the nonmalignant tumor microenvironment (TME), instigate polyfunctional antitumor CD8 T cell responses[27]. This leads to upregulation of the PD1 immune checkpoint on tumor-infiltrating T cells. Thus, lerapolturev's mechanism of action is synergistic with blockade of PD-1/L1 interaction.

- Synergy of lerapolturev and anti-PD-1 therapy has been demonstrated in a mouse glioma model (refer to lerapolturev IB).

Lerapolturev was subjected to extensive dose-range finding, toxicology, biodistribution, shedding and neutralizing antibody tests with intrathalamic inoculation of up to 5×10^9 $TCID_{50}$ of lerapolturev in M. Fascicularis[28]. These revealed: (i) absence of morbidity and mortality; (ii) absence of neuropathological signs consistent with virus-induced central nervous system (CNS) damage; (iii) absence of virus dissemination from the brain or viremia; (iv) absence of extraneural replication; (v) absence of shedding with saliva, urine or stool; (vi) presence of a neutralizing antibody response.

1.2.2 Clinical Studies

1.2.2.1 Lerapolturev in patients with recurrent glioblastoma (rGBM)

The safety and efficacy of lerapolturev monotherapy and lerapolturev in combination with pembrolizumab in patients with recurrent glioblastoma multiforme (rGBM) is currently under investigation. Lerapolturev monotherapy has been administered intratumorally to more than 190 rGBM participants via convection enhanced delivery (CED). The first-in-human Phase I (NCT01491893) evaluated 7 lerapolturev doses, ranging from 1×10^7 and

1 x 10^{10} $TCID_{50}$[18]. Across all doses tested, one dose limiting toxicity (DLT) of intracranial hemorrhage due to removal of the infusion catheter post infusion was observed in a single participant. No other DLTs were reported. The most commonly (≥30% of total participants) reported treatment-related AEs were consistent with the signs and symptoms expected to be observed with increased peritumoral edema resulting from lerapolturev induced inflammation after intratumoral infusion of the brain tumor. There was no evidence of viral encephalomyelitis, poliomyelitis, meningitis, or systemic autoimmune reactions in any participant treated at any dose level.

For the follow-on Phase 2 study of lerapolturev monotherapy in rGBM (NCT02986178), a dose of 5 x 10^7 $TCID_{50}$ delivered via CED was selected. The most commonly (≥30% of total participants) reported treatment-related AEs were headache (62.0%), hemiparesis (57.9%), seizure (40.5%), and aphasia (30.6%). This AE profile is consistent with the treatment-related AEs observed during Phase 1, which are signs and symptoms expected to be observed with increased peritumoral edema resulting from lerapolturev-induced inflammation after intratumoral infusion of the brain tumor. With a median follow-up of 17.1 months (range: 13.1 to 46), six participants had PRs per investigator determination and median OS was 12.0 months. Landmark OS at 12 and 24 months were 50% and 17%, which taken together is consistent with the efficacy results observed in Phase 1.

LUMINOS-101 (NCT04479241) is an ongoing single-arm Phase 2 study investigating the safety and efficacy of a single intratumoral infusion of lerapolturev (5 x 10^7 $TCID_{50}$) in combination with intravenous pembrolizumab (200mg every three weeks) in adults with rGBM. No DLTs were reported during the safety run-in (n=3). As of 16 September 2021, 15 participants were enrolled and the most common AEs (≥25% of total participants) regardless of relatedness were headache (66.7%), nausea (33.3%), fatigue (26.7%), and peritumoural oedema (26.7%). The most common lerapolturev-related AEs (≥15% of total participants) were headache (66.7%), peritumoural oedema (26.7%), cognitive disorder (20%), dizziness (20.0%), fatigue (20.0%), and hemiparesis (20.0%). These data suggest that the addition of anti-PD-1 therapy (ie, pembrolizumab) does not significantly affect the safety profile of lerapolturev, and the combination of lerapolturev and anti-PD-1 therapy is tolerable. Furthermore, there was no evidence of viral encephalomyelitis, poliomyelitis, meningitis, or systemic autoimmune reactions in any participant receiving lerapolturev combined with pembrolizumab. For more information, consult the IB.

1.2.2.2 Lerapolturev in patients with melanoma

Lerapolturev has been administered via intratumoral injection (1 x 10^8 $TCID_{50}$ per lesion per visit) to 12 participants in a Phase 1 open-label study with unresectable and/or metastatic anti-PD-1 refractory melanoma (as defined by the investigator) as of 23 March 2021 (NCT03712358). No serious adverse events (SAEs) or dose-limiting toxicities (DLTs) were observed, and administration of 1 x 10^8 $TCID_{50}$ lerapolturev to one lesion/visit

for up to three successive visits was well tolerated. This included 5 participants that received 3 injections of lerapolturev who had received prior anti-PD-1 therapy within 30 days prior to administration of lerapolturev (and thus had anti-PD1 therapy on board during lerapolturev); none of these participants experienced systemic toxicities. All AEs were Grade 1 or 2 in severity (pruritis [58%] and erythema [50%]) and primarily localized to the injected lesion(s).

Based on the favorable tolerability profile, and encouraging preliminary anti-tumor activity (33% ORR per irRC) noted in Phase 1, this study (NCT04577807) was designed to test the safety and efficacy of lerapolturev alone or in combination with anti-PD-1 therapy, in participants with unresectable melanoma who meet the more rigorous and objective definition for anti-PD-1 resistance, as described in the SITC Immunotherapy Resistance Task Force Guidance[7]. Despite a 6-fold increase in the total maximum lerapolturev dose relative to Phase 1 (ie, 6 x 10^8 $TCID_{50}$ versus 1 x 10^8 $TCID_{50}$ in Phase 1) and/or injection of up to 6 lesions per treatment visit (compared to 1 lesion/visit in Phase 1), lerapolturev remains well tolerated, whether given alone or when combined with anti-PD-1 therapy. As summarized in Table 3 and Table 4, 17 participants were treated with lerapolturev monotherapy (n=11) or lerapolturev combined with anti-PD-1 therapy (n=6) as of the 10 December 2021 data cutoff. Additionally, 7 lerapolturev monotherapy participants crossed over to receive the lerapolturev/anti-PD-1 combination.

An overall summary of AEs can be found in Table 3. No DLTs, lerapolturev AEs of special interest, treatment-related Grade ≥3 AEs, treatment-related SAEs, or Grade 5 AEs regardless of relationship were reported. The most common (of total participants) AEs regardless of relationship were vomiting (17.6%), constipation (11.8%), fatigue (11.8%), hypertension (11.8%), nausea (11.8%), pain in extremity (11.8%), pyrexia (11.8%), and tumour pain (11.8%). The only AE related to lerapolturev that was reported in more than 1 participant was fatigue, which was observed in 2 participants (11.8%) (Table 4). There were no Grade ≥3 AEs that occurred in more than 1 participant and none were related to either lerapolturev or anti-PD-1 therapy. No participant reported symptoms of CRS or immune effector cell associated neurotoxicity syndrome (ICANS). (Table 3). With regards to efficacy, the best anti-tumor responses observed as of the data cutoff have been stable disease. Together, these data suggest further optimization of the lerapolturev dose and/or schedule may be required to rekindle immunotherapy responsiveness in the truly refractory population. For more information, consult the IB.

Table 3. LUMINOS-102 Overall Summary of Adverse Events as of 10DECEMBER2021

Summary	Lerapolturev Monotherapy, Safety Run-in[1] (N=6)	Lerapolturev Monotherapy (Arm 1)[1] (N=5)	All Lerapolturev Monotherapy[1] (N=11)	Lerapolturev/ Anti-PD-1 (Arm 2) (N=6)	All Participants[1] (N=17)	Crossover[2] Lerapolturev Monotherapy to Lerapolturev/Anti-PD-1 (N=7)	All Lerapolturev/ Anti-PD-1 (N=13)
Participants with one or more AEs, n (%)	6 (100.0%)	2 (40.0%)	8 (72.7%)	2 (33.3%)	10 (58.8%)	5 (71.4%)	7 (53.8%)
Participants with one or more treatment-related[3] AEs, n (%)	4 (66.7%)	1 (20.0%)	5 (45.5%)	2 (33.3%)	7 (41.2%)	2 (28.6%)	4 (30.8%)
Lerapolturev-related AEs, n (%)	4 (66.7%)	1 (20.0%)	5 (45.5%)	1 (16.7%)	6 (35.3%)	2 (28.6%)	3 (23.1%)
Anti-PD-1-related AEs, n (%)	NA	NA	NA	1 (16.7%)	1 (5.9%)	2 (28.6%)	3 (23.1%)
Participants with one or more serious AEs, n (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (16.7%)	1 (5.9%)	0 (0.0%)	1 (7.7%)
Total number of serious AEs, n	0	0	0	3	3	0	3
Participants with one or more treatment-related[3] serious AEs, n (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Lerapolturev-related serious AEs, n (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Anti-PD-1-related serious AEs, n (%)	NA	NA	NA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Total number of treatment-related[3] serious AEs, n	0	0	0	0	0	0	0
Lerapolturev-related serious AEs, n	0	0	0	0	0	0	0
Anti-PD-1-related serious AEs, n	NA	NA	NA	0	0	0	0
Participants with one or more dose-limiting toxicities AEs, n (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Participants with one or more Grade 3 or 4 AEs, n (%)	2 (33.3%)	0 (0.0%)	2 (18.2%)	1 (16.7%)	3 (17.6%)	1 (14.3%)	2 (15.4%)
Participants with any Grade 3 or 4 Treatment-related[3] AE, n (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Lerapolturev-related Grade 3 or 4 AEs, n (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Anti-PD-1-related Grade 3 or 4 AEs, n (%)	NA	NA	NA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Participants with one or more Grade 5 AEs, n (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Summary	Lerapolturev Monotherapy, Safety Run-in[1] (N=6)	Lerapolturev Monotherapy (Arm 1)[1] (N=5)	All Lerapolturev Monotherapy[1] (N=11)	Lerapolturev/ Anti-PD-1 (Arm 2) (N=6)	All Participants[1] (N=17)	Crossover[2] Lerapolturev Monotherapy to Lerapolturev/Anti-PD-1 (N=7)	All Lerapolturev/ Anti-PD-1 (N=13)
Participants with any Grade 5 treatment-related[3] AE, n (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Lerapolturev-related Grade 5 AEs, n (%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Anti-PD-1-related Grade 5 AEs, n (%)	NA	NA	NA	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Participants with one or more lerapolturev AESIs	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Participants with one or more AEs with an ISR	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Participants with one or more AEs with signs of CRS/ICANs	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

AESI = adverse event of special interest; CRS = cytokine release syndrome; ICANS = immune effector cell associated neurotoxicity syndrome; ISR = injection site reaction; NA = not applicable; PD-1 = programmed cell death protein 1; AE = adverse event

[1] Data shown do not include data collected during crossover.

[2] Crossover starts with first administration of first crossover cycle. Pooled data presented for all crossover participants.

[3] Treatment-related based on definite, possible, and probable relatedness to lerapolturev and/or anti-PD-1.

Table 4. LUMINOS-102 Lerapolturev-Related Adverse Events by Preferred Term in Descending Frequency as of 10DECEMBER2021

Summary	Lerapolturev Monotherapy, Safety Run-in[1] (N=6)	Lerapolturev Monotherapy (Arm 1)[1] (N=5)	All Lerapolturev Monotherapy[1] (N=11)	Lerapolturev/ Anti-PD-1 (Arm 2) (N=6)	All Participants[1] (N=17)	Crossover[2] Lerapolturev Monotherapy to Lerapolturev/Anti-PD-1 (N=7)	All Lerapolturev/ Anti-PD-1 (N=13)
Participants with one or more Lerapolturev-Related[3] AEs, n (%)	4 (66.7%)	1 (20.0%)	5 (45.5%)	1 (16.7%)	6 (35.3%)	2 (28.6%)	3 (23.1%)
Preferred term, n (%)							
Fatigue	1 (16.7%)	1 (20.0%)	2 (18.2%)	0 (0.0%)	2 (11.8%)	1 (14.3%)	1 (7.7%)
Anaemia	1 (16.7%)	0 (0.0%)	1 (9.1%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	0 (0.0%)
Decreased appetite	1 (16.7%)	0 (0.0%)	1 (9.1%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	0 (0.0%)
Dyspnoea	1 (16.7%)	0 (0.0%)	1 (9.1%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	0 (0.0%)
Groin pain	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (16.7%)	1 (5.9%)	0 (0.0%)	1 (7.7%)
Hyperglycaemia	1 (16.7%)	0 (0.0%)	1 (9.1%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	0 (0.0%)
Hypoalbuminaemia	1 (16.7%)	0 (0.0%)	1 (9.1%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	0 (0.0%)
Hypochloraemia	1 (16.7%)	0 (0.0%)	1 (9.1%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	0 (0.0%)
Hyponatraemia	1 (16.7%)	0 (0.0%)	1 (9.1%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	0 (0.0%)
Lymphadenopathy	1 (16.7%)	0 (0.0%)	1 (9.1%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	0 (0.0%)
Nausea	1 (16.7%)	0 (0.0%)	1 (9.1%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	0 (0.0%)
Oncologic complication	1 (16.7%)	0 (0.0%)	1 (9.1%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	0 (0.0%)
Pain in extremity	1 (16.7%)	0 (0.0%)	1 (9.1%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	0 (0.0%)
Paraesthesia	1 (16.7%)	0 (0.0%)	1 (9.1%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	0 (0.0%)
Peripheral swelling	1 (16.7%)	0 (0.0%)	1 (9.1%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	0 (0.0%)
Pyrexia	1 (16.7%)	0 (0.0%)	1 (9.1%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	0 (0.0%)
Tumour pain	1 (16.7%)	0 (0.0%)	1 (9.1%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	0 (0.0%)
Vomiting	1 (16.7%)	0 (0.0%)	1 (9.1%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	0 (0.0%)
Wound haemorrhage	1 (16.7%)	0 (0.0%)	1 (9.1%)	0 (0.0%)	1 (5.9%)	0 (0.0%)	0 (0.0%)
Headache	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (14.3%)	1 (7.7%)
Pruritus	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (14.3%)	1 (7.7%)

, PD-1 = programmed cell death protein 1; AE = adverse event
[1] Data shown do not include data collected during crossover.
[2] Crossover starts with first administration of first crossover cycle. Pooled data presented for all crossover participants.
[3] Includes AEs considered definitely, probably or possibly related to lerapolturev.

1.3 Benefit/Risk Assessment

1.3.1 Known Potential Risks

Risks related to lerapolturev administration: For detailed risks regarding use of lerapolturev, refer to the IB. Based on available data from ongoing Phase 1 and Phase 2 trials in rGBM, lerapolturev infusion into CNS tumors can result in peritumoral edema during the first 12 months post-infusion. This peritumoral inflammation can manifest as transient neurologic changes or exacerbate existing neurologic signs and symptoms, which are best managed with low dose dexamethasone and/or bevacizumab. In contrast, lerapolturev injection into solid tumors outside the brain has resulted in treatment-related AEs that are only Grade 1 or 2. For the Phase 1 melanoma study in anti-PD-1 refractory melanoma, lerapolturev-related AEs were primarily localized to the injected lesion(s), with pruritis (45.5%) and erythema (27.3%) at the injection site most commonly reported. As discussed in Section 1.2.2, all treatment-related AEs in participants enrolled in this study as of the 10 December 2021 data cutoff continue to be Grade 1 or 2, whether lerapolturev is given as monotherapy or in combination with anti-PD-1 therapy. Fatigue was the only lerapolturev-related AE reported in >10% of participants (11.8%).

After oral uptake, poliovirus replicates in the gastrointestinal tract and is excreted by infected individuals in stool. Per the Istari IB, analysis of viral shedding in non-human primates have shown that excretion of lerapolturev does not occur after intracerebral administration, implying the absence of dissemination and subsequent gastrointestinal replication. In addition, prior immunization against poliovirus, as well as administration of a poliovirus booster 1 to 6 weeks prior to lerapolturev administration, is required for study participation. Immunity to poliovirus following proper immunization is believed to be lifelong[29], which is supported by a study from Rumke et al, who showed that 92% (22 of 24) of participants previously immunized against poliovirus with an undetectable poliovirus titer had a four-fold increase in neutralizing antibodies within 1 week of DT-IPV administration[30]. Therefore, lerapolturev dissemination in participants treated with lerapolturev is less likely given the high-titer immune response to poliovirus that is present following immunization and pre- lerapolturev boosting with the Salk poliovirus vaccine (Istari data on file). Lerapolturev has not been detected in the stool of participants with rGBM treated with intratumoral injection. However, lerapolturev excretion within stool following subcutaneous injection of melanoma tumors cannot be categorically excluded, although viral shedding data from the Phase 1 melanoma study indicate lerapolturev was undetectable in the stool of the tested melanoma participants treated with lerapolturev (Istari data on file). Again, high-titer poliovirus immunity was demonstrated both pre- and post-IPOL® vaccination in these participants. Stool specimens and swabs of injected lesions and dressings will be collected in the current study following lerapolturev administration to further rule out risk of virus transmission, systemic dissemination, and gastrointestinal excretion during the study.

Given current clinical and non-clinical data demonstrate the lack of systemic dissemination or replication and the absence of virus shedding through stool, the likelihood of unintended exposure of participant household contacts is low. If accidental exposure occurred, it would equal the risk of exposure to any type 1 Sabin vaccine virus or vaccine virus derivatives. Thus, exposure to lerapolturev is equal to oral immunization with a safer version of type 1 Sabin. Because type 1 Sabin vaccine virus or vaccine virus derivatives have to be considered part of the human environment, exposure to lerapolturev would not represent an added risk beyond the possibility for exposure that already exists. Study participants will be advised of the risks of exposure to unvaccinated household contacts (such as babies), or those who might be immunocompromised.

Risks related to modifying the dose/schedule of lerapolturev: Review of all available safety data for this study (Section 1.2.2.2) indicates that lerapolturev injected into multiple lesions/visit up to a maximum dose of 6 x 10^8 $TCID_{50}$ every 3 weeks is safe and tolerable when administered as monotherapy or in combination with anti-PD-1 therapy. In addition, review of the published literature (Table 5) for other innate immune agonists (including oncolytic viruses) delivered by intratumoral injection, indicates that increasing the maximum possible dose of lerapolturev to 1.6 x 10^9 $TCID_{50}$ and changing the schedule of administration to an “induction” phase of weekly injections x 7 followed by a maintenance phase of less frequent administration is a common approach that is safe and tolerable and not predicted to substantially change the safety profile of the drug.

Table 5. Oncolytic Virus Doses and Schedules

Innate Immune Agonist	Total maximum dose delivered by intratumoral injection	Schedule	Safety	Reference
BO-112 (double-stranded RNA in carrier)	2mg	Weekly x 7 followed by every 3 week maintenance	No treatment-related Grade ≥3 AEs	Marquez-Rodas et al 2021[31]
Vidutolimod (TLR9 agonist in virus-like particle)	10mg	Weekly x 7 followed by every 3 week maintenance	No treatment-related Grade ≥4 AEs	Kirkwood et al 2021[32]
CAVATAK (coxsackievirus)	3×10^8 $TCID_{50}$	Day 1, 3, 5, 8, and 22 followed by every 3 week maintenance	No DLTs or treatment-related Grade ≥3 AEs	Andtbacka et al 2021[33]
ONCOS-102 (adenoviral vector with payload)	3×10^{11} viral particles	Day 1, 4, 8, 15, and 19 followed by every 4 week maintenance	No DLTs	Ranki et al 2016[34]
NG-350A (adenoviral vector with payload)	2×10^{12} viral particles	Weekly x 4	No DLTs	Nain et al 2021[35]
ORCA-010 (adenoviral vector)	1.5×10^{12} viral particles	One dose	No DLTs or treatment-related Grade ≥3 AEs	Brachtlova et al 2021[36]
ONCR-177 (HSV-1 vector with payload)	4×10^8 pfu	Every 2 weeks	No DLTs or treatment-related Grade ≥3 AEs	Park et al 2021[37]
IMLYGIC (HSV-1 vector with payload)	4×10^8 pfu	Every 2 weeks	No DLTs	Hu et al 2006[38]

Risks related to anti-PD-1 administration: Anti-PD-1 administration carries the risk of immune-related AEs (irAEs) in various organ systems, including the skin. Consult the current pembrolizumab or nivolumab package insert for the most current information related to administration and safety, such as AE management and dose modifications. Per the respective package inserts for nivolumab and pembrolizumab, administration of either anti-PD-1 can be associated with rash, and pruritis has been reported in melanoma patients treated with nivolumab. Given lerapolturev's mechanism of action, and the monotherapy safety profile observed in melanoma participants treated to date, it is possible that concurrent use of lerapolturev with anti-PD-1 therapy could increase the frequency or severity of cutaneous irAEs like rash or pruritis. However, review of the available safety data for participants treated with the combination of lerapolturev and anti-PD-1 therapy in this study suggests there is not an increased risk of cutaneous irAEs for

the combination (Table 3 and Table 4). Because co-administration of lerapolturev with anti-PD-1 therapy has a theoretical risk for CRS and/or immune effector cell-associated neurotoxicity syndrome (ICANS)[20], this study will monitor participants for such events, and will obtain blood samples to determine the cytokine profile associated with any such events (if applicable) in order to inform treatment algorithms in subsequently treated study participants. In addition, the study will employ a safety run-in (Section 3.1.2) to establish the safety of multiple lerapolturev intratumoral injections prior to proceeding the randomized portion of the trial.

1.3.2 Known Potential Benefits

Lerapolturev has demonstrated evidence of meaningful anti-tumor response in patients with rGBM, which is a nearly universally fatal disease. For participants treated with lerapolturev in the Phase 1 study, based on an interim data summary, overall survival reached a plateau of 21% (95% CI: 11 to 33) at 24 months that was sustained at 36 months; this was a significant improvement relative to criteria-matched historical controls[18]. Lerapolturev monotherapy is currently being investigated in an ongoing multi-center Phase 2 in rGBM, with preliminary evidence supporting the observations seen in Phase 1. Lerapolturev is also being evaluated in combination with pembrolizumab in participants with rGBM (LUMINOS-101); the data from this study further suggest the combination of lerapolturev and anti-PD-1 therapy is safe and well tolerated.

Lerapolturev has also shown anti-tumor activity in participants with anti-PD-1 refractory melanoma in a Phase 1 study. Four of twelve participants with anti-PD-1 refractory (and BRAF/MEK refractory, for those with BRAF-mutated melanoma) treated with intratumoral lerapolturev achieved an objective response with lerapolturev, despite dosing being limited to one injection of a single lesion/visit every three weeks for a maximum of three visits. Of those who received 3 lerapolturev injections (maximum dose administered), responses were observed in 4/6 (67%). Responses included two participants with in-transit melanoma who had complete pathologic responses.

The current study was designed to test the safety and efficacy of lerapolturev administered as monotherapy and in combination with anti-PD-1 therapy where the dose of lerapolturev was increased relative to the Phase 1 study by varying the injected volume with the lesion size, and increasing the number of injected lesions/visit. While this approach has been shown to be safe and tolerable (Section 1.2.2.2), the level of clinical activity observed in the Phase 1 study has not been duplicated. The most likely explanation for this observation is differences in the enrolled patient populations ie, the LUMINOS-102 protocol includes a more rigorous definition of anti-PD-1 resistance than what was implemented for the Phase 1 study. Therefore, to improve the probability of participant benefit and the resultant benefit/risk profile, both the eligibility criteria and dosing regimen are being updated in this amendment. The changes in eligibility criteria will exclude those patients with a lower probability of receiving benefit (eg, patients with mucosal melanoma or who are more heavily pretreated) while maintaining the more clinically relevant and rigorous definition of anti-PD-1 resistance.

In addition, both the schedule and dose of lerapolturev injections will be altered to potentially improve the probability of response in this more anti-PD-1 resistant patient population (compared to the Phase 1). Both the change in schedule frequency, and increase in dose, were chosen based on review of published literature for other innate immune agonists (including oncolytic viruses). For example, as outlined in Table 5, the majority of innate immune agonists employ an “induction/maintenance” type of approach to the schedule of injections where more frequent injections are given in the first 1 to 2 months of the treatment period to ignite the immune system, followed by transition to a less frequent schedule where the goal is to maintain the immune response. It is expected that the increase in injection frequency will result in an improvement in participant response without a significant increase in toxicity.

The data summarized in Table 5 also indicates that virus doses of ≈10^8 $TCID_{50}$ or pfu are safe and well tolerated with no dose limiting toxicities when delivered by intratumoral injection. Given anti-PD-1 refractory melanoma participants have received up to 6 x 10^8 $TCID_{50}$ lerapolturev without clinically significant toxicity, and that all participants will have high levels of circulating neutralizing anti-poliovirus antibodies after being boosted prior to enrollment, the proposed study dose of up to 1.6 x 10^9 $TCID_{50}$ lerapolturev is not expected to cause clinically significant increases in toxicity.

With respect to the potential benefits of combining with anti-PD-1 therapy, the nonclinical data summarized in Section 1.2.1 suggest that lerapolturev injection upregulates the compensatory anti-PD-1/L1 pathway, such that concurrent blockade of that pathway is predicted to increase the potential benefit to patients. This hypothesis has been tested in nonclinical experiments where the combination of a mouse adapted version of lerapolturev (mRIPO) administered in combination with a murine anti-PD-1 antibody results in more significant effects on tumor growth as compared to either drug alone (see lerapolturev IB).

1.3.3 Benefit-Risk Summary

Clinical evidence gathered to date suggests that intratumoral injection of lerapolturev in patients with cutaneous, subcutaneous, or nodal unresectable melanoma is well tolerated. Lerapolturev has been associated with clinically relevant peritumoral edema when administered to patients with rGBM. However, AEs related to injection of lerapolturev into melanoma lesions have been mainly local, and remain Grade 1 or 2 in severity in all participants treated to date, regardless of treatment group. This suggests that systemic dissemination of lerapolturev does not occur (likely due to pre-existing anti-polio immunity and CD155 tropism), which distinguishes lerapolturev from other oncolytic viruses such as T-VEC that are associated with systemic dissemination and systemic AEs such as CRS.

Given that all currently available safety data indicate that lerapolturev-related AEs in participants with melanoma are mild to moderate despite administration of a lerapolturev dose of up to 6×10^8 $TCID_{50}$ (alone and when combined with anti-PD-1 therapy), it is reasonable to assume a lerapolturev dose of up to 1.6×10^9 $TCID_{50}$, and a weekly dosing regimen following by Q3/4W maintenance dosing would not result in DLT. In addition, because participants receiving lerapolturev monotherapy are allowed to crossover to the combination regimen, those participants randomized to lerapolturev monotherapy have an opportunity to maximize their potential benefit upon crossover.

2 OBJECTIVES AND ENDPOINTS

2.1 Objectives and Endpoints

Objectives	Endpoints/Evaluation Criteria
Primary	
To evaluate the anti-tumor activity of lerapolturev with and without anti-PD-1, in participants who have failed anti-PD-1/L1-based therapy	• Overall Response Rate (ORR): the proportion of participants achieving confirmed complete (CR) or partial response (PR), per RECIST 1.1 criteria.
To evaluate the safety/tolerability of lerapolturev with and without anti-PD-1, in participants who have failed anti-PD-1/L1-based therapy	• The frequency and severity of treatment-emergent adverse events (AE) via Common Terminology Criteria for Adverse Events (CTCAE, v5.0) • Changes in laboratory (hematology, chemistry) and vital sign parameters • Lerapolturev AESIs • Anti-PD-1 irAEs • Study treatment discontinuation due to AEs
To evaluate the effect of lerapolturev on the TME of injected and non-injected lesions when administered with and without anti-PD-1 therapy	• Changes from baseline in the number of CD8+ tumor infiltrating lymphocytes (TILs) • Changes from baseline in PD-L1 expression
Secondary	
To evaluate survival and disease control outcomes of lerapolturev with and without anti-PD-1, in participants who have failed anti-PD-1/L1-based therapy	• Overall survival (OS): time from treatment group assignment until death from any cause • Duration of Response (DOR): time from confirmed objective response (CR or PR per RECIST 1.1) until unequivocal disease progression or death, whichever occurs first • Disease control rate (DCR): the proportion of participants achieving confirmed CR, confirmed PR, or stable disease (SD) per RECIST 1.1, as best response • Disease control rate-6months (DCR-6mo): the proportion of participants achieving confirmed CR (any duration), confirmed PR (any duration), or SD (≥ 6 months) per RECIST 1.1 as best response • Durable Response Rate (DRR): the proportion of participants with confirmed CR or PR (per RECIST 1.1) lasting at least 6 months

	• Progression-free survival (PFS): time (number of months) from treatment group assignment until date of documented radiologic disease progression per RECIST 1.1 or death due to any cause, whichever comes first
Exploratory:	
To explore lerapolturev mechanism of action and predictors of response to lerapolturev with or without anti-PD-1 in participants who have failed anti-PD-1/L1-based therapy	• Assessment and identification of genetic, cytologic, histologic and/or other markers in tumor biopsies and PBMC samples that may correlate with response. • Assessment of changes over time in immune markers, including, but not limited to, immune cell density, T cell receptor repertoire, and chemokine and/or cytokine profile in blood samples and/or tissue
To evaluate anti-tumor response to lerapolturev with and without anti-PD-1, based on iRECIST criteria in participants who have failed anti-PD-1/L1-based therapy	• ORR/DOR, DRR, DCR, and DCR-6mo based on iRECIST criteria
To evaluate anti-tumor response outcomes to lerapolturev with and without anti-PD-1, based on subgroup in participants who have failed anti-PD-1/L1-based therapy	• ORR, DOR, DRR, DCR, and DCR-6mo in the following subgroups: o Acquired versus primary PD-1/L1 resistance, as defined by Kluger, et al[14] o BRAF wild type and mutant o LDH levels at baseline o Time since last dose of anti-PD-1/L1 therapy prior to randomization (≤ or > 6 weeks) o Crossover to combination arm from lerapolturev monotherapy
To evaluate survival outcomes to lerapolturev with and without anti-PD-1, based on subgroup in participants who have failed anti-PD-1/L1-based therapy	• OS and PFS in the following subgroups: o According to treatment arm and AJCC stage at baseline o Primary versus acquired resistance, as defined by Kluger et al[14] o BRAF wild type and mutant o LDH levels at baseline o Time since last dose of anti-PD-1/L1 therapy prior to randomization (≤ or > 6 weeks) o Crossover to combination from lerapolturev monotherapy

2.2 Hypothesis

Efficacy: Lerapolturev administered in combination with anti-PD-1 therapy using the induction/maintenance schedule of injections (weekly x 7 followed by injections every 3 to

4 weeks concurrent with anti-PD-1 therapy) to participants with anti-PD-1 refractory melanoma will result in an ORR of ≥ 33%.

Translational: Lerapolturev administered in combination with anti-PD-1 therapy will result in an increase in CD8+ T cells and PD-L1 expression in TME.

3 TRIAL DESIGN

3.1 Overall Design

The purpose of this randomized, multicenter, open-label Phase 2 study is to test the safety and efficacy of lerapolturev alone and in combination with anti-PD-1 therapy in participants with unresectable advanced anti-PD-1/L1 refractory melanoma. Participants will be randomized 1:1 to receive lerapolturev alone (Arm 1) or lerapolturev and an FDA-approved anti-PD-1 therapy (Arm 2). Prior to starting the randomized portion of the study, a safety run-in cohort of at least six participants treated with lerapolturev monotherapy was to have been enrolled to demonstrate the safety and tolerability of the administration of multiple lerapolturev injections per visit.

As of July 2021, the safety run-in portion of the study has been completed. Six participants were enrolled and received total lerapolturev doses ranging from 1 x 10^8 to 6 x 10^8 $TCID_{50}$ injected into between 1 and 6 lesions/visit. The schedule of administration was for participants to receive a single lerapolturev injection into the largest lesion on Day 1 followed by initiation of multiple lesion injections/visit on Day 10. For participants in Arm 2, anti-PD-1 therapy was started on Day 10 concurrent with initiation of multiple lerapolturev injections. Upon initiation of multiple injections on Day 10, the timing of future injections was dependent upon anti-PD-1 dosing, ie, for pembrolizumab the schedule was every 3 weeks starting from Day 10, and for nivolumab the schedule was every 4 weeks starting from Day 10. For participants in Arm 1, the timing of future injections was every 3 weeks. This schedule of injections will be abbreviated as the **Q3/4W** schedule.

Upon review of the safety profile for lerapolturev administered as monotherapy or in combination with anti-PD-1 therapy (Section 1.2.2), and in agreement with the DSMC, the total lerapolturev dose to be administered will be a maximum of 1.6 x 10^9 $TCID_{50}$/visit, and the maximum number of lesions injected/visit will remain 6. The schedule of administration will include weekly injections x 7 followed by either Q3W dosing (lerapolturev monotherapy or in combination with pembrolizumab) or Q4W dosing (combination with nivolumab); this schedule of injections will be abbreviated as the **QW** schedule.

Participants receiving the **Q3/4W** schedule of injections will be given the option to change to the **QW** schedule (Section 3.1.2). Upon commencement of enrollment using the **QW** schedule, the DSMC will continue to review all available safety data at a minimum of every 4 months to ensure independent oversight of the safety of participants enrolled in

this study (Section 9.1.5). This includes a meeting to review all safety data once the first 12 participants receiving the new lerapolturev dose and **QW** schedule of injections clear the DLT period.

Participants randomized to receive the **Q3/4W** schedule of injections were stratified based on time since prior anti-PD-1/L1 exposure (≤6 weeks versus >6 weeks) and baseline LDH levels (normal vs >ULN). With the change in eligibility criteria, participants randomized to the QW dosing schedule will be stratified based on the type of anti-PD-1/L1 resistance (primary versus secondary as defined in Kluger et al[7]), while the baseline LDH (normal versus >ULN) stratification factor will be retained.

3.1.1 Crossover

Participants receiving lerapolturev monotherapy (Safety Run-In Cohort or Arm 1) may crossover to combination (lerapolturev and anti-PD-1) therapy if any of the following criteria are met

- radiologic disease progression per RECIST v1.1 (progression does not need to be confirmed prior to crossover)
- have not had progression or confirmed partial response per RECIST v1.1 by week 26 on study
- partial response ≥ 6 months in duration

All participants crossing over to combination therapy should have a complete assessment of tumor burden (ie, scans and caliper/ruler measurement of skin lesions with photographic documentation) ≤ 28 days prior to starting the combination. The dose of lerapolturev administered in combination will be the same as monotherapy and is detailed in Table 7. The schedule of lerapolturev injections at the time of initiation of combination therapy will depend on the timing of crossover (Table 6).

Table 6. Lerapolturev Injection Schedule After Crossover

Schedule of lerapolturev monotherapy injections at the time of crossover	Timing of when criteria for crossover are met	Recommended lerapolturev dosing schedule for combination
Participant is receiving weekly injections	< 6 months from Cycle 1 Day 1	Continue weekly injections for a total of 7 (monotherapy + combination) followed by every 3 week dosing (pembrolizumab) or every 4 week dosing (nivolumab)*
Participant is receiving injections every 3 weeks (pembrolizumab) or every 4 weeks (nivolumab)	< 6 months from Cycle 1 Day 1	Continue concurrent dosing schedule
Participant is receiving injections every 3 weeks (pembrolizumab) or every 4 weeks (nivolumab)	≥ 6 months from Cycle 1 Day 1	Weekly x 7 followed by every 3 week dosing (pembrolizumab) or every 4 week dosing (nivolumab)

***NOTE:** Anti-PD-1 dosing begins Day 1 of crossover. Following the 7th weekly injection, lerapolturev should be administered Q3w when in combination with pembrolizumab (given Q3 or 6w), or Q4w when in combination with nivolumab (given Q2 or 4w).

3.1.2 Considerations for Modification of Dose and/or Dosing Schedule for Participants receiving the Q3/4W Schedule of Injections.

Lerapolturev Dose: All participants receiving lerapolturev on the **Q3/4W** schedule will have their lerapolturev dose adjusted as described in Section 5.3.

Lerapolturev Schedule: Participants receiving treatment using the **Q3/4W** schedule will have the option to receive the "induction" series of weekly x 7 injections (ie, **QW** schedule). If the participant chooses to receive the weekly x 7 injections, the timing and schedule of assessments as shown in Table 1 (or Table 2 if receiving lerapolturev + nivolumab) beginning with Cycle 1, Day 1 should be followed. Note that the on-study biopsy and blood collection timepoints outlined in the Table 1 and Table 2 will remain mandatory for those participants who choose to undergo "induction" injections.

Participants who choose not to receive the "induction" injections will continue on the every 3 week (pembrolizumab) or every 4 week (nivolumab) schedule of injections.

3.1.3 Determination of the Recommended Lerapolturev Dose

As a primary endpoint of this Phase 2 study, the safety and tolerability of lerapolturev alone or in combination with anti-PD-1 will be continually assessed.

*Lerapolturev Dose, **Q3/4W** Schedule* To more fully characterize the safety and tolerability of lerapolturev monotherapy, the Sponsor will enroll a safety run-in cohort of 6 participants. Participants enrolled in the cohort will receive a lerapolturev injection into a maximum of 6 lesions (maximum dose of 6 x 10^8 $TCID_{50}$) on Day 10 (or after, depending on resolution of treatment-limiting AEs) following the single lesion "test" injection. The decision to adjust the lerapolturev dose upon completion of the cohort will be based on the presence of dose-limiting toxicities (DLTs) observed during the 21 days following administration of Cycle 2, in consultation with the independent DSMC.

Provided the DLT rate for the safety run-in cohort is ≤33%, it will be considered that the maximum tolerated dose (MTD) for lerapolturev has not been exceeded. However, should the DLT rate in the safety run-in cohort be >33%, then the recommended dose for single-agent lerapolturev will be the maximum number of lesions injected (and/or $TCID_{50}$ dose) where the DLT rate is ≤33%. If needed, the number of participants enrolled in the safety run-in cohort may be increased up to a maximum of 18 to establish the recommended lerapolturev dose prior to starting the randomized portion of the study. The recommended lerapolturev dose from this cohort will be used in both arms of the randomized portion of the trial (ie, single-agent lerapolturev or lerapolturev in combination with anti-PD-1). Once 12 participants have been treated and on study approximately 31 days (ie, once 6 participants have completed the first cycle of multi-lesion lerapolturev injection with concurrent anti-PD-1), the DSMC will perform an additional safety review to evaluate the prevalence of any untoward safety signals in either treatment arm. Should an excessive DLT rate (ie, >33%) be detected, the lerapolturev dose (ie, the maximum number of lesions and/or maximum $TCID_{50}$ dose) may be decreased in one or both treatment arms, in conjunction with DSMC recommendations. See Section 3.1.4 for the DLT definitions.

Any participant from the safety run-in cohort is allowed to crossover to combination therapy after completion of the safety run-in, as described in Section 3.1.1. However, these participants will be analyzed separately, and will not contribute to the population enrolled to establish the anti-tumor activity of the lerapolturev/anti-PD-1 regimen.

*Lerapolturev Dose, **QW** Schedule:* Given the slight increase in the lerapolturev dose and increased frequency of dosing during the first 2 months on treatment, the DSMC will review all safety data once 12 participants have started the weekly x 7 "induction" series of injections (ie, n=6 monotherapy and n=6 combination with anti-PD-1) and have cleared the DLT evaluation period (ie, 21 days after Cycle 1, Day 1). Applying the same rules as used for the original safety run-in, should an excessive DLT rate (ie, >33%) be detected, the lerapolturev dose (ie, the maximum number of lesions and/or maximum $TCID_{50}$ dose) and/or the number of weekly injections may be decreased in one or both treatment arms, based on DSMC recommendations. See Section 3.1.4 for the DLT definitions.

NOTE: For Arm 2 (lerapolturev/anti-PD-1 combination), AEs should only be considered a DLT if judged by the investigator to be related to lerapolturev and of increased severity/frequency compared to what is expected with administration of single-agent anti-PD-1 therapy in this population, or if it is refractory to common management practices due to lerapolturev.

3.1.4 Definition of Dose-Limiting Toxicity

The DLT evaluation period begins on the day of first lerapolturev injection (ie, Cycle 1, Day 1) and ends 21 days later. The definition of a DLT includes any of the following treatment-emergent events that are deemed at least possibly related to lerapolturev administration:

- Any Grade 4 non-hematologic toxicity (not laboratory)
- Any Grade 3 non-hematologic toxicity (not laboratory), EXCEPT:
  - Grade 3 fatigue lasting ≤3 days or Grade 3 fatigue in a participant with Grade 1 fatigue at baseline
  - Grade 3 diarrhea, nausea, or vomiting without use of anti-emetics or anti-diarrheals per standard of care
  - Grade 3 adrenal insufficiency, hypothyroidism, or Type 1 diabetes mellitus due to suboptimal hormone replacement therapy
  - Grade 3 rash without use of corticosteroids or anti-inflammatory agents per standard of care
- Grade 3 or Grade 4 non-hematologic laboratory values ONLY if:
  - Clinically significant medical intervention is required to treat the participant, or
  - The abnormality leads to hospitalization, or
  - The abnormality persists for >1 week after intervention
  - The abnormality results in a diagnosis of drug-induced liver injury (DILI)
  - Exceptions: Clinically nonsignificant, treatable, or reversible laboratory abnormalities including liver function tests, uric acid, etc.
- Any Grade 4 hematologic toxicity lasting ≥7 days, EXCEPT:
  - Grade 4 thrombocytopenia of any duration
  - Grade 3 thrombocytopenia associated with bleeding that requires a platelet transfusion
- Febrile neutropenia Grade 3 or Grade 4 ONLY if:
  - Grade 3 is defined as ANC <1000/mm^3 with a single temperature of >38.3 °C (101 °F) or a sustained temperature of ≥38 °C (100.4 °F) for more than 1 hour
  - Grade 4 is defined as ANC <1000/mm^3 with a single temperature of >38.3 °C (101 °F) or a sustained temperature of ≥38 °C (100.4 °F) for more than 1 hour, with life-threatening consequences and urgent intervention indicated
- Prolonged delay (>2 weeks) in initiating the second cycle of anti-PD-1 therapy while on study due to lerapolturev-related toxicity.
- Prolonged delay (> 15 weeks) in initiating the second cycle of anti-PD-1 therapy while on study due to anti-PD-1-related toxicity (12 weeks for recovery to ≤ Grade

1 and up to an additional 3 weeks for steroid taper of oral prednisone or equivalent to ≤ 10 mg/day)
- Any treatment-related toxicity that causes the participant to discontinue treatment during the DLT evaluation period
- Grade 5 treatment-related toxicity

3.2 Evaluation of Lerapolturev Shedding

As described in Section 1.2.1 and in the IB, lerapolturev has been subjected to extensive non-clinical and clinical viral shedding studies. Non-human primate studies in Cynomolgus macaques were unable to detect lerapolturev in saliva, stool or urine up to 56 days post intracerebral inoculation, with a lerapolturev dose of 5×10^9 $TCID_{50}$[28]. Likewise, lerapolturev shedding was undetected in the stool (the main excretory pathway for polio) of participants treated with lerapolturev for rGBM following administration of intracerebral doses of up to 1×10^{10} $TCID_{50}$ or in melanoma participants receiving 3 telerapolturev injections (1×10^8 $TCID_{50}$ each). These results combined with the requirement that all participants must receive a CDC-recommended immunizing vaccination against PV, as well as a PV booster prior to study entry (which has resulted in extremely high levels of neutralizing anti-PV antibodies in patients tested to date) the risk of extra-tumoral lerapolturev dissemination and shedding is predicted to be extremely low.

However, because lerapolturev shedding in melanoma participants receiving repeat injections of lerapolturev at the dose levels allowed in this study has not been fully characterized, the Sponsor will test for the presence of lerapolturev in stool and on the surface of lerapolturev-injected lesions (as well as on the internal and external surfaces of any occlusive dressings applied post-lerapolturev injection) for up to 25 participants who received lerapolturev injections using the **Q3/4W** dosing schedule. For those participants enrolled using **QW** schedule of lerapolturev injections, viral shedding studies will be done in up to 25 participants as described in the appropriate SOA (Table 1 or Table 2 [Arm 2: lerapolturev + nivolumab]).

All samples related to the analysis of viral shedding may be collected during clinic visits, where feasible. Otherwise, samples may be collected at home and brought to the next clinic visit, as appropriate. Refer to the laboratory manual for additional details related to sample collection and storage.

3.3 Justification for Lerapolturev Dose and Schedule

The initial dose of lerapolturev evaluated in this trial was selected based on IND-directed toxicity studies and experience from ongoing studies in participants with rGBM or anti-PD-1 refractory melanoma (Section 1.2.2). Dose-range finding and toxicology studies in non-human primates documented the absence of viral encephalomyelitis, poliomyelitis and meningitis with intracerebral injection of lerapolturev up to a dose of 5×10^9 $TCID_{50}$[39].

In the adult Phase 1 rGBM study, 4 participants were dosed at the maximum intended dose of 10^{10} $TCID_{50}$, injected intracerebrally, without signs of viral encephalomyelitis, poliomyelitis, or meningitis. However, due to persistent peri-tumoral inflammation and the continuing requirement for steroids in all participants, the recommended intracerebral dose was eventually reduced to 5 x 10^7 $TCID_{50}$.

In subcutaneous melanoma, the effect of inflammation is not expected to be as significant because cutaneous swelling is more easily managed than edema within the confines of the brain/skull. Data from the Phase 1 trial in anti-PD-1 refractory melanoma, as well as the currently available data generated during this study, has shown that a lerapolturev dose of up to 6 x 10^8 $TCID_{50}$ is very well tolerated when given alone or in combination with anti-PD-1 therapy. All treatment-related AEs remain Grade 1 or 2, with no treatment-related SAEs, DLTs or evidence of CRS or ICANS in participants treated at any dose level (Section 1.2.2.2).

Therefore, this study has been amended to explore an approximately 2-fold higher lerapolturev dose using a dosing schedule (**QW**) with more frequent injections during the first 2 months of treatment followed by transition to a less frequent “maintenance” schedule. The rationale and data supporting this change are summarized in Section 1.3.

3.4 End of Study Definition

The study is considered completed with the last study assessment for the last participant participating in the study. The final data from the site will be sent to the Sponsor after completion of the final participant assessment at that study site, in the time frame specified in the clinical trial agreement.

4 STUDY POPULATION

The inclusion and exclusion criteria for enrolling participants in this study are described below. If there is a question about these criteria, the investigator must consult with the Sponsor or designee to resolve any issues prior to enrollment.

Screening for eligible participants will be completed within 28 days prior to Day 1. Refer to Section 4.3 for conditions under which repeat screening procedures are permitted.

4.1 Inclusion Criteria

1. ≥ 18 years of age
2. Prior CDC-recommended vaccination series against poliovirus (PV), and has received a boost immunization with trivalent IPOL® (Sanofi-Pasteur SA) at least 1 week, but less than 6 weeks, prior to Day 1
    a. NOTE: Patients who are unsure of their vaccination status must provide evidence of anti-PV immunity prior to enrollment, as applicable

3. Has biopsy proven unresectable cutaneous melanoma and is willing to undergo tumor biopsy prior to the first dose of study drug(s) and at prespecified intervals during the study. Note the following details:
    a. Patients with ocular, acral or mucosal melanoma are not eligible
    b. Patients with M1c or M1d disease are NOT eligible
    c. Submission of an archival biopsy sample is allowed in lieu of the baseline tumor biopsy, provided the tissue is ≤4 months old and the participant received no intervening systemic/intratumoral anti-cancer therapy since the biopsy was acquired.
    d. Must have at least 1 lesion that is amenable to biopsy. The lesion must be safely accessible as determined by the investigator and should not be located at sites that require significant risk procedures to biopsy. Examples of sites considered to be of significant risk include but are not limited to the following: the brain, lung, mediastinum, pancreas, or endoscopic procedures extending beyond the esophagus, stomach, or bowel wall.

4. Has ≥ 2 melanoma lesions that are accurately measurable by caliper or a radiological method according to RECIST 1.1 criteria
    a. One lesion must be injectable--defined as a visible or palpable cutaneous, subcutaneous, or nodal melanoma lesion ≥ 10mm in longest diameter or multiple injectable melanoma lesions which in aggregate have a longest diameter of ≥ 10mm and where the minimal lesion size is ≥ 5mm.
    b. Note that visceral lesions (eg, liver, lung, retroperitoneal, subpleural lesions) are not considered injectable for the purposes of this trial.
5. Has had confirmed progression of disease (PD) while receiving at least 6 weeks (> 1 dose) of an FDA-approved anti-PD-1/L1 therapy (as monotherapy or in combination) for the treatment of melanoma. Note the following details:
    a. Initial PD as defined by RECIST v1.1
    b. Confirmation of PD per iRECIST must occur by repeat assessment ≥ 4 weeks from initial evidence of PD, in the absence of rapid clinical progression.
    c. Those who discontinue anti-PD-1/L1 therapy after at least 6 weeks (> 1 dose) and have confirmed PD per iRECIST within 12 weeks of their last anti-PD-1/L1 dose are also eligible, provided the anti-PD-1/L1 was not stopped due to toxicity requiring permanent discontinuation
    d. Those treated with anti-PD-1/L1 in the adjuvant setting and who have biopsy-confirmed progression either while receiving anti-PD-1/L1-based therapy or ≤ 12 weeks after their last dose of anti-PD-1/L1 therapy are allowed

NOTE: Adjuvant is defined as therapy received after surgical resection of disease such that the patient has no evidence of disease when the anti-PD-1/L1 therapy is initiated. Patients with known BRAF mutation must have also failed or refused to receive BRAF-targeted therapy (alone or in combination with MEK inhibitor) to be eligible

6. Eastern Cooperative Oncology Group (ECOG) status of 0-1
7. Serum lactate dehydrogenase (LDH) levels ≤3 x upper limit of normal (ULN)
8. Adequate bone marrow, liver and renal function as assessed by the following:
    a. Hemoglobin ≥9.0 g/dl, patients may be transfused
    b. Lymphocyte count ≥0.5 x 10^9/L (500 µL)
    c. Absolute neutrophil count (ANC) ≥1.5 x 10^9/L (1500 µL)
    d. Platelet count ≥100 x 10^9/L (100,000 µL) without transfusion
    e. AST, ALT, and alkaline phosphatase (ALP) ≤2.5 x upper limit of normal (ULN), with the following exceptions:
        i. Patients with documented liver metastases: AST and ALT ≤5 x ULN
        ii. Patients with documented liver or bone metastases: ALP ≤5 x ULN
    f. Serum bilirubin ≤1.5 x ULN with the following exception:
        i. Patients with known Gilbert disease: serum bilirubin level ≤3 x ULN
    g. Measured or calculated (per institutional standards) creatinine clearance ≥30 ml/min (GFR can also be used in place of creatinine clearance)
    h. For patients not receiving therapeutic anticoagulation: INR, PT, PTT (or aPTT) ≤1.5 x ULN
9. Life expectancy of >12 weeks
10. Signed informed consent form (ICF) indicating that participant understands the purpose of, and procedures required for the study, and is willing/able to participate in the study

4.2 Exclusion Criteria

1. Has biopsy-proven ocular, acral or mucosal melanoma
2. Has M1c or M1d disease
3. No more than one prior systemic anti-cancer regimen (monotherapy or combination) for management of melanoma. Additional details noted below:
    a. Adjuvant anti-cancer therapy administered ≥ 6 months prior to the first injection of lerapolturev does NOT count as a line of treatment.
    b. Patients with BRAF mutant melanoma may enroll if they have received ≤ 2 prior lines of systemic anti-cancer therapy only if one of those lines of therapy was a BRAF-targeted regimen (alone or in combination with MEK inhibitor).
    c. A line of therapy is defined as a regimen in which at least 2 doses of systemic anti-cancer therapy (monotherapy or combination) was administered, and the regimen was discontinued because of progressive disease.

4. Uncontrolled tumor-related pain. Participants requiring pain medication must be on a stable regimen at study entry
    a. Symptomatic lesions amenable to palliative radiotherapy (eg, bone metastases or metastases causing nerve impingement) should be treated prior to enrollment. Patients should be recovered from the effects of radiation. There is no required minimum recovery period
    b. Asymptomatic metastatic lesions that would likely cause functional deficits or intractable pain with further growth (eg, epidural metastasis that are not currently associated with spinal cord compression) should be considered for loco-regional therapy if appropriate prior to enrollment
5. Grade ≥2 pleural effusion, pericardial effusion, or ascites
6. Active or history of autoimmune disease or immune deficiency within previous 2 years, with the following exceptions:
    a. History of autoimmune-related endocrinopathy (e.g. adrenal insufficiency, hypothyroidism, Type 1 diabetes mellitus, etc.) that is managed by hormone replacement therapy (e.g. hydrocortisone, thyroid hormone, insulin, etc.)
    b. Eczema, psoriasis, or lichen simplex chronicus with dermatologic manifestations only (eg, patients with psoriatic arthritis are excluded), provided all of the following conditions are met:
        i. Rash must cover <10% of body surface area
        ii. Disease is well-controlled at baseline and requires only low-potency topical corticosteroids
        iii. No occurrence of acute exacerbations of the underlying condition requiring psoralen plus ultraviolet A radiation, methotrexate, retinoids, biologic agents, oral calcineurin inhibitors, or high potency or oral corticosteroids within 12 months of Day 1
7. History of idiopathic pulmonary fibrosis, organizing pneumonia (eg, bronchiolitis obliterans), drug-induced or idiopathic pneumonitis, or evidence of active pneumonitis on screening chest computed tomography (CT) scan
    a. History of radiation pneumonitis in the radiation field (fibrosis) is allowed
8. History of a positive HIV RNA test (HIV 1 or 2 RNA by PCR)
9. Known active hepatitis B virus (HBV) infection (chronic or acute)
    a. NOTE: Participants with a negative HBsAg test and a positive total hepatitis B core antibody (HBcAb) test are allowed
10. Known active hepatitis C virus (HCV) infection
    a. NOTE: History of a positive HCV antibody test, but negative HCV RNA test, is allowed
11. Active tuberculosis

12. Significant cardiovascular disease, such as New York Heart Association Class II or greater cardiac disease, myocardial infarction, or cerebrovascular accident within 3 months of Day 1, unstable arrhythmia, or unstable angina
13. Has received prior systemic anti-cancer therapy including investigational agents within 4 weeks or 5 elimination half-lives—whichever is shorter—prior to treatment or has not recovered from all AEs due to previous therapies to ≤Grade 1 or baseline. Participants with ≤Grade 2 neuropathy are eligible
    a. Note: Anti-PD-1/L1 within 4 weeks prior to Day 1 is allowed
    b. If participant received major surgery, they must have recovered adequately from the toxicity and/or complications from the intervention prior to starting study treatment
14. History of other malignancy within 2 years prior to Day 1, with the exception of those with a negligible risk of metastasis or death (eg, resected cutaneous basal cell carcinoma, or other cancers with 5-year OS of >90%)
15. Severe infection within 4 weeks prior to Day 1, including, but not limited to, hospitalization for complications of infection, bacteremia, or severe pneumonia
    a. Prophylactic antibiotics (eg, to prevent a urinary tract infection or chronic obstructive pulmonary disease exacerbation) are allowed
16. Prior allogeneic stem cell or solid organ transplantation
17. Treatment with a live, attenuated vaccine within 4 weeks prior to Day 1
18. Treatment with systemic immunosuppressive medication within 4 weeks prior to Day 1, with the following exceptions:
    a. Participants who received acute, low-dose systemic immunosuppressant medication or a one-time pulse dose of systemic immunosuppressant medication (eg, 48 hours of corticosteroids for a contrast allergy) are eligible
    b. Patients receiving mineralocorticoids (eg, fludrocortisone), or systemic prednisone equivalent corticosteroid doses of <10mg per day are eligible for the study
19. Known hypersensitivity to pembrolizumab, nivolumab, or any of the respective excipients
20. Requires therapeutic anticoagulation and cannot discontinue anticoagulation safely during the day prior, day of, and day after each lerapolturev injection
    a. NOTE: Participants receiving anticoagulation with warfarin at the time of study entry are allowed if they can be transitioned to an alternative anticoagulant (eg, low molecular weight heparin or direct oral anticoagulants) prior to the first dose of lerapolturev. Anyone transitioned from warfarin to an oral anticoagulant prior to the first dose of lerapolturev should have an INR <1.5x upper limit of normal in order to participate. Antiplatelet agents (eg, aspirin, clopidogrel, etc.) are not considered anticoagulants for the purposes of this study (ie, are allowed)

21. A pregnant or nursing female, or women of child-bearing potential and men who are sexually active and not willing/able to use medically acceptable forms of contraception starting from signed ICF through 150 days after last anti-PD-1 dose
22. History of human serum albumin allergy
23. History of neurological complications due to polio virus infection
24. History of agammaglobulinemia
25. Concurrent participation in a separate interventional clinical trial during this study
26. Any underlying medical condition for which, in the opinion of the investigator, participation would not be in the best interest of the participant (eg, compromises the participant's well-being) or that could prevent, limit, or confound protocol-specified assessments

4.3 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently enrolled. A minimal set of screen failure information (demography, screen failure details, eligibility criteria, and any SAEs) will be collected to ensure transparent reporting.

Individuals who do not meet the criteria for participation in this study may be rescreened. Rescreening is at the discretion of the Investigator. Participants who are to be rescreened based on a laboratory exclusion are not required to reconsent. Otherwise, a new ICF is required before rescreening and the participant must be assigned a new participant number.

5 STUDY DRUG (LERAPOLTUREV, NIVOLUMAB OR PEMBROLIZUMAB)

5.1 Lerapolturev: Packaging, labeling and how supplied

Lerapolturev is a modified version of the serotype 1 live-attenuated (Sabin) PV vaccine (PV1S) and is classified as an oncolytic viral immunotherapeutic.

Lerapolturev is formulated in 50 mM sodium phosphate in 0.9% sodium chloride, pH 7.4 with 0.2% human serum albumin (HSA) in phosphate buffered saline (PBS). Lerapolturev is provided in sterile, single use glass vials with a flip off top containing approximately 0.5 mL of stock lerapolturev (2.24×10^9 $TCID_{50}$).

5.2 Preparation/Handling/Storage/Accountability

For complete lerapolturev storage, handling, and preparation instructions, please refer to the pharmacy manual. Lerapolturev preparation with Sponsor-supplied vehicle must occur in a biosafety cabinet or similarly contained environment in the institution's

investigational pharmacy or equivalent. All handling of the study agent should occur under appropriate biosafety measures.

Any materials in contact with the study agent, eg, pipettes, vials, etc., should be disposed of as biological waste. The minimum final desired volume of the study agent for injection is 0.5 ml, and the maximum volume will be 3.0 ml. The final volume should be drawn into the intended delivery device(s) (See pharmacy manual for list of needles/syringes that are acceptable for lerapolturev administration).

5.3 Lerapolturev Dose, Schedule, and Administration

Dose: Lerapolturev should be administered at a fixed concentration of 5.33×10^8 $TCID_{50}$/ml in accordance with the pharmacy manual (PM). Please refer to the PM for complete information regarding lerapolturev administration. The maximum volume injected at an individual treatment visit will be 3 ml, which is dependent on the number and size of injectable lesions. This corresponds to a maximum lerapolturev dose of 1.6×10^9 $TCID_{50}$. The minimum injection volume is 0.5 ml (ie, minimum lerapolturev dose 2.67×10^8 $TCID_{50}$). Up to 6 lesions may be injected at an individual treatment visit. It is possible that more than 1 syringe may be required to treat a single lesion, due to large lesion size. See Table 7 for recommended lerapolturev injection volumes. See Section 3.1.2 for instructions regarding lerapolturev dose for participants receiving the **Q3/4W** schedule of injections.

Table 7. Recommended Lerapolturev Injection Volumes

Lesion Size (longest dimension [cm])	Maximum Injection Volume Per Lesion (ml)	Lerapolturev Concentration ($TCID_{50}$/ml)
≥ 5	3.0	5.33×10^8
≥ 4 and < 5	2.0	
≥ 3 and < 4	1.5	
≥ 2 and < 3	1.0	
0.5 to <2.0	0.5	

Schedule: Participants will receive lerapolturev injections using the **QW** schedule which is defined as weekly x 7 followed by every 3 weeks (pembrolizumab) or every 4 weeks (nivolumab). See Section 3.1.1 for instructions of what injection schedule to use upon crossover. See Section 3.1.2 for instructions of what injection schedule to use for participants receiving the **Q3/4W** schedule.

Key Considerations Regarding Lerapolturev Injection:

- Where possible, at least one RECIST1.1/iRECIST target lesion should remain uninjected for as long as feasible while on study to facilitate the anti-tumor response assessment of non-injected target lesions.
- Participants with lesions known to be sensitive to intratumoral injection (ie, are painful, such as in transit lesions with limited subcutaneous tissue on the shin) may receive reduced injection volumes relative to the recommended volume for a given tumor size, per investigator's discretion, to manage tumor-related pain. However, the minimum allowable injection volume for a given lesion is 0.5 ml.
- Any lesion that is visible or palpable may be injected; lesions located in sites considered to be of more significant risk for complications from lerapolturev injection and associated inflammation (eg brain, lung, mediastinum, liver, pancreas, other visceral organs, lesions subjected to curative levels of radiation where radiation-associated toxicity has not healed, lesions encasing or immediately adjacent to major blood vessels including the carotid artery) are not to be injected in this study. Ultrasound may be used to better visualize the lesion during lerapolturev injection to optimize participant safety.
- Participants may stop receiving lerapolturev injections upon confirmation of CR or upon resolution of all injectable lesions (participants in Arm 2 should continue anti-PD-1 maintenance per manufacturer's prescribing instructions) and should be observed until progression/recurrence, at which point retreatment with lerapolturev is allowed. This process of injection, discontinuing injections and retreatment with injection may occur for a maximum of three times.
    - Participants receiving single-agent lerapolturev with a CR or complete resolution of all injectable disease (ie, other non-injectable disease persists) can crossover to Arm 2 upon progression. If upon progression, there are no

injectable lesions, the participant should begin anti-PD-1 therapy monotherapy upon crossover.

- Lerapolturev injections should be administered concurrently with anti-PD-1 therapy during the “maintenance” schedule, (ie, Q3W for pembrolizumab and Q4W for nivolumab). Note that lerapolturev should be continued Q3W in the event pembrolizumab is administered on a Q6W cycle or Q4W in the event nivolumab is administered on a Q2W cycle.
- Where possible, participants should receive the same anti-PD-1 that they failed prior to study entry, administered in accordance with the respective package insert with respect to length of exposure and/or management (eg, dose delay or discontinuation) of irAEs.

Administration: Lerapolturev administration should be performed in accordance with the Pharmacy Manual. Anticoagulant and antiplatelet medications will be managed by the treating physician, per local institutional guidelines, to optimize the safety of intratumoral injection of lerapolturev.

5.4 Prior/Concomitant Therapy

There are no currently known contraindications or drug-drug interactions for lerapolturev (per the IB), pembrolizumab, or nivolumab as per FDA-approved package labeling. All medical conditions should be treated by the investigator per current standard of care. Participants may also receive medications for symptomatic relief (ie, analgesics, laxatives, anti-emetics).

- **NOTE:** Lerapolturev administration in combination with an anti-PD-L1 therapy is not allowed during this study

Medications the participant has received within 28 days of Day 1 until 30 days after the last dose of study drug(s) should be recorded in the eCRF. This includes, but is not limited to, prescription medications, over-the-counter medications, injected medications, natural/herbal remedies, biological products, blood products, imported drugs, or “street” drugs. Subsequent anticancer therapy should be collected until the end of study.

Prohibited Therapies:

Concomitant treatments that could prevent or mask potential AEs related to the investigational treatment or confound the assessment of its anti-tumor efficacy are not permitted, as far as the participant’s safety is protected.

Administration of other systemic concomitant non-protocol anticancer therapies prior to progression is not permitted while on this study, with the exception of adjuvant endocrine therapy for breast cancer or prostate cancer defined as M0 disease or PSA persistence/recurrence without metastatic disease. Local treatment of isolated lesion(s) with palliative radiation therapy or surgery is permitted to control disease symptoms but

not to aid in the response of the tumor. If the lesion(s) treated is being followed for evaluation by RECIST (target or nontarget lesion), then “not evaluable” should be reported in the eCRF for this lesion at subsequent disease assessments following palliative radiotherapy or surgery. Participants requiring palliative radiation may continue receiving study drug until documented disease progression (radiographic or clinical) if, in the Investigator’s opinion, the participant is continuing to receive clinical benefit and they meet the requirements described in Section 6.2. However, for participants who have not had disease progression at the time of the need for palliative radiation therapy or surgery, the requirement for intervention will be regarded as disease progression in the study’s analyses and will be entered as such in the eCRF.

The following medications are also not permitted:

- Non-protocol specified investigational drugs.
- Use of topical corticosteroids with occlusive dressings.
- Anticoagulant use the day prior to, day of and the day following lerapolturev injection. Warfarin use is not allowed at any time during study participation. Antiplatelet agents, such as aspirin, clopidogrel, etc., are not considered anticoagulants for the purposes of this study and are allowed.
- Live virus vaccines (beyond lerapolturev)
    - Examples of live virus vaccines include, but are not limited to, measles, mumps, rubella, varicella/zoster, yellow fever, rabies, and typhoid vaccine.
    - Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (eg, FluMist®) are live attenuated vaccines and are not allowed.

COVID-19 Vaccination:

Vaccination against COVID-19 is allowed on study, provided the vaccine is not based on a live virus (eg, the Johnson and Johnson single-dose vaccine or AstraZeneca vaccine). While on study, it is recommended that participants vaccinated with non-live virus vaccines (eg, mRNA-based vaccines) receive the vaccine at least 14 days before or after lerapolturev administration, if possible. The COVID-19 vaccine should be given in an anatomic area as far as possible from melanoma lesions that were recently injected with lerapolturev or that are planned for future injection. Participants receiving a COVID-19 vaccine based on a live virus may begin Cycle 1, Day 1, once they are at least 4 weeks removed from vaccine administration.

5.5 Dose Modification/Toxicity Management Guidelines

5.5.1 Lerapolturev

For AEs related to lerapolturev, lerapolturev may be withheld or permanently discontinued based on the severity of the adverse reaction.

Holding Lerapolturev Dosing
If a participant experiences any of the following toxicities deemed related to lerapolturev, lerapolturev administration should be held until the toxicity has resolved to at least CTCAE Grade 1 or has returned to baseline:

- Grade 2 or greater irAE, with the exception of vitiligo
- Grade 2 or greater allergic reactions
- any other Grade 3 or greater hematologic or non-hematologic toxicity

For participants receiving concurrent lerapolturev and anti-PD-1 therapy, lerapolturev and the anti-PD-1 therapy can be administered independently of each other where appropriate. Should the investigator determine an AE is related to anti-PD-1 therapy and not lerapolturev, continuation of lerapolturev injections is allowed (or vice versa). However, if the AE is determined to be related to the combination, this should be noted and treatment with both therapies should be held until resolution, as described above.

Permanent Discontinuation:
Lerapolturev should be permanently discontinued if any lerapolturev-related Grade 3 or greater hematologic or non-hematologic toxicity fails to resolve to Grade 1 or baseline OR occurs more than twice (ie, three episodes = permanent discontinuation).

5.5.2 Anti-PD-1 Therapy

For suspected irAEs related to anti-PD-1 therapy, the anti-PD-1 therapy may be withheld or permanently discontinued based on the severity of the adverse reaction, in accordance with FDA-approved package labeling and physician discretion.

Withholding anti-PD-1 Therapy:
The anti-PD-1 must be withheld for any of the following:

- Grade 2 pneumonitis
- Grade 2 or 3 colitis
- Grade 3 or 4 endocrinopathies
- Grade 2 nephritis
- Grade 3 severe skin reactions or suspected Stevens-Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN)
- Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) greater than 3 and up to 5 times upper limit of normal (ULN) or total bilirubin greater than 1.5 and up to 3 times ULN
- Any other severe or Grade 3 treatment-related AE

Lerapolturev is not required to be withheld for participants enrolled in Arm 2, provided the investigator determines the AE requiring holding of the anti-PD-1 dose is not related to or

worsened by lerapolturev. The anti-PD-1 therapy can resume in participants whose adverse reactions recover to Grade ≤1 or baseline.

Permanent Discontinuation:
Anti-PD-1 therapy should be permanently discontinued for any of the following AEs

- Any life-threatening adverse reaction (excluding endocrinopathies controlled with hormone replacement therapy)
- Grade 3 or 4 pneumonitis or recurrent pneumonitis of Grade 2 severity
- Grade 3 or 4 nephritis
- Grade 4 severe skin reactions or confirmed SJS or TEN
- AST or ALT greater than 5 times ULN
    - For participants with liver metastasis who begin treatment with Grade 2 AST or ALT, discontinue if AST or ALT increases by greater than or equal to 50% relative to baseline and lasts for at least 1 week
- Total bilirubin greater than 3 times ULN.
- Grade 3 or 4 infusion-related reactions
- Inability to reduce corticosteroid dose to 10 mg or less of prednisone or equivalent per day within 12 weeks
- Persistent Grade 2 or 3 adverse reactions (excluding endocrinopathies controlled with hormone replacement therapy) that do not recover to Grade 0-1 within 12 weeks after last dose of the anti-PD-1
- Any severe or Grade 3 treatment-related adverse reaction that recurs

Management of anti-PD-1-related irAE:
Always refer to the manufacturer's prescribing information for the most current recommendations for management of irAE. Additional information can also be found in the NCCN Guidelines for Management of Immunotherapy-Related Toxicities[40].

- *Suspected pneumonitis:* Participants should be evaluated with radiographic imaging. They will receive corticosteroids (initial dose of 1 to 2 mg/kg/day prednisone or equivalent followed by a taper) for Grade 2 or greater pneumonitis. The anti-PD-1 must be withheld for moderate (Grade 2) pneumonitis, and permanently discontinued for severe (Grade 3), life-threatening (Grade 4), or recurrent moderate (Grade 2) pneumonitis.
- *Colitis:* Participants must be monitored for signs and symptoms of colitis and administered corticosteroids (initial dose of 1 to 2 mg/kg/day prednisone or equivalent followed by a taper) for Grade 2 or greater colitis. The anti-PD-1 must be withheld for moderate (Grade 2) or severe (Grade 3) colitis, and permanently discontinued for life-threatening (Grade 4) colitis.
- *Hepatitis:* Participants must be monitored for changes in liver function tests. They must receive corticosteroids (initial dose of 0.5 to 1 mg/kg/day [for Grade 2 hepatitis] and 1 to 2 mg/kg/day [for Grade 3 or greater hepatitis] prednisone or

equivalent followed by a taper) and, based on severity of liver enzyme elevations, the anti-PD-1 must be discontinued.

- *Hypophysitis (including hypopituitarism and adrenal insufficiency):* Participants must be monitored for signs and symptoms of endocrine origin. They will receive corticosteroids and hormone replacement as clinically indicated. The anti-PD-1 must be withheld for moderate (Grade 2) and severe (Grade 3) hypophysitis and must be discontinued for severe (Grade 3) hypophysitis that does not recover or life-threatening (Grade 4) hypophysitis.
- *Thyroid disorders:* Anti-PD-1 products can cause thyroid disorders, including hyperthyroidism, hypothyroidism, and thyroiditis. Participants must be followed for changes in thyroid function (at the start of treatment, periodically every 4 to 6 weeks during treatment, and as indicated based on clinical evaluation) and for clinical signs and symptoms of thyroid disorders per the SOA (Table 1 or Table 2 [Arm 2 lerapolturev + nivolumab]). Hypothyroidism will be treated by replacement hormones. Hyperthyroidism will be managed with thionamides and beta-blockers as appropriate. The anti-PD-1 must be withheld or discontinued for severe (Grade 3) or life-threatening (Grade 4) hyperthyroidism.
- *Hyperglycemia:* Participants must be regularly monitored for blood glucose or other parameters of Type 1 diabetes. They must be treated with insulin and the anti-PD-1 withheld. In case of severe (Grade 3) hyperglycemia, they will receive anti-hyperglycemics.
- *Nephritis:* Participants must be evaluated for renal function. They will be given corticosteroids (initial dose of 1 to 2 mg/kg/day prednisone or equivalent followed by a taper) for Grade 2 or greater nephritis. The anti-PD-1 must be withheld for moderate (Grade 2) nephritis and must be permanently discontinuation for severe (Grade 3) or life-threatening (Grade 4) nephritis.
- *Skin disorders:* Immune-mediated rashes, including Stevens-Johnson Syndrome (SJS) and Toxic Epidermal Necrolysis (TEN) (some cases with fatal outcome), exfoliative dermatitis, and bullous pemphigoid, can occur. Participants must be monitored for suspected severe skin reactions and exclude other causes. Based on the severity of the adverse reaction, the anti-PD-1 must be withheld or permanently discontinued. Participants with Grade 2 or higher dermatologic toxicity will receive topical corticosteroids and/or systemic corticosteroids (initial dose 0.5 – 1.0 mg/kg/d prednisone or equivalent). For signs or symptoms of SJS or TEN, the anti-PD-1 must be withheld, and the participant must be referred to specialized care for assessment and treatment. In case SJS or TEN is confirmed, the anti-PD-1 must be permanently discontinued.
- *Infusion related reactions:* During infusion, monitor patients for signs and symptoms of infusion-related reactions including rigors, chills, wheezing, pruritus, flushing, rash, hypotension, hypoxemia, and fever. For severe (Grade 3) or life-threatening (Grade 4) infusion-related reactions, stop infusion and permanently discontinue pembrolizumab. Follow the recommend management practices noted

in the current package insert or in keeping with current medical practices when managing reactions noted during or after anti-PD-1 agent infusion. For severe or life-threatening infusion related reactions, this may include IV dexamethasone followed by oral steroids. Other immunosuppressive treatment should also be initiated if the reaction cannot be controlled by corticosteroids alone.

Management of AEs related to CRS or ICANS:

Administration of lerapolturev has not been associated with CRS or ICANS in the limited number of melanoma patients treated to date, or in the 190 patients treated with lerapolturev in other indications (Istari data on file). However, the current study is investigating lerapolturev in conjunction with concurrent anti-PD-1 therapy, which given the immune-mediated mechanism of action, could potentially affect the safety profile of lerapolturev. Therefore, monitoring of CRS or ICANS as AEs of special interest (AESI) will occur in this trial, which are each defined in Section 9.2. This includes reporting any AESI of CRS or ICANS within 24 hours of awareness of the event, regardless of severity or seriousness.

NOTE: Per the American Society for Transplantation and Cellular Therapy (ASTCT) consensus guidelines[41], symptoms of CRS or ICANS are primarily experienced in the first two weeks of therapy.

Participants with suspected CRS should have lerapolturev and/or anti-PD1 therapy held, and a blood sample should be obtained and sent to the central laboratory for cytokine profiling. Therapy may resume upon resolution of CRS-related AEs to Grade 1 or baseline, per investigator discretion. ASTCT[41] and/or institutional guidelines for treatment should be applied for management of CRS, including the use of corticosteroids and tocilizumab for Grade 3 or higher CRS, including whether or not to resume study therapy upon AESI resolution.

Participants with neurological symptoms of suspected ICANS should have lerapolturev and/or anti-PD-1 therapy held until resolution of the ICANS-related AEs to Grade 1 or baseline. In addition, each participant should have a neurological exam that includes the Immune Effector Cell-Associated Encephalopathy (ICE) screening tool (Appendix I) per ASTCT guidelines, as well as a blood sample obtained for cytokine profiling as above. ASTCT[41] and/or institutional treatment guidelines should be followed for ICANS-related AE management, including the administration of corticosteroids, or whether or not to resume study therapy upon AESI resolution.

6 DISCONTINUATION OF STUDY TREATMENT, TREATMENT BEYOND PROGRESSION, PARTICIPANT COMPLETION AND WITHDRAWAL, AND STUDY TERMINATION

6.1 Discontinuation of Study Treatment

Study drug(s) (defined as lerapolturev, nivolumab or pembrolizumab) will be discontinued if any of the following events occur during the study:

- A participant suffers an AE that, in the judgment of the Investigator, Sponsor, or Medical Monitor, presents an unacceptable risk to the participant
- General or specific changes in the participant's condition (eg, a significant intercurrent illness or complication) that, in the judgment of the Investigator, are unacceptable for further administration of study drug
- Occurrence of pregnancy during the study
- Significant noncompliance with protocol requirements
- The Sponsor or legal representative of the Sponsor requests the participant to withdraw
- Withdrawal of consent
- Lost to follow-up
- Participant has radiologically documented disease progression and the physician determines the participant is not deriving clinical benefit as defined in Section 6.2
- Where permanent discontinuation of all study drugs is indicated in the dose modifications guidelines (see Section 5.5)

At the time of study drug discontinuation, an EOT visit should be completed with assessments performed as shown in appropriate General Schedule of Assessments (Table 1 or Table 2 depending on treatment group). The Investigator or Designee will document the reason for study drug discontinuation on the applicable electronic case report form (eCRF). When discontinuation is due to a SAE or a Grade 3 or 4 toxicity considered to be related to study drug(s), the Investigator should follow the event until resolution, return to baseline, or it is deemed that further recovery is unlikely. Data on these events should be collected in the eCRF. In the event a participant discontinues due to pregnancy, the Investigator or designee should notify the Medical Monitor by telephone within 24 hours of pregnancy confirmation (see Section 7.3.5).

6.2 Treatment Beyond Progression

Evidence indicates some patients treated with immunotherapy may derive clinical benefit after initial evidence of apparent disease progression[42,43]. Therefore, study drug administration may be continued until loss of clinical benefit, provided the participant appears to be deriving clinical benefit and the Investigator believes it is in the best interest of the participant to continue. Treatment past disease progression per RECIST 1.1 should only be considered if a participant is clinically stable and has the following:

- Evidence of clinical benefit as assessed by the Investigator; assessment of clinical benefit should take into account whether the participant is clinically deteriorating and unlikely to receive further benefit from continued treatment
- Absence of symptoms and signs (including worsening of laboratory values; eg, new or worsening hypercalcemia) indicating unequivocal progression of disease
- No decline in ECOG performance status that can be attributed to disease progression
- Absence of tumor growth at critical anatomical sites (eg, leptomeningeal disease) that cannot be managed by protocol-allowed medical interventions

Decisions to continue treatment beyond initial progression per RECIST 1.1 may be discussed with the medical monitor and should be documented in the study records.

Participants with radiographic disease progression confirmed at a subsequent tumor assessment (ie, iCPD) may be considered for continued study treatment at the discretion of the investigator if they continue to meet the criteria above and have evidence of clinical benefit.

6.3 Participant Withdrawal from the Trial

Participants may be withdrawn from the study under any of the following circumstances:

- Withdrawal of consent
- Lost to follow-up
- Administrative reasons (eg, Sponsor decision)
- Major violation of the protocol
- If in the opinion of the investigator, it is in the best interest of the participant
- Non-compliance
- Termination of the study

Participant follow-up should continue as specified in the protocol until the scheduled date of study completion, or until recovery or stabilization of an AE, whichever comes last. All

participants that permanently discontinue study treatment should be followed for survival and disease progression outcomes; these assessments may be made via telephone contact if local follow-up is not possible. At minimum, all participants should have an EOT visit that includes tumor measurements (imaging and cutaneous measurements) and a focused physical assessment prior to study withdrawal. Chemistry and hematology should be collected, if possible. Investigators should also provide a final and best overall anti-tumor response assessment with the eCRF.

Lost to Follow-up

A participant will be considered lost to follow-up if they fail to return for 2 scheduled visits and are unable to be contacted by the study site. The following actions must be taken if a participant fails to return to the clinic for a required study visit:

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

Participants who are lost to follow-up may be replaced at the Sponsor's discretion.

6.4 Study Termination

The entire study may be terminated by the Sponsor in the event of any of the following:

- Upon discussion with the Data and Safety Monitoring Committee, the occurrence of AEs unknown to date with respect to their nature, severity, and duration, or an unexpected increase in the incidence of known AEs.
- Medical or ethical reasons affecting the continued performance of the study
- Difficulties in the recruitment of participants
- Cancellation of the drug development program
- Sponsor decision for other reasons

7 STUDY ASSESSMENTS AND PROCEDURES

Study procedures and their timing are summarized in the SOAs (Table 1 and Table 2 [Arm 2: lerapolturev + nivolumab]). Adherence to the study design requirements, including those specified in the SOA, is essential and required for study conduct. Immediate safety concerns should be discussed with the Sponsor immediately upon occurrence/awareness to determine if the participant should continue or discontinue the study drug treatments.

All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria prior to randomization. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.

Procedures conducted as part of the participant's routine clinical management (eg, blood count, scans) and obtained before signing of the ICF may be utilized for screening purposes provided the procedures met the protocol-specified criteria and were performed within the time frame defined in the SOA.

7.1 Efficacy Evaluations

Anti-tumor evaluations will be conducted as specified in the appropriate SOA (Table 1 and Table 2 [Arm 2: lerapolturev + nivolumab]). Unscheduled assessments should be considered if clinically indicated, and results collected in the eCRF. The anti-tumor activity evaluations include the following:

- *Tumor measurements:* All participants will undergo an extent of disease evaluation consisting of a chest, abdomen and pelvis CT, a brain MRI and skin caliper measurement (with photograph) of target lesions. Designation of which lesions are to be target lesions for assessment of anti-tumor response should be based on RECIST 1.1 guidelines. All participants will undergo repeat evaluations of their extent of disease per the SOA, or as clinically indicated. Imaging modalities should remain consistent throughout study. Evaluation of treatment response by imaging and/or caliper/ruler measurement will be performed according to RECIST 1.1 criteria (NOTE: evaluation of tumor response per iRECIST is exploratory). Where possible, target lesions for assessment of tumor response should be separate from lesions chosen for biopsy (injected or non-injected). RECIST/iRECIST target lesions are not required to be lesions injected with lerapolturev. Participants without evidence of CNS disease at screening are not required to have brain MRI while on study, provided they remain without clinical evidence of progression in the CNS space.
    - **NOTE:** All MRIs should be performed with and without contrast, provided the participant can tolerate the contrast agent. A CT of the brain (with IV contrast if not contraindicated) may be performed in lieu of an MRI for those participants unable to receive an MRI.

- *Long term follow-up:* Study personnel will obtain survival information and radiographic scans (if the participant discontinued study for any reason other than radiographic progression or death) per SOC during the follow-up phase. Personnel should collect this information (including post-study anti-cancer therapies) during a participant's routine clinic visit, review of the local medical records, by communicating with referring healthcare providers for participants who do not return to the study site for their subsequent care, or review of the Social Security Death Index.

7.2 Safety Assessments

7.2.1 Physical Examination and Vital Signs

The screening physical examination will be a complete physical exam of major body systems and include, the general appearance of the participant, height and weight, vital signs (temperature, blood pressure (systolic and diastolic blood pressure [mmHg]), respiratory rate, and heart rate (bpm)), examination of the skin, ears, nose, throat, lungs, heart, abdomen, extremities, musculoskeletal system, lymphatic system, and nervous system. During the Treatment Phase and at the EOT visit, focused and symptom-directed physical examination and weight assessment should be performed per the corresponding SOA (Table 1 and Table 2 [Arm 2: lerapolturev + nivolumab]) or as clinically indicated. In addition, an evaluation of all skin lesions, including those injected with lerapolturev at the previous treatment visit, should be performed prior to subsequent lerapolturev injection while on study. Only clinically relevant abnormalities found during the screening physical examination should be recorded and reported in the medical history eCRF. Clinically relevant abnormalities that present during study participation should be reported as AEs, where appropriate.

7.2.2 Clinical Safety Laboratory Assessments

Laboratory assessments that will be conducted during the study are listed below:

- Hematologic assessments include complete blood count with differential, which measures the following:
    - Hemoglobin
    - WBC count with differential
    - Platelet count
- Chemistry assessments including the following analytes:
    - LDH
    - Glucose
    - Calcium
    - Sodium
    - Potassium
    - Bicarbonate

    - Chloride
    - BUN
    - Creatinine
    - Total protein
    - Albumin
    - ALP
    - AST
    - ALT
    - Total bilirubin
    - Amylase
    - Lipase
- INR, PT, PTT (or aPTT), as performed/reported per institutional standards
- Beta-hCG, if applicable, within 2 days prior to Day 1 and approximately every 12 weeks thereafter, while on study
- TSH, free T4, and free T3

All protocol-required laboratory assessments, as outlined above, must be conducted in accordance with the laboratory manual and the appropriate SOA (Table 1 and Table 2 [Arm 2: lerapolturev + nivolumab]). The investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study as AEs in the eCRF. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition.

All laboratory tests with values considered clinically significantly abnormal after the first lerapolturev injection through EOT visit should be repeated approximately weekly until the values return to Grade 1 or baseline, or are no longer considered clinically significant by the investigator or medical monitor.

If such values do not return to ≤Grade 1 or baseline within a period of time judged reasonable by the investigator, the etiology should be identified and entered as an AE (as applicable), and the Sponsor notified.

If laboratory values from non-protocol specified laboratory assessments performed at the institution’s local laboratory require a change in participant management or are considered clinically significant by the investigator (eg, SAE, AE, or dose modification), then the results must be recorded in the eCRF.

NOTE: Only report clinically significant laboratory abnormalities as AE when a diagnosis for the condition resulting in the laboratory abnormality is not available/not possible.

7.3 Adverse Events, Immune-Related Adverse Events, Adverse Events of Interest and Serious Adverse Events

Potential AEs will be spontaneously solicited from the participant (or, when appropriate, by a caregiver, surrogate, or the participant's legally acceptable representative) for the duration of the study.

The definitions of an AE, irAE, AESI, or SAE can be found in Section 9.2.

The investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE, irAE, AESI, or SAE and remain responsible for following up AEs that are serious, considered related to lerapolturev and/or lerapolturev in combination with anti-PD-1 therapy or study procedures that caused the participant to discontinue the study.

7.3.1 Time Period and Frequency for Collecting Information on Adverse Events, Immune-Related Adverse Events, Serious Adverse Events and Adverse Events of Special Interest

Table 4 provides an overview of the time periods and frequency for AE, irAE, SAE, and AESI reporting. Any AEs and SAEs related to screening procedures that are not SOC (eg, PV vaccination booster) will be collected from signing of the ICF. All other AEs/AESI/SAEs will be collected upon commencement of lerapolturev administration (ie, events that are treatment emergent). Collection of AEs will continue until 30 days and SAEs and AESIs for 90 days after completion of the participant's last dose of study drug(s), including any contact for follow-up of safety (Note: treatment-related SAEs will be followed until resolution or stabilization). The investigator will immediately report to the Sponsor any SAE (whether or not considered study drug-related, including those listed in the protocol or IB) and must include an assessment of whether there is a reasonable possibility that lerapolturev caused the event.

Disease progression is not to be reported as an AE/SAE.

Table 8. Time Windows and Frequency for Reporting Based on AE Type

	Reporting		
	Start	**End**	**Timeframe**
SAE	Signing of ICF	≤90 days of last dose of lerapolturev or anti-PD-1: all SAE	Within 24 hours of awareness
AE	• During screening, only AE deemed related to PV booster or mandatory per protocol biopsy • All other AE after 1st lerapolturev injection	≤30 days of last dose of lerapolturev or anti-PD-1	Per study agreement
AESI & irAE	• During screening, only if deemed related to PV booster • After 1st lerapolturev injection	≤90 days of last dose of lerapolturev or anti-PD-1	• For irAE: Per study agreement • For CRS or ICANS ONLY: ○ Within 24 hours of awareness • For ISR: ○ Grade 3/4: Within 24 hours of awareness ○ Grade1/2 ISR: Per study agreement

All SAEs will be recorded and reported to the Sponsor or designee within 24 hours, as indicated in Section 9.2. The investigator will submit any updated SAE data to the Sponsor within 24 hours of it being available. In addition, any AESI of CRS, ICANS, or Grade 3 or 4 injection site reaction (ISR), should also be reported to the Sponsor within 24 hours, regardless of seriousness per the study's safety reporting guidelines. An AESI of Grade 1 or 2 ISR should be reported in accordance with the site's study agreement.

Investigators are not obligated to actively seek AEs, irAEs, AESIs or SAEs after conclusion of the study participation. However, if the investigator learns of any SAE, including a condition resulting in death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to lerapolturev, lerapolturev in combination with anti-PD-1 or study participation, the investigator must promptly notify the Sponsor or designee.

The method of recording, evaluating, and assessing causality of AEs, irAEs, AESIs and SAEs, and the procedures for completing and transmitting SAE reports, are provided in Section 9.2.

7.3.2 Method of Detecting Adverse Events, Immune-Related Adverse Events, Adverse Events of Special Interest and Serious Adverse Events

Care will be taken not to introduce bias when detecting AEs, irAEs, AESIs and/or SAEs. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrences.

7.3.3 Follow-up of Adverse Events, Immune-Related Adverse Events, Adverse Events of Special Interest and Serious Adverse Events

After the initial AE/irAE/AESI/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs and non-serious irAEs and AESIs (see Section 9.2) will be followed until satisfactory resolution, the investigator deems the event to be chronic, the participant is stable, or the participant is lost to follow-up (as defined in Section 6.3). Supporting documentation of the event may be requested by the Sponsor and should be provided as soon as possible. Further information on follow-up procedures is provided in Section 9.2.

7.3.4 Regulatory Reporting Requirements for Serious Adverse Events

The investigator is required to promptly (ie, within 24 hours) notify the Sponsor of the occurrence of an SAE, so that legal and ethical obligations towards the safety of participants and the safety of a study drug under clinical investigation are met.

The Sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study drug under clinical investigation. The Sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and investigators.

Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSARs) according to local regulatory requirements and Sponsor policy and forwarded to investigators as necessary.

An investigator who receives an investigator safety report describing an SAE or other specific safety information (eg, summary or listing of SAEs) from the Sponsor will review and then file it along with the IB and will notify the IRB/IEC, if appropriate, according to local requirements.

7.3.5 Notification of Pregnancy

Because all participants are required to have a boost immunization of trivalent inactivated IPOL™, risk of lerapolturev transmission from mother to fetus after intratumoral injection is expected to be limited. Because risk is unknown, all participants will be required to utilize contraception during receipt of lerapolturev injections. In addition, use of PD-1 inhibitors may increase risk of fetal harm when administered to pregnant women. Women should be advised of the potential risk to a fetus and to use effective contraception during treatment with anti-PD1 therapy and for 150 days after the last dose received.

Details of all pregnancies in female participants and female partners of male participants will be collected after the start of study drugs and until 150 days after the last dose. If a pregnancy is reported, the investigator should inform the Sponsor within 24 hours of learning of the pregnancy.

Abnormal pregnancy outcomes (eg, spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs.

7.4 Tissue and Blood-Based Biomarker and Cytokine Analyses

Tissue Biopsy Collection: Participants are required to submit a baseline tumor biopsy to enroll in the study. Submission of archival tissue is allowed if collected within 4 months prior to Cycle 1, Day 1, and the participant received no intervening systemic/intratumoral anti-cancer therapy between biopsy collection and Cycle 1, Day 1 (eg, focal RT of non-biopsied lesions is allowed). If no archival tissue meeting these requirements is available, a baseline tumor biopsy should be collected during the Screening Period. During study participation, mandatory biopsies should be collected from injected lesions, non-injected lesions, and draining lymph nodes per the corresponding SOA (Table 1 and Table 2 [Arm 2: lerapolturev + nivolumab]). For participants in Arm 1 that crossover to Arm 2, additional mandatory biopsies of an injected lesion, non-injected lesion, and draining lymph node should be performed at the time of crossover and on treatment as described in the treatment group's SOA (Table 1 and Table 2 [Arm 2: lerapolturev + nivolumab]).

At all timepoints, biopsies from all three sites (injected lesion, noninjected lesions and draining lymph nodes) should be collected if the biopsy is technically feasible and does not put the participant at significant risk. Examples of biopsy sites considered to be of significant risk include, but are not limited to the following: biopsies of the brain, lung, mediastinum, pancreas, or endoscopic procedures extending beyond the esophagus, stomach, or bowel wall. For those situations where the investigator determines biopsy of a lesion would (1) lead to a significant decrease in the size of the lesion such that response assessment by RECIST 1.1 would be confounded, or (2) increase the risk of lerapolturev leakage from the lesion, the mandatory biopsy for that timepoint can be waived. In addition, complete resection of target lesions is prohibited unless necessary for participant's safety as determined by the investigator.

Tissue samples may be analyzed by immunohistochemical staining, RNA and/or DNA sequencing, and other molecular techniques to confirm the presence of lerapolturev, and to determine the presence or absence of biomarkers that may (1) predict response to lerapolturev, (2) measure the associated immune response and/or changes in immune cell function over time, or (3) to further characterize lerapolturev's mechanism of action. Samples (blood and tissue) may be stored and used for future research for up to 10 years.

Blood Biopsy Collection: Mandatory blood samples should be drawn during the screening period and while on study, as described in the appropriate SOA (Table 1 and Table 2

[Arm 2: lerapolturev + nivolumab]), to facilitate evaluation of immune cell function and cytokine profiles before and after study treatment administration. Participants in Arm 1 who crossover to Arm 2 should also have blood samples collected at the time of crossover and on treatment as described in the requisite SOA (Table 1 and Table 2 [Arm 2: lerapolturev + nivolumab]). In addition, an additional sample is to be drawn in the event any participant (regardless of treatment arm) experiences an AESI of CRS or ICANS. This sample should be drawn as soon as possible after learning of the event, to facilitate the interrogation of potentially relevant changes in circulating cytokine levels.

Blood samples may be interrogated by cytokine and flow cytometric analyses, RNA and/or DNA sequencing, and other molecular techniques to evaluate signatures of anti-tumor immune response to lerapolturev in circulation. In addition, various immune cell types, including peripheral blood mononuclear cells, may be isolated and assayed via genetic and proteomic techniques to evaluate any changes in cell function following treatment with lerapolturev with or without concurrent anti-PD-1. These samples will be stored under the appropriate conditions as described in the laboratory manual and analyzed as a group by the Sponsor or designee at designated intervals. Samples (blood and tissue) may be stored and used for future research for up to 10 years.

8 STATISTICAL CONSIDERATIONS

8.1 Stratification Factors

Participants receiving lerapolturev injections using the **Q3/4W** schedule ± anti-PD-1 therapy will be stratified based on time since prior anti PD-1/L1 exposure (≤6 weeks versus >6 weeks) and baseline LDH (normal versus >ULN).

Participants receiving lerapolturev injections using the **QW** schedule ± anti-PD-1 therapy will be stratified based on type of anti-PD-1/L1 resistance (primary versus secondary as defined in Kluger et al[7]) and baseline LDH (normal versus >ULN).

8.2 Statistical Hypotheses

Lerapolturev administered in combination with anti-PD-1 therapy on the **QW** injection schedule to participants with anti-PD-1 refractory melanoma will result in an ORR of ≥ 33%.

8.3 Sample Size Determination

The determination of sample size in this two-arm, randomized open-label Phase 2 trial was based on feasibility and logistical considerations. As discussed in Section 1.1, the Sponsor estimates that the true activity of anti-PD-1 re-challenge in the anti-PD-1 refractory population eligible for this study is less than 10%. Therefore, lerapolturev administered in combination with anti-PD-1 therapy using the **QW** injection schedule, will be considered to have anti-tumor activity in the anti-PD-1 refractory melanoma population

if the lower bound of the 95% CI is above 10%. We estimate the sample size for participants receiving lerapolturev (**QW** schedule) ± anti-PD-1 therapy to be approximately 30 participants (n=15 per arm). Therefore, 5 of 15 participants (ie, 33%) in Arm 2 treated with the lerapolturev/anti-PD-1 combination (**QW** schedule) must have an objective response for the 95% CI to be greater than 10% (95% CI: 11.8% to 61.6%).

Additional comparisons (eg, Arm 1 versus Arm 2; Q3/4W schedule vs QW schedule for each Arm etc.) are planned. However, no formal hypothesis will be tested. Additional detail will be provided in the statistical analysis plan (SAP).

8.4 Populations for Analyses

For participants receiving the **QW** schedule of lerapolturev injections, enrollment will be stratified based on baseline LDH levels (normal versus >ULN) and type of anti-PD-1/L1 resistance (primary versus secondary), primarily for the purpose of balancing key participant descriptors between the treatment arms. For purposes of analysis, the following key populations are defined; additional populations may be defined in the SAP:

Population	Description
Intent-to-treat	All participants who sign the ICF and are randomized
Efficacy--**QW** Schedule	Participants receiving at least one lerapolturev injection on the **QW** schedule and at least 1 post-baseline evaluation
Efficacy—**Q3/4W** Schedule	Participants receiving at least one lerapolturev injection on the **Q3/4W** schedule and at least 1 post-baseline evaluation
Crossover	Participants from Arm 1 receiving at least one lerapolturev injection with concurrent anti-PD-1 and at least 1 post-crossover evaluation
Safety—**QW** Schedule	Participants receiving at least one lerapolturev injection on the **QW** schedule
Safety—**Q3/4W** Schedule	Participants receiving at least one lerapolturev injection on the **Q3/4W** schedule

8.5 Statistical Analyses

The SAP will be developed and finalized before database lock and will describe the participant populations to be included in the analyses, and procedures for accounting for missing data. The statistical approach described in the SAP will supersede the statistical approach described here, in the event of discrepancy. This section is a summary of the planned statistical analyses for key endpoints of interest.

8.5.1 Efficacy Analyses

The primary endpoint for measuring anti-tumor activity will be ORR, which will be evaluated once participants have reached at least Week 26, based on RECIST 1.1. ORR will be described as the overall number (%) of participants in each treatment arm with CR

or PR and will be presented along with the respective 95% confidence intervals (CI) for each arm. For Arm 1, the primary analysis will include participant response prior to anti-PD-1 re-challenge (eg, before crossover to Arm 2). ORR by treatment stratum and lerapolturev dosing schedule will be conducted, as will other pre-determined subgroups defined in the SAP.

Additional secondary endpoints, including DOR, DRR, DCR, PFS and OS will also be summarized. DRR will be presented as the number (%) of participants with CR or PR lasting ≥6 months, along with 95% CI. DCR will be shown as the number (%) of participants with SD, PR, or CR together with the 95% CI. DOR, PFS and OS will be described using the Kaplan-Meier method. Additional details will be included in the SAP.

8.5.2 Translational Analyses

As a primary objective, markers of immune activation in response to lerapolturev treatment (with or without anti-PD-1) will be evaluated in both injected and non-injected lesions. Participants with a baseline and at least 1 post-treatment biopsy will be analyzed for changes in the number of CD8+ TILs located within the TME, as well as for changes in PD-L1 expression. Additional exploratory analyses will be performed to characterize changes in other important markers of immune function following treatment with single-agent lerapolturev or with the lerapolturev/ anti-PD-1 combination. In general, pre- and post-treatment tissue biopsies will be assayed for changes in immune cell density by histology or other molecular techniques (eg, flow cytometry). Changes in T cell receptor repertoire, and chemokine and/or cytokine profiles in blood samples and/or tissue will also be assayed at the gene and/or protein expression level, as appropriate. All results will be described by summary statistics as outlined in the SAP.

8.5.3 Safety Analyses

Participants receiving at least one lerapolturev injection will be analyzed for safety. Any AEs reported after the initiation of study treatment and ≤30 days after the last dose of study treatment are defined as treatment emergent. For each treatment-emergent AE, the maximum grade experienced by each participant will be summarized with frequency distributions. Adverse event tabulations will include all treatment emergent events (regardless of attribution), and a tabulation for all study-related interventions that are at least possibly related to lerapolturev. Adverse events occurring during screening due to lerapolturev-related procedures that are not standard of care (eg, PV booster) will be considered related to lerapolturev and summarized separately.

8.5.4 Exploratory Analyses

Exploratory objectives evaluating subgroup analyses, such as outcomes for participants based on BRAF status, primary versus secondary resistance, time since prior anti-PD-1 administration, responses following Arm 1 to Arm 2 crossover (ie, anti-PD-1 re-challenge),

baseline LDH levels or others (as described in Section 2.1) will be summarized by endpoints, including DOR, DRR, DCR, PFS and OS. DRR will be presented as the number (%) of participants with CR or PR lasting ≥6 months, along with 95% CI. DCR will be shown as the number (%) of participants with SD, PR, or CR together with the 95% CI. DOR, PFS and OS will be described using the Kaplan-Meier method. For the analysis of response following crossover from Arm 1 to Arm 2, the baseline for anti-tumor response in this subgroup will based on the size of the tumor at the time of crossover and will be summarized separately. Anti-tumor response (ie, ORR, DOR, and DRR) based on iRECIST will also be summarized with descriptive statistics, as appropriate.

In addition, a per-protocol population (no major protocol deviations, etc.) and predictors of lerapolturev response and/or changes in markers of immune function will be summarized by summary statistics, as appropriate. Additional details regarding these and other analyses will be described in the SAP.

8.6 Interim Analysis (IA)

No interim analysis will be conducted for this study. Rather, efficacy results for participants receiving the **Q3/4W** schedule and **QW** schedule will be summarized separately for participants receiving either lerapolturev monotherapy or lerapolturev in combination with anti-PD-1 therapy, as described in the SAP.

9 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

9.1 Regulatory, Ethical, and Trial Oversight Considerations

9.1.1 Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with the following:
  - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and applicable ICH Good Clinical Practice (GCP) Guidelines
  - Applicable federal, state, and local regulations
- The protocol, ICF, IB, and other relevant documents (eg, advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
- The investigator will be responsible for the following:
  - Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, and all other applicable local regulations.
  - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC.
  - Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures.

9.1.2 Financial Disclosure

Investigators and sub-investigators will provide the Sponsor with sufficient, accurate financial information as requested to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the study and for 1 year after completion of the study.

9.1.3 Informed Consent Process

Participants must be informed that their participation is voluntary. The investigator or his/her representative will provide full and adequate written and oral explanation of the nature of the study to the participant or his/her legally authorized representative (LAR) and answer all questions regarding the study.

Participants or their LAR will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or trial center.

The medical record must include a statement that written informed consent was obtained before the participant was screened for the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.

Participants must be re-consented to the most current version of the ICF(s) during their participation in the study, when required.

A copy of the ICF(s) must be provided to the participant or the LAR.

9.1.4 Data Protection

All personal information for participants in the study will be kept strictly confidential and will be protected by the investigators, investigational site staff, the Sponsor, and any third parties working on behalf of the Sponsor. Every effort will be made to maintain the confidentiality of any personal information that identify the participant by name (eg, signed ICF, laboratory reports, clinic charts), except to the extent necessary to allow monitoring or auditing by the Sponsor or designee, and auditing by the FDA, or other regulatory authorities.

To protect participant privacy, coded information will be labeled in a way that will not identify the participant to the Sponsor and others who may see the information at the Sponsor location. The participant must be informed that his/her medical records may be examined by study monitors, Clinical Quality Assurance auditors, other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities. The participant must also be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant.

This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants.

9.1.5 Data and Safety Monitoring Committee

An external DSMC has been engaged to closely monitor the safety of the interventions under investigation during this study. The DSMC was tasked to review the data during (1) the safety run-in, (2) once 12 participants treated with the **Q3/4W** schedule were randomized and completed the DLT period (eg, after approximately six participants have been treated with lerapolturev plus anti-PD1 therapy), (3) at the previously planned interim analysis (IA) and (4) approximately every 4 months after the IA.

As mentioned in Section 3.1, the DSMC met on 20 December 2021 to review all available safety data collected following clearance of the DLT monitoring period of the 12th participant randomized (all receiving **Q3/4W** schedule) (18 participants total: n=6 from the safety run-in and n=12 randomized [n=6 per arm]), as well as to discuss the feasibility of increasing the lerapolturev dose and changing the dosing schedule (ie, max dose of 1.6×10^9 $TCID_{50}$ administered using the **QW** schedule). Given the favorable tolerability profile of lerapolturev, when given alone or in combination with anti-PD-1, the DSMC supported the Sponsor's recommendation to change the lerapolturev dose and schedule, with the agreement that the next DSMC meeting should occur once 12 participants (n=6 per arm) have received the **QW** lerapolturev dosing schedule and have cleared the DLT period. As before, the DSMC will make recommendations regarding whether adjustments to lerapolturev dose are warranted, as well as whether to continue the study as planned. Based on this agreement with the DSMC and other logistical considerations, the DSMC will no longer meet to review data at the previously planned IA. Once the planned QW schedule safety evaluation is complete, the DSMC will continue to meet approximately every 4 months thereafter, as previously described.

The DSMC will also review any DLTs that are deemed at least possibly related to lerapolturev and any SUSARs on an ongoing basis during the conduct of the trial; and may provide additional recommendations with regard to lerapolturev dose and/or other modifications to study conduct. For example, dosing of current participants may be held to evaluate SUSARs that have not been described to date with respect to their nature, severity, and duration, or if there is an unexpected increase in the incidence of known AEs in excess of expectations (with lerapolturev and/or anti-PD-1 therapy). The DSMC may also formulate recommendations relating to the selection, recruitment, and retention of participants and their management. Additional details regarding the responsibility of the DSMC/its chair will be provided in a DSMC charter document, as applicable.

9.1.6 Data Quality Assurance

All participant data relating to the study will be recorded on eCRF unless transmitted to the Sponsor or designee electronically (eg, imaging data). The investigator is responsible for verifying that all data entries are correct and must maintain accurate documentation (source data) that supports the information entered in the eCRF. The investigator must also permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents. Investigators will keep personal medical records and a list that links each participant's name to his or her code number as part of the study requirements according to local regulatory and legal requirements.

The Sponsor or designee is responsible for the data management of this study including quality checking of the data. Study monitors will perform ongoing source data verification to confirm that data entered into the eCRF by authorized site personnel are accurate, complete, and verifiable from source documents. Ultimately, the study monitors will help ensure that the safety and rights of participants are being protected, that the study is being conducted in accordance with the currently approved protocol, as well as any other study agreements, ICH GCP, and all applicable regulatory requirements.

All essential records and documents pertaining to the conduct of this study must be retained by the investigator for the following time periods:

- At least 2 years after the last marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region; or
- At least 2 years have elapsed since the formal discontinuation of clinical development of the study drug

These documents should be retained for a longer period, if required by applicable regulatory requirements or by the executed clinical trial agreement with the Sponsor. It is the responsibility of the Sponsor to inform the investigator/institution as to when these documents no longer need to be retained. The executed clinical trial agreement will govern if language differs from that contained herein. No records may be transferred to another location or party without written notification to the Sponsor.

9.1.7 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol or ICH GCP. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions may be developed by the site and implemented promptly.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations according to ICH and/or Sponsor requirements. All deviations must be addressed in study source documents and reported to the Sponsor. Protocol deviations must be sent to the reviewing IRB/IEC, per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB/IEC requirements.

9.1.8 Source Documents

Source documents are necessary to provide evidence for the existence of the participant and to substantiate the integrity of the data collected. Source documents are filed at the investigator’s site. Source data includes (but is not limited to): in-patient hospital charts, clinic notes, out-patient records, original tests results, pathology reports, laboratory data, worksheets, investigational product accountability records, consent forms, etc.

Data reported on the CRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available. Remote methods to access source documents may be used throughout the conduct of the study. For example, remote access to study charts and regulatory files may be required throughout the conduct of the study. In addition, remote monitoring visits may also be performed, as needed.

9.1.9 Study and Site Closure

The Sponsor or designee reserves the right to temporarily suspend or close the study, either at a single site or all sites, at any time for any reason. If such action is taken, the Sponsor will discuss this with the investigator (including the reasons for taking such action) at that time. The Sponsor will promptly inform all other investigators and institutions conducting the study if the study is suspended or terminated for safety reasons. The Sponsor will also inform necessary regulatory authorities of the suspension or termination of the study and the reason(s) for the action. If required by applicable regulations, the investigator must inform the IRB/IEC promptly and provide the reason for the suspension or termination.

Study sites will be closed upon study completion. A study site is considered closed when all required documents/records are complete, study supplies (including study drug) have been collected and/or are accounted for, remaining data issues are clarified or resolved, and study-site closure visit has been performed.

Reasons for the early closure of a study site by the Sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the Sponsor's procedures/SOPs, or GCP guidelines.
- Inadequate recruitment of participants by the investigator.
- Discontinuation of further lerapolturev development.

9.1.10 Confidentiality and Publication Policy

The investigator and other study site personnel will keep confidential any information provided by the Sponsor related to this study (including this protocol) and all data and records generated while conducting the study. The investigational site will not use the information, data, or records for any purpose other than conducting the study, in keeping with all provisions related to such in the executed clinical trial agreement.

- The results of this study may be published or presented at scientific meetings, only with documented Sponsor approval. The investigator is required to submit all manuscripts or abstracts to the Sponsor for review or approval before submission.
- In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

9.2 Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

AE Definition
• An AE is any untoward medical occurrence in a patient or clinical study participant, temporally associated with the use of study drug(s), whether or not considered related to the study drug(s). • NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study drug(s).

Events Meeting the AE Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (ie, not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition, including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study drug(s) administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study drug(s) or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae. • "Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfil the definition of an AE or SAE.

Events NOT Meeting the AE Definition

- Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition.
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition.
- Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that leads to the procedure is the AE.
- Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital, including elective/planned medical procedures).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

Definition of SAE:

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (eg, hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

An SAE is defined as any untoward medical occurrence that, at any dose:

- **Results in death**
- **Is life-threatening**

The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

- **Requires inpatient hospitalization or prolongation of existing hospitalization**

In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.

Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

- **Results in persistent disability/incapacity**

The term disability means a substantial disruption of a person's ability to conduct normal life functions. This includes abnormal birth outcomes (spontaneous abortion, fetal death, stillbirth, congenital anomalies, etc.) from pregnancies of participants who became pregnant (either female participants or female partners of male study participants).This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

- **Is a congenital anomaly/birth defect**

Other situations:

Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.

Examples of such events include intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Definition of Immune-Related Adverse Events (irAEs):

An irAE is defined as:

- Any adverse event affecting an organ system through an autoimmune or inflammatory mechanism, whereby the immune system is acting against normal tissue. Examples of irAEs, as well as recommendations for study drug management in the event of an irAE are described in Section 5.5.

Definition of Adverse Events of Interest (AESI):

An AESI is defined as:

- Any AE (serious or non-serious) of scientific and medical concern for which ongoing monitoring and communication by the investigator to the Sponsor or designee may be appropriate. Such events may require further investigation to characterize and understand them. Any AESI should be entered in the eCRF.

Because lerapolturev is an immunotherapeutic administered by intratumoral injection, AESIs fall into 2 categories: 1.) AEs related to lerapolturev administration (ie, ISR), and 2.) AEs related to CRS and ICANS, which can occur when the body's immune cells release a bolus of inflammatory cytokines in response to an immunotherapeutic[44]. AESI related to CRS and ICANS should be reported to the Sponsor within 24 hours, regardless of severity or seriousness. AESI related to ISR does not require expedited reporting (ie, within 24 hours), unless considered an SAE. A brief overview of each AESI is outlined below.

AEs associated with ISR:

To more completely describe the signs and symptoms associated with ISR, additional information related to the parameters outline below should be collected, which have been adapted from FDA guidance for evaluating ISR in vaccine trials[45] (see Appendix I), as well as past clinical experience with lerapolturev in melanoma.

- Erythema
- Induration
- Pruritus
- Ecchymosis/bruising/skin discoloration
- Pain

Each symptom will be graded on a scale of 1 to 4, as described in Appendix I, which may represent a measure of intensity, *not seriousness*. Thus, a Grade 3 or Grade 4 sign or symptom may be severe, but not necessarily serious. Therefore, although all Grade 3/4 AEs of ISR should be reported within 24 hours, only AEs of ISR meeting the criteria for seriousness (see SAE definition above for a definition of seriousness) should be reported as an SAE. Where appropriate, reporting of symptoms may also require a measurement recorded as a continuous variable (ie, diameter of erythema).

AEs associated with CRS and ICANS:

The management of signs and symptoms of CRS and ICANS are described in Section 5.5 (Management of CRS and ICANS). CRS has been associated with administration of other immunotherapeutics, including oncolytic viruses, such as T-VEC[19] and CAR T cell therapies, but has not been detected in any participants treated with lerapolturev to data (Istari data on file). The ASTCT consensus guidelines describe that the onset of symptoms of CRS typically occur within 2 weeks of treatment[41], and may include (but are not limited to):

- Fever (required; ≥ 38°C) not attributable to any other cause or underlying condition
- Hypoxia
- Tachycardia

- Hypotension
- Dyspnea

Per the ASTCT consensus guidelines[41], symptoms of ICANS are defined as AEs that result in "a pathologic process involving the CNS following any immune therapy that results in activation or engagement of endogenous or infused T cells and/or other immune effector cells." AEs related to ICANS can include (but may not be limited to) the following:

- Cerebral edema
- Seizure
- Motor weakness
- Aphasia
- Altered level of consciousness
- Impaired cognition

As stated, administration of lerapolturev has not been associated with CRS or ICANS in any participant treated with lerapolturev to date, regardless of indication (Istari data on file). Because the current study is investigating lerapolturev in conjunction with concurrent anti-PD-1 therapy, additional steps are being taken to characterize the occurrence of, duration of, and response to interventions for signs and symptoms of CRS and/or ICANS, should they occur. This includes reporting any AESI of CRS or ICANS within 24 hours of awareness of the event, regardless of severity or seriousness, as well as acute testing of plasma for cytokine profile, as outlined in Section 5.5. Suspected ICANS also requires a neurological exam which should include the ICE screening assessment, per Appendix I. All AESI of CRS and ICANS will be graded on a scale of 1 to 5 according the ASTCT consensus guidelines (see Appendix I).

If any individual sign or symptom meets the criteria for seriousness (ie, results in new hospitalization, prolonged hospitalization, death, persistent or significant disability/incapacity, a life-threatening condition, or an event of medical significance), it should be reported as an SAE, as described in Section 7.3.4.

Recording and Follow-Up of AE, AESI and/or SAE

AE, irAE, AESI and SAE Recording

- When an AE/irAE/AESI/SAE occurs, it is the responsibility of the investigator to review all documentation (eg, hospital progress notes, laboratory reports, and diagnostics reports) related to the event and report the event within the appropriate timeframe.
- The investigator will then record all relevant AE/irAE/AESI/SAE information in the eCRF.
- It is not acceptable for the investigator to send photocopies of the participant's medical records to the Sponsor/designee in lieu of completion of the necessary eCRF page and/or any other safety event reporting form (if applicable) noted in the study's safety reporting guidelines.

  NOTE: AESI of CRS or ICANS should be recorded in eCRF with the verbatim term of CRS or ICANS, rather than the individual signs and symptoms related to the AESI. The underlying signs and symptoms associated with the AESI should be reported per the study's safety reporting guidelines and/or as part of the descriptive narrative, rather than as individual AEs.
- There may be instances when copies of medical records for certain cases are requested the Sponsor/designee. In this case, all participant identifiers, except for the participant number, will be redacted on the copies of the medical records before submission.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/AESI/SAE.

Assessment of Intensity

With the exception of AESIs related to CRS or ICANS, the investigator will assess severity grade for each AE, irAE, AESI (ISR only) and SAE reported during the study using the National Cancer Institute – Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 5 or later. AESI related to CRS or ICANS should be graded according to the ASTCT guidelines[41] (see Appendix I). Any AE not listed in the NCI-CTCAE will be graded according to the investigator's clinical judgment using the standard grades as follows:

- Grade 1 (Mild): Awareness of symptoms that are easily tolerated, causing minimal discomfort and not interfering with everyday activities.
- Grade 2 (Moderate): Sufficient discomfort is present to cause interference with normal activity.
- Grade 3 (Severe): Extreme distress, causing significant impairment of functioning or incapacitation. Prevents normal everyday activities.

AE, irAE, AESI and SAE Recording

- Grade 4, (Life-threatening): Urgent intervention indicated.
- Grade 5: Death.

The investigator should use clinical judgment in assessing the severity of events not directly experienced by the participant (eg, laboratory abnormalities).

Assessment of Causality

- The investigator is obligated to assess the relationship between study drug(s) and each occurrence of each AE/irAE/AESI/SAE and to record this determination in the eCRF. The AE must be characterized as unrelated, unlikely to be related, possibly related, probably related, or definitely related.
  - "Definitely related" conveys the adverse event has a timely relationship to administration of study treatment and there is no apparent, potential alternate etiology.
  - “Probably related” conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
  - “Possibly related” suggests that the association of the AE with the study treatment is unknown; however, the AE is not reasonably supported by other conditions.
  - “Unlikely to be related” suggests that only a remote connection exists between the study treatment and the AE. Other conditions, including chronic illness, progression or expression of the disease state or reaction to concomitant therapy, appear to explain the reported AE.
  - “Unrelated” is used if there is not a reasonable possibility that the study treatment caused the AE
- The investigator should use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study drug(s) administration will be considered and investigated.
- The investigator should consult the IB during his/her assessment.
- For each AE/irAE/AESI/SAE, the investigator **must** document in the medical notes that he/she has reviewed the AE/irAE/AESI/SAE and has provided an assessment of causality, along with indication of the key pieces of evidence that factored into that determination.
- There may be situations in which an AESI or SAE occurs, and the investigator has minimal information to include in the initial report to the Sponsor/designee. However, **it is very important that the investigator always make an assessment of causality for every SAE before the initial transmission of the AESI or SAE to the Sponsor.**

Assessment of Causality

- The investigator may change his/her opinion of causality in light of follow-up information and may submit the relevant follow-up information with the updated causality assessment for the AESI or SAE.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AEs, irAEs, AESIs and SAEs

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the Sponsor to elucidate the nature and/or causality of the AE, irAEs, AESI or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- New or updated information will be recorded in the originally completed eCRF.
- The investigator will submit any updated AESI (for AESI related to CRS, ICANS, or Grade 3/4 ISR ONLY) or SAE data to the Sponsor within 24 hours of receipt of the information.

10 ABBREVIATIONS

AE	Adverse event
AESI	Adverse events of special interest
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
aPTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
ASTCT	American Society for Transplantation and Cellular Therapy
BPM	Beats per minute
CBC	Complete blood count
CI	Confidence interval
CNS	Central nervous system
CR	Complete response
CRF	case report form(s) (paper or electronic as appropriate for this study)
CRS	Cytokine release syndrome
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTL	Cytotoxic T lymphocytes
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
DC	Dendritic cell
DCR	Disease control rate
DILI	Drug-induced liver injury
DLT	Dose limiting toxicity
DOR	Duration of response
DSMC	Data safety monitoring committee
ECG	Electrocardiogram
ECOG	Eastern cooperative oncology group
eCRF	Electronic case report form
EOT	End of treatment
FDA	Food and Drug Administration
GBM	Glioblastoma multiforme
GCP	Good Clinical Practice
HBsAg	hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitic C virus
HIPAA	Health Insurance Portability and Accountability Act
HSA	Human serum albumin
IA	Interim analysis
IB	Investigator’s brochure
ICANS	Immune effector cell-associated neurotoxicity syndrome
ICF	informed consent form
ICH	International Council for Harmonization
IEC	Independent Ethics Committee
IgG	Immunoglobulin G
INR	International normalized ratio

IPHP	Investigational product handling plan
IPOL	Immunization with PV Inactivated
irAE	Immune-related adverse event
IRB	Institutional Review Board
iRECIST	Immune response evaluation criteria in solid tumors
IRES	Internal ribosome entry site
ISR	Injection site reaction
LAR	Legally authorize representative
LDH	Lactose dehydrogenase
MDRD	Modification of diet in renal disease
MRI	Magnetic resonance imaging
ORR	Overall response rate
OS	Overall survival
PBS	Phosphate buffered saline
pCR	Pathologic complete response
PD	Progressive disease
PD-1	Program death receptor - 1
PD-L1	Programmed death-ligand 1
PK	pharmacokinetic(s)
PQC	Product Quality Complaint
PR	Partial response
PT	Prothrombin time
PTT	Partial thromboplastin time
PV	Poliovirus
PV1S	Poliovirus serotype 1
PVR	Polio virus receptor
rGBM	Recurrent glioblastoma multiforme
RECIST	Response evaluation criteria in solid tumors
SAE	serious adverse event
SAP	Statistical analysis plan
SD	Stable disease
SITC	Society for Immunotherapy of Cancer
SJS	Stephen Johnson Syndrome
SOA	Schedule of assessments
SUSAR	suspected unexpected serious adverse reaction
TBP	Treatment beyond progression
TCID	Tissue culture infectious dose
TEN	Toxic epidermal necrolysis
TME	Tumor microenvironment
T-VEC	Talimogene laherparepvec
ULN	Upper limit of normal
WBC	White blood cell

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12 APPENDIX I

ASTCT Consensus Grading for CRS adapted from Lee et al., 2019[41]

CRS Parameter	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Fever[1]	Temperature ≥38°C	Temperature ≥38°C	Temperature ≥38°C	Temperature ≥38°C	Death due to CRS, where another factor is not the principle cause
With					
Hypotension	None	Not requiring vasopressors	Requiring a vasopressor with or without vasopressin	Requiring multiple vasopressors (excluding vasopressin)	
And/or[2]					
Hypoxia	None	Requiring low-flow nasal cannula[3] or blow-by	Requiring high-flow nasal cannula[3], facemask, nonrebreather mask, or Venturi mask	Requiring positive pressure (eg, CPAP, BiPAP, intubation and mechanical ventilation)	

Organ toxicities associated with CRS may be graded according to CTCAE v5.0, but do not influence CRS grading.

1. Fever is defined as temperature ≥38°C not attributable to any other cause. In patients who have CRS then receive antipyretic or anticytokine therapy such as tocilizumab or steroids, fever is no longer required to grade subsequent CRS severity. In this case, CRS grading is driven by hypotension and/or hypoxia.
2. CRS grade is determined by the more severe event: hypotension or hypoxia not attributable to any other cause. For example, a patient with temperature of 39.5°C, hypotension requiring 1 vasopressor, and hypoxia requiring low-flow nasal cannula is classified as grade 3 CRS.
3. Low-flow nasal cannula is defined as oxygen delivered at ≤6 L/minute. Low flow also includes blow-by oxygen delivery, sometimes used in pediatrics. High-flow nasal cannula is defined as oxygen delivered at >6 L/minute.

ASTCT Consensus Grading for ICANS adapted from Lee et al., 2019[41]

Neurotoxicity Domain	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
ICE score[1]	7 to 9	3 to 6	0 to 2	0 (patient is unarousable and unable to perform ICE)	Death due to ICANS where another cause is not the principle factor leading to this outcome
Depressed level of consciousness[2]	Awakens spontaneously	Awakens to voice	Awakens only to tactile stimulus	Patient is unarousable or requires vigorous or reptitive tactile stimuli to arouse. Stupor or coma	
Seizure	N/A	N/A	Any clinical seizure focal or generalized that resolves rapidly or nonconvulsive seizures on EEG that resolve with intervention	Life-threatening prolonged seizure (>5min); or repetitive clinical or electrical seizures without return to baseline in between	
Motor findings[3]	N/A	N/A	N/A	Deep focal motor weakness such as hemiparesis or paraparesis	
Elevated ICP/cerebral edema	N/A	N/A	Focal/local edema on neuroimaging[4]	Diffuse cerebral edema on neuroimaging; decerebrate or decorticate posturing; or cranial nerve VI palsy; or papilledema; or Cushing's triad	

ICANS grade is determined by the most severe event (ICE score [see below], level of consciousness, seizure, motor findings, raised ICP/cerebral edema) not attributable to any other cause; for example, a patient with an ICE score of 3 who has a generalized seizure is classified as grade 3 ICANS. N/A indicates not applicable.

1. A patient with an ICE score of 0 may be classified as grade 3 ICANS if awake with global aphasia, but a patient with an ICE score of 0 may be classified as grade 4 ICANS if unarousable.
2. Depressed level of consciousness should be attributable to no other cause (eg, no sedating medication).
3. Tremors and myoclonus associated with immune effector cell therapies may be graded according to CTCAE v5.0, but they do not influence ICANS grading.
4. Intracranial hemorrhage with or without associated edema is not considered a neurotoxicity feature and is excluded from ICANS grading. It may be graded according to CTCAE v5.0.

ASTCT ICE Screening Tool and Scoring adapted from Lee et al., 2019[41]

Category	Task	Score
Orientation	Orientation to year, month, city, hospital	4 points
Naming	Ability to name 3 objects (eg, point to clock, pen, button)	3 points
Following Commands	Ability to follow simple commands (eg, “show me 2 fingers” or “Close your eyes and stick out your tongue”)	1 point
Writing	Ability to write a standard sentence (eg, “Our national bird is the bald eagle”)	1 point
Attention	Ability to count backwards from 100 by 10	1 point

ISR Grading adapted from FDA Guidance for Vaccine Clinical Trials[45]

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-Threatening (Grade 4)
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever >24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Erythema/Redness[1]	2.5 to 5cm	5.1 to 10cm	>10cm	Necrosis or exfoliative dermatitis
Induration/Swelling[2]	2.5 to 5cm and does not interfere with activity	5.1 to 10cm or interferes with activity	>10cm or prevents daily activity	Necrosis

1. In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.
2. Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement

13 APPENDIX II

Amendment Summary of Changes

Amendment 4.0, 14January2022
Protocol Amendment 4 includes the below major changes:

- Updated PVSRIPO to lerapolturev throughout
- Modified lerapolturev dose and dosing schedule
  - Modified other study assessment timepoints impacted by new dosing schedule
  - Added DSMC review once 12 participants have been treated on new dose and schedule (6 to each arm) and clear the DLT period
- Modified the stratification factor of time since prior anti-PD-1/L1 exposure (≤6 weeks versus >6 weeks) to type of anti-PD-1/L1 resistance (primary versus secondary)
- Modified crossover criteria
- Modified study participant inclusion and exclusion criteria as per the below:
  - Patients with ocular, acral or mucosal melanoma are now excluded
  - Patients with M1c or M1d disease are now excluded
  - Inclusion criterion 5 was modified to clarify definition of resistance to anti-PD-1/L1-based therapy
  - Patients must have not failed more than one prior anti-PD-1/L1-based regimen
- Removed study interim analysis after 20 participants randomized

Amendment 3.0, 23April2021
Protocol Amendment 3 includes the below major changes:

- Added a new primary objective: To evaluate the effect of PVSRIPO on the TME of injected and non-injected lesions when administered with and without anti-PD-1 therapy.
- The primary safety endpoint of “anti-PD-1 AESI” was changed to “anti-PD-1 irAEs” and an irAE definition was added to Section 9.2.
- Modified study participant inclusion and exclusion criteria in Section 4 and the synopsis as per the below:
  - Inclusion criterion 3 was modified to clarify the melanoma subtypes eligible for study participation, requirements for qualifying biopsy and/or archival tissue, and to add the requirement that at least one melanoma lesion is amenable to biopsy while on study.
  - Inclusion criterion 8 no longer includes a minimum serum albumin requirement.
  - Exclusion criterion 1 was added to state exclusion of ocular melanoma
  - Exclusion criteria related to uncontrolled/symptomatic hypercalcemia and worsening steroid myopathy were removed.
  - Added exclusion criterion 20 to clarify that participants unable to interrupt anticoagulant use the day prior, the day of, and the day after each PVSRIPO injection are ineligible for the study.

- Modified pregnancy testing for women of childbearing potential from approximately every 6 months to approximately every 12 weeks during study participation.
- Clarified the timing of thyroid function monitoring (along with allowable time windows) around were clarified.
- Added mandatory on-study tissue biopsies of injected and non-injected lesions, as well as draining lymph nodes, at multiple timepoints. The types of lesions and conditions under which lesions should be biopsied was clarified throughout.
- Added mandatory blood samples at multiple timepoints on-study. The Day 31 blood sample was removed.
- Updated the Cycle 2 lesion swabbing timepoint from Study Day 24 to Study Day 22 to align with tissue biopsy and blood collection timepoints.
- Clarified tumor imaging requirements throughout the document
- Clarified crossover requirements for participants enrolled in the safety run-in.
- Added clarification to Sections 5.2 and 5.3 to reflect the updated IPHP and device compatibility standards.
- Added clarification in Section 5.4 that COVID vaccination with mRNA-based vaccines is allowed on study, provided the vaccine is administered as far away as possible from injected or injectable melanoma lesions and is given at least 2 weeks before or after PVSRIPO injection.
- Updated Section 5.4 to reflect prohibition of use of steroids with occlusive dressings, warfarin use at any time on study, as well as the use of other anticoagulants the day prior, the day of, or the day after PVSRIPO injection.
- Updated Sections 7.3 and 9.2, along with other areas of the protocol to reflect the requirement to report Grade 3 or 4 ISR within 24 hours of awareness of the event.
- Added Section 8.4.3 to outline the statistical approach to analysis of primary and exploratory translational medicine objectives.

Amendment 2.0, 08November2020

Protocol Amendment 2 includes the below major changes:

- Added additional supporting information regarding the PV booster timing in Section 1.3.1.
- Clarified or modified study participant inclusion and exclusion criteria in Section 4 as per the below:
    - Inclusion criterion 4 modified to require that one melanoma lesion must be injectable and minimal lesion size is 5mm. Clarified that visceral lesions (eg liver, lung, retroperitoneal, subpleural lesions) are not considered injectable for the purposes of this trial.
    - Inclusion criterion 5 modified to require disease progression per iRECIST and added allowance of failure on anti-PD-L1 therapy.
    - Exclusion criterion 13 clarified permissible timeframe for receiving received prior systemic anti-cancer therapy to within 4 weeks or 5 elimination half-lives, whichever is shorter- prior to treatment.

- For participants crossing over from Arm 1 to Arm 2, added requirement for complete tumor burden assessment (ie, scans or measurements) within 28 days of first treatment in Arm 2 (PVSRIPO/anti-PD-1 combination)
- Clarified that qualifying scans prior to and during screening should be submitted to central imaging vendor, where possible
- Expanded Section 6 to include information on discontinuation of study treatment, treatment beyond progression, and study termination.
- Modified Section 7.2 Safety Assessments timeframe for reporting events to extend reporting periods through 30 days after the last dose of study therapy for AEs and 90 days after the last dose of study therapy or upon resolution/stabilization for AESI and SAEs.
- Added amylase testing to biochemistry laboratory assessments in Section 7.2.2.
- Modified DSMC meeting frequency in Section 9.1.5 to clarify IA meeting and allow for continued regular meetings approximately every 4 months following the IA.

In addition, the study protocol number was modified from PVSRIPO ICI M201 to LUMINOS-102.

Amendment 1.0, 09October2020
The PVSRIPO ICI M201 study protocol Amendment 1 includes the below major changes:

- Addition of a safety run-in of at least 6 subjects receiving single-agent PVSRIPO to characterize safety and tolerability of PVSRIPO when injected into multiple lesions per treatment visit.
- Additional shedding samples to be collected from the PVSRIPO-injected lesions to assess for PVSRIPO poliovirus shedding, in addition to stool shedding testing for PVSRIPO poliovirus.
- Modifying Inclusion Criterion 4 to require at least 2 measurable melanoma lesions according to RECIST 1.1 criteria.
- Modified Inclusion Criterion 6 to specify radiographic confirmation of disease progression on prior anti-PD-1.
- Additional safety monitoring for CRS and ICANS.
- Mandatory blood biopsy samples at screening, prior to study treatment administration at Cycle 3, and upon crossover to Arm 2, where applicable.

In addition, the nomenclature of study visits was updated from “Day” to “Cycle” for clarity and additional clarifications were added to various study visits and procedures to clarify requirements.