Protocol

A Phase 2 Study of Intratumoral Injection of LTX-315 in Combination with Pembrolizumab in Patients with Percutaneously Accessible Lesions with Advanced Melanoma Refractory to PD-1/PD-L1 Inhibitor Therapy

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I have read the following and approve it:

[redacted], PhD

Chief Executive Officer

Certain information in this protocol has been redacted, and is shown as solid black boxes, to protect personally identifiable information (PII) and/or confidential commercial information (CCI)

Title of study:
A Phase 2 Study of Intratumoral Injection of LTX-315 in Combination with Pembrolizumab in Patients with Percutaneously Accessible Lesions with Advanced Melanoma Refractory to PD-1/PD-L1 Inhibitor Therapy.

Indication:
Advanced (inoperable locally advanced or metastatic) melanoma.

Number of investigators and study centers:
Approximately 10 sites in the United States and European Union.

Development phase:
Phase 2.

Objectives:
Primary
- to evaluate preliminary antitumor activity of LTX-315 in combination with pembrolizumab in the treatment of advanced melanoma in patients who have failed or are refractory to anti-programmed death-protein 1 (PD-1)/anti-programmed death-ligand 1 (PD-L1) therapy.

Secondary
- to evaluate the safety and tolerability of LTX-315 in combination with pembrolizumab in patients with advanced melanoma who have failed or are refractory to anti-PD-1/PD-L1 therapy.
- to explore the pharmacokinetics (PK) of LTX-315 in plasma following intratumoral injection in 20 patients enrolled with advanced melanoma who have failed or are refractory to anti-PD-1/PD-L1 therapy.

Exploratory
- to evaluate preliminary antitumor activity of LTX-315 in combination with pembrolizumab in the treatment of advanced melanoma in patients who have failed or are refractory to anti-PD-1/PD-L1 therapy.
- to evaluate the immune response in injected and noninjected lesions.
- to perform biomarker analyses in all patients.

Methodology/study design:
This will be an open-label, single-arm study in patients with advanced melanoma accessible for injection (cutaneous, subcutaneous, lymph node, or intramuscular tumors) and who have either exhausted treatment options or are not eligible for, suitable for, or willing to undergo such treatments. Patients will be recruited, as follows:
- Patients with unresectable Stage III B, C, D and Stage IVm1a, m1b metastatic melanoma, Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, who have received an approved anti-PD-1/PD-L1 therapy and have progressed on or after prior anti-PD-1 or anti-PD-L1 therapy, alone or in combination with systemic therapy.

Patients must have a minimum of two lesions. All patients will have at least one lesion with a mean longest perpendicular diameter ≤2 cm available for injection with LTX-315, and at least one additional measurable per Response Evaluation Criteria in Solid Tumors (RECIST) (non-injected) lesion; the additional lesion may be located in any place and must be a measurable target lesion with a longest diameter ≥1.0 cm (or ≥1.5cm short diameter if a target lesion is a

lymph node) according to RECIST version 1.1. Any lesion classed as target for RECIST should not be biopsied and should not be injected with LTX-315.

Treatment will be administered in two phases:

1. **Phase A**: LTX-315 in combination with pembrolizumab (through to Day 29).
2. **Phase B**: Pembrolizumab monotherapy every 6 weeks until discontinuation from the study or for a maximum of 24 months' total therapy, whichever comes first.

Patients will be discontinued from the study when they experience disease progression, unacceptable toxicity, or withdraw for another reason such as investigator's decision or withdrawal of consent, whichever comes first.

Number of patients:

A minimum of 40 patients.

Diagnosis and main criteria for inclusion and exclusion:

The following are the main inclusion criteria:

1. Have one of the following confirmed histologically:
   Patients with Stage III B, C, D or Stage IV m1a, m1b unresectable metastatic melanoma who have received an approved anti-PD-1/PD-L1 therapy and have progressed on or after prior anti-PD-1 or anti-PD-L1 therapy, alone or in combination with systemic therapy.
   For patients who have refused prior standard-of-care treatment other than anti-PD-1/PDL-1 therapy, the patient's reason for refusing standard therapy for their disease shall be clearly documented in the study electronic case report form prior to study participation.
   All patients must have received anti-PD-1 or anti-PD-L1 in addition to complying with the relevant criteria below. Melanoma patients with v-raf murine sarcoma viral oncogene homolog B1 (BRAF) mutation who are eligible and suitable for BRAF inhibitor therapy should have received specific BRAF inhibitor therapy before enrolling in this study and must have completed treatments at least 3 weeks prior to starting treatment and have no signs of rapidly progressive disease (PD). Patients who have refused BRAF inhibitor therapy are also eligible for the study.
2. The patients should have had radiologically PD after the most recent line of systemic therapy (no more than two prior lines in the metastatic setting). Adjuvant or Neoadjuvant therapy is not considered as a prior line of treatment for eligibility purposes. Anti-PD-1 or anti-PD-L1 does not need to be the most recent line of therapy prior to study entry.
3. Disease that is not amenable to further radiotherapy or surgery for cancer treatment.
4. Have at least one superficial, non-visceral tumor lesion accessible for injection via cutaneous, subcutaneous, or intramuscular route. Note, lymph nodes with metastatic disease may be selected for injection if they are superficial, but not if deep-seated; visceral lesions must not be selected for injection. The lesion must not be located close to airways, defined as close enough to jeopardize the patient's safety, in the opinion of the Investigator, in the event of a local reaction to LTX-315 injection (for example, if such a reaction has the potential to interfere with swallowing or result in hemorrhaging into the airways). The size of the lesion(s) selected for injection must be $\leq$2 cm in the mean longest perpendicular diameter. For larger lesions or conglomerate lesions, approval from the sponsor's Medical Monitor is needed prior to enrollment.
5. At least one measurable target lesion evaluable according to RECIST version 1.1 that is not planned to be injected with LTX-315 or biopsied. The location of this noninjected tumor may be superficial, deep-seated, or visceral.
6. Have a life expectancy $\geq$3 months.

7. Are males or females aged 18 years or older.
8. Have an ECOG performance status of 0 or 1.
9. Resolution of all disease or prior treatment-related toxicities to Grade ≤1, with the exception of alopecia, <Grade 2 neuropathy and laboratory abnormalities (parameters below apply). If the patient underwent major surgery or received radiation therapy, they must have fully recovered from the intervention.
10. Have a left ventricular ejection fraction (LVEF) that is above the institution's lower limit of normal (by echo scan assessment).
11. Meet the following laboratory requirements:
    a. Absolute neutrophil count ≥1.00 × 10^9/L,
    b. Absolute lymphocyte count that is ≥0.5 k/μL or equivalent,
    c. Platelet count ≥75.0 × 10^9/L,
    d. Hemoglobin ≥9.0 g/dL,
    e. Prothrombin time/partial thromboplastin time ≤1.5 x upper limit of normal (ULN), if the patient is receiving oral anticoagulation international normalized ratio ≤1.5 x ULN, activated partial thromboplastin time ≤1.5 x ULN,
    f. Total bilirubin ≤1.5 x ULN (≤2 x ULN if associated with hepatobiliary metastases or Gilbert's syndrome) AND associated to the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) 2.5 x ULN,
    g. AST and ALT ≤2.5 × ULN,
    h. Calculated creatinine clearance ≥30 mL/min using Cockcroft-Gault formula,
    i. Lactate dehydrogenase (LDH) ≤ULN,
    j. Serum albumin ≥30g/L.
12. Are willing and able to comply with the protocol and agree to return to the clinical site for follow-up visits and examinations.
13. Are willing to undergo tumor biopsy procedures.
14. Are fully informed about the study and have signed the informed consent form (ICF).
15. Are willing to use contraceptive measures as prescribed by the protocol. Female patients of childbearing potential and their partners who are sexually active must agree to the use of two highly effective forms of contraception starting from the screening visit, throughout their participation in the study, and for at least 120 days after their last dose of pembrolizumab or at least 180 days after their last dose of LTX-315, whichever is longer. Male patients with partners who are pregnant or of childbearing potential must use a barrier method of contraception starting from the screening visit, throughout their participation in the study and for at least 120 days after their last dose of pembrolizumab or at least 180 days after their last dose of LTX-315, whichever is longer. A woman is considered of childbearing potential, that is, fertile, following menarche and until becoming postmenopausal unless permanently sterile.
16. Female patients must not be pregnant or breastfeeding. Female patients of childbearing potential must have a negative pregnancy test result during screening and at Day 1.

The following are the main exclusion criteria:

1. Patients with primary ocular melanoma or mucosal melanoma are not eligible
2. Has excessive tumor burden. The patient has more than 10 lesions available for injection, or has any injected lesion >2cm, or in the opinion of the Investigator. For larger lesions or conglomerate lesions, approval from the sponsor's Medical Monitor is needed prior to enrollment. Patients with more than 10 lesions may be deemed eligible after discussion with the sponsor's Medical Monitor, based on assessment of overall tumor burden.

3. Known bone-only or active central nervous system metastases and/or carcinomatous meningitis.
   Patients with untreated brain metastases ≤3 mm that are asymptomatic, do not have significant edema, and do not require steroids or anti-seizure medications are eligible after discussion with the sponsor's Medical Monitor. Patients with previously treated brain metastases may participate provided they are stable after treatment and without evidence of progression by imaging for at least 4 weeks prior to the first dose of study drug administration, and are not using corticosteroids for at least 7 days prior to study drug administration.
4. Have a history of cerebrovascular or cardiac disorders (e.g., Class III or IV New York Heart Association cardiac failure) and in the investigators' opinion the patient would be at particular risk of sequelae following a short hypotensive episode.
5. Have a marked baseline prolongation of QT interval corrected for heart rate using Fridericia's formula (i.e., repeated demonstration of a QTc interval >480 ms National Cancer Institute Common Terminology Criteria for Adverse Events [NCI-CTCAE] Grade 2).
6. Are currently taking immunosuppressive agents or use of systemic corticosteroids or other systemic immunosuppressive drugs within 28 days prior to study drug administration (see Appendix 4 for examples). Patients who require corticosteroids should have been on a stable dose (up to 10 mg daily prednisone or equivalent) for at least 2 weeks prior to study drug administration. Topical and inhaled corticosteroids are also permitted.
7. Have a history of systemic autoimmune disease requiring anti-inflammatory or immunosuppressive therapy within 3 months prior to Day 1, with the following exceptions:
   a. Patients with a history of autoimmune thyroiditis are eligible provided they require only thyroid hormone replacement therapy and their disease has been stable for ≥1 year.
   b. Patients with well-controlled type I diabetes (in the opinion of the Investigator) are eligible.
   c. Patients with a history of chronic disease, who have been stable for the preceding 6 months without treatment.
8. Patients who are allergic or have hypersensitivity to [redacted].
9. Have a history of severe hypersensitivity to another monoclonal antibody; are receiving immunosuppressive therapy; have a history of severe immune-related adverse reaction from treatment with a monoclonal antibody, defined as any Grade ≥3 toxicity requiring corticosteroid treatment (>10 mg/day prednisone or equivalent) for >12 weeks.
10. Have had (non-infectious) pneumonitis Grade ≥3 in the past or current pneumonitis.
11. History of interstitial lung disease.
12. Have a known hypersensitivity to pembrolizumab or LTX-315 or any of their excipients (for pembrolizumab those listed in the prescribing information).
13. Have any other serious illness or medical condition, such as, but not limited to:
    a. Uncontrolled infection or infection requiring systemic antibiotics,
    b. Uncontrolled cardiac failure: Class III or IV New York Heart Association,
    c. Uncontrolled hypertension or risk factors for uncontrolled hypertension (>160 mmHg

systolic and/or >100 mmHg diastolic), despite appropriate antihypertensive medication,
d. Uncontrolled systemic or gastrointestinal inflammatory conditions,
e. Known bone marrow dysplasia,
f. History of positive tests for human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome or active hepatitis B or C (based on serology); positive serology will be confirmed by a viral load test. Patients with treated HIV and a viral load test result consistent with treated HIV, as well as patients with treated hepatitis B or C with an undetectable viral load test result, are eligible,
g. History of or current mastocytosis.

14. Have received external radiotherapy or cytotoxic chemotherapy within 4 weeks prior to Day 1 or have not recovered (to NCI-CTCAE Grade ≤1; alopecia of any grade is allowed, and peripheral neuropathy of up to Grade 2 is allowed) from adverse events (AEs) due to such agents being administered more than 4 weeks prior to Day 1.
15. Have received cancer immunotherapy within 4 weeks prior to Day 1 or have not recovered from AEs (to NCI-CTCAE Grade ≤1) due to such agents being administered more than 4 weeks prior to Day 1.
16. Have received an investigational drug within 4 weeks prior to Day 1 or are scheduled to receive one during the treatment or post-treatment periods.
17. Are expected to need any other anticancer therapy or immunotherapy to be initiated during the study period. Note: Palliative radiotherapy or surgery are allowable under certain conditions in Phase B.
18. Receipt of any BRAF and/or mitogen-activated protein kinase (MEK) inhibitor (including any investigational inhibitor) within 3 weeks before first dose of study drug and/or with signs of rapidly progressive disease.

Test products, dose, and mode of administration:

Phase A (LTX-315 Plus Pembrolizumab):

LTX-315 is administered as 5 mg per intratumoral injection. Depending on tumor size (see table below), multiple injections should be given per lesion. More than one lesion should be injected where possible per dosing day. The largest lesion must be injected first with the maximum number of injections allowed per the table below before injection of the next lesion occurs. The aim is to achieve complete necrosis of each injected lesion. Only viable tissue should be injected. The Investigator should use all 8 injections on a dosing day when possible, but the total number of injections across all lesions must not exceed eight per day (40 mg total)

The Investigator will select nonvisceral tumor lesion(s) accessible for injection via cutaneous, subcutaneous, or intramuscular route. Selected tumor lesion(s) must be ≤2 cm in mean longest perpendicular diameter. For larger lesions or conglomerate lesions, approval from the sponsor's Medical Monitor is needed prior to enrollment. Selected lesions must not be close to the airway.

Patients should not be dosed further if the entire lesion appears to be necrotized, in the opinion of the Investigator, upon clinical examination. In addition, LTX-315 treatment may

[redacted]

[redacted]

Pembrolizumab administered as 200 mg by intravenous (IV) infusion over 30 minutes. Pembrolizumab treatment should start at least 30 minutes after the last LTX-315 injection for that day is completed (safety evaluation permitting) and after resolution or improvement to a severity of ≤Grade 1 of LTX-315 related AEs.
Phase B (Pembrolizumab Monotherapy):
Pembrolizumab administered as 400 mg by intravenous infusion over 30 minutes.

Reference therapy, dose, and mode of administration:
Not applicable.

Duration of patient participation in study:
Planned screening duration: maximum 28 days from signing informed consent.
Planned treatment duration: maximum of 24 months total therapy, or treatment until disease progression, unacceptable toxicity, or withdrawal for another reason such as Investigator decision or withdrawal of consent.
Planned follow-up duration: up to 90 days after the End of Study Visit.

Analysis populations:
Safety/Intention-to-Treat (ITT) Population: all patients who receive at least one injection of LTX-315 and/or one infusion of pembrolizumab and have at least one post-dose safety evaluation.
Efficacy Population: all patients who received at least five out of seven injection days of LTX-315 (or fewer, if injections were stopped early due to complete necrosis of the injected lesion), at least one infusion of pembrolizumab, and have at least one post-dose efficacy evaluation.
Pharmacokinetic Population: all patients who received at least one injection of LTX-315 and have evaluable PK data.

Evaluation: efficacy

Primary efficacy endpoints:

- objective response rate (ORR), defined as the proportion of patients who achieved partial response (PR) and/or complete response (CR) per local Investigator assessment using RECIST version 1.1.
- clinical benefit rate (CBR), defined as the proportion of patients who respond to treatment, estimated as the proportion of patients who achieve stable disease (SD), PR, or CR per local Investigator assessment using RECIST version 1.1.
- overall survival (OS), evaluated as time from baseline until death.

Exploratory efficacy endpoints:

- ORR, defined as the proportion of patients who achieved PR and/or CR per local Investigator assessment using immune-related Response Evaluation Criteria in Solid Tumors (iRECIST).
- CBR, defined as the proportion of patients who respond to treatment, estimated as the proportion of patients who achieve SD, PR, or CR per local Investigator assessment using iRECIST.
- regression of injected tumor lesion as assessed by caliper measurement or ultrasound, per local Investigator assessment.
- progression-free survival (PFS), evaluated as time from baseline until objective tumor progression using RECIST version 1.1 and iRECIST or death, whichever comes first, up to 90 days after end of treatment per local Investigator assessment.
- duration of response (DoR), defined as the interval between the date of the first observation of tumor response (CR or PR) and the first date that progressive disease is objectively documented per local Investigator assessment using RECIST version 1.1 and iRECIST.
- time to response (TTR), defined as the time between the first intratumoral injection and the first observation of tumor response (CR or PR) per local Investigator assessment using RECIST version 1.1 and iRECIST.
- to evaluate biomarkers of immune response in injected and noninjected lesions at cellular and molecular levels.
- to evaluate efficacy based on specific biomarkers (such as PD-1/PD-L1 with different immunohistochemistry cut-off values) in tumor tissues, and/or additional biomarkers to be defined in the future.

Evaluation: safety

Secondary endpoints:

- incidence and severity of AEs (including physical examination findings) related to LTX-315 or to the combination of LTX-315 and pembrolizumab from baseline to end of treatment.
- incidence of clinical laboratory abnormalities based on clinical chemistry, hematology, urinalysis, and coagulation test results.
- change from baseline in vital signs measurements (pulse rate, body temperature, respiration rate).
- change from baseline in blood pressure measurements.
- change from baseline in 12-lead electrocardiogram (ECG) parameters, including Fridericia's corrected QT interval.
- incidence of LTX-315 injection site assessment findings (injection site pain,

swelling, and redness).

Evaluation: pharmacokinetics

Secondary PK endpoints of LTX-315 in plasma in 20 patients enrolled in the study:

- area under the plasma concentration-time curve from time zero extrapolated to infinity (AUC 0-∞).
- maximum observed plasma concentration (C_{max}).
- apparent plasma terminal elimination half-life ($t_{1/2}$).

Plasma PK parameters of LTX-315 will be calculated using standard noncompartmental methods.

Renal function will be documented at baseline and at subsequent time points for each patient enrolled in the study. The effect of renal impairment on the PK of LTX-315 may be evaluated via population PK modeling and will be reported separately.

Statistical methods:

Efficacy analysis:

- The objective tumor response (CR, PR, SD, PD) will be determined separately according to RECIST version 1.1 and iRECIST.
- Best overall tumor response (from screening until disease progression) will be listed and summarized (including 80% and 90% exact confidence intervals for proportions) by RECIST version 1.1 and iRECIST.
- ORR, CBR, PFS, DoR, and TTR will be listed and summarized by RECIST version 1.1 and iRECIST.
- Time-to-event data, such as OS, PFS, and DoR, will be summarized using the Kaplan-Meier method.

Size assessments details of lesion(s) for injection will be listed. Summary statistics of the size of the injected lesion(s) and the percentage change from baseline will be presented by visit.

Safety analysis:

- AEs will be listed and summarized. The incidence of AEs, AEs ≥NCI-CTCAE Grade 3, related AEs, serious AEs, and AEs leading to discontinuation of the study treatment will be presented by Medical Dictionary for Regulatory Activities system organ class (SOC) and preferred term. In addition, the incidence of AEs by severity will be presented by SOC and preferred term.
- Laboratory test variables will be summarized by visit using descriptive statistics (number of patients, mean, standard deviation, minimum, maximum, and mean change from baseline). Shift tables and categorical tables by NCI-CTCAE grade between baseline and post baseline time points will be presented by laboratory test. Laboratory tests without NCI-CTCAE grading will be presented in shift tables by relationship to the normal range (low, normal, high).
- Descriptive statistics of vital signs, blood pressure monitoring, and ECG results at each visit will be presented, including changes from baseline. If echo assessments are performed as clinically indicated, LVEF results for such patients will be listed and may be summarized if appropriate. Physical examination findings, ECOG performance status, and LTX-315 injection site assessment findings will be listed for each patient.

Sample size assumptions:

A sample size of 40 patients provides approximately 99,4% power to detect a response rate of 30% for the combination treatment (LTX-315 plus pembrolizumab) assuming pembrolizumab alone would deliver a response rate of 5% or less in this population utilizing a 1-sided test with

an alpha spend of 0.05. No estimation for dropouts was included due to this being a study in an advanced cancer population where patients are expected to continue on therapy until progression.
Pharmacokinetic analysis:
No inferential statistical analysis will be performed on the PK parameter data.

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Abbreviation	Definition
ACTH	adrenocorticotropic hormone
ADA	antidrug antibodies
AE	adverse events
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the plasma concentration-time curve
AUC0-∞	area under the plasma concentration-time curve from time zero extrapolated to infinity
AUC0-t	area under the plasma concentration-time cure from time zero to t where t
BMI	body mass index
BRAF	v-raf murine sarcoma viral oncogene homolog B1
BUN	blood urea nitrogen
CBC	complete blood count
CBR	clinical benefit rate
CFR	Code of Federal Regulations
C_{max}	maximum observed plasma concentration
CONSORT	Consolidated Standards of Reporting Trials
CR	complete response
CRO	Contract Research Organization
CSA	clinical study agreement
CT	computed tomography
CTLA-4	anti-cytotoxic T-lymphocyte antigen-4
DoR	duration of response
DPA	data protection agreement
DPIA	Data Protection Impact Assessment
DPO	Data Protection Officer
ECG	Electrocardiogram
Echo	Echocardiograms
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic case report form
EDC	electronic data capture
EOT	end of treatment
ET	early termination
FSH	follicle-stimulating hormone
GCP	good clinical practice
GDPR	General Data Protection Regulation
GGT	gamma glutamyl transferase
HBcAB	hepatitis B core antibody
HBsAB	hepatitis B surface antibody
HBsAg	hepatitis B surface antigen
HCC	Hepatocellular carcinoma
HIV	human immunodeficiency virus

Abbreviation	Definition
HRT	hormone replacement therapy
I	Injected
ICF	informed consent form
ICH	International Council for Harmonization
ICI	Immune checkpoint inhibitor
iCPD	Immune Confirmed Progressive Disease
iCR	immune complete response
iPR	immune partial response
IgE	immunoglobulin E
IMP	investigational medicinal product
IRB	institutional review board
iRECIST	immune-related Response Evaluation Criteria in Solid Tumors
irRC	immune-related response criteria
iSD	immune stable disease
IT	Intratumoral
ITT	intention-to-treat
IUD	intrauterine device
iUPD	Immune Unconfirmed Progressive Disease
IUS	intrauterine hormone-releasing system
IV	Intravenous
LDH	Lactate dehydrogenase
LPD	Longest perpendicular distance
LVEF	left ventricular ejection fraction
Lytix	Lytix Biopharma AS
MAP	mean arterial blood pressure
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MedDRA	Medical Dictionary for Regulatory Activities
MEK	Mitogen-activated protein kinase
Mrgx2	Mas-related gene X2
MRI	magnetic resonance imaging
MTD	maximum tolerated dose
N	number of patients
NA	not applicable
NCCN	National Comprehensive Cancer Network
NCI-CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
NE	not evaluable
NI	Noninjected
NL	new lesion
NLNT	new lesion – nontarget
NLT	new lesion – target
NOAEL	no-observed-adverse-effect level

Abbreviation	Definition
NT-I	nontarget injected
NT-NI	nontarget noninjected
ORR	objective response rate
OS	overall survival
PBMC	peripheral blood mononuclear cells
PD	programmed death / progressive disease
PD-1	programmed death-1
PD-L1	programmed death-ligand 1
PD-L2	programmed death-ligand 2
PFS	progression-free survival
PK	Pharmacokinetic
PR	partial response
QTcF	QT interval corrected for heart rate using Fridericia's formula
RBC	red blood cell
RECIST	Response Evaluation Criteria in Solid Tumors
RNA	ribonucleic acid
SAE	serious adverse event
SD	stable disease
SERP	subject eligibility registration package
SJS	Stevens Johnson syndrome
SOC	system organ classification (MedDRA classification)
SOD	sum of diameters
SOM	sum of measures
T-I	target injected
T-NI	target noninjected
T	Target
$t_{1/2}$	apparent plasma terminal elimination half-life
TEAE	treatment-emergent adverse event
TEN	toxic epidermal necrolysis
TIL	tumor-infiltrating lymphocytes
T_{max}	time of maximum observed plasma concentration
Treg	regulatory T cell
TTR	time to response
ULN	upper limit of normal
WBC	white blood cell
WOCBP	women of childbearing potential

1.0

1.1 STUDY RATIONALE

This study aims to assess the safety and efficacy of LTX-315 in combination with the anti-programmed death-protein 1 (PD-1)/anti- programmed death-ligand 1 (PD-L1) therapy pembrolizumab in patients with superficial advanced melanoma who have failed or are refractory to anti-PD-1/PD-L1 therapy and have unresectable Stage III, B, C, D or IVm1a, m1b metastatic melanoma.

LTX-315 has been administered with pembrolizumab in a previous Phase 1/2 study (Study C12-315-03), and there were clear indications that LTX-315 + pembrolizumab was a clinically active combination. Furthermore, the addition of LTX-315 to pembrolizumab dosing did not appear to increase the overall incidence or severity profile of toxicities. The present study will document the preliminary efficacy, clinical safety, and tolerability of LTX-315 in combination with pembrolizumab, in a dose regimen that is considered to be safe, in patients with unresectable Stage III, B, C, D or Stage IV m1a, m1b metastatic melanoma, Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 that have received an approved anti-PD-1/PD-L1 therapy and have progressed after prior anti-PD-1 or anti-PD-L1 therapy, alone or in combination with systemic therapy. The rationale of including this is to test the ability of intratumoral injection of LTX-315 to prime the immune system and thus overcome the resistance to anti-PD-1/PD-L1 therapy.

1.2 BACKGROUND ON LTX-315

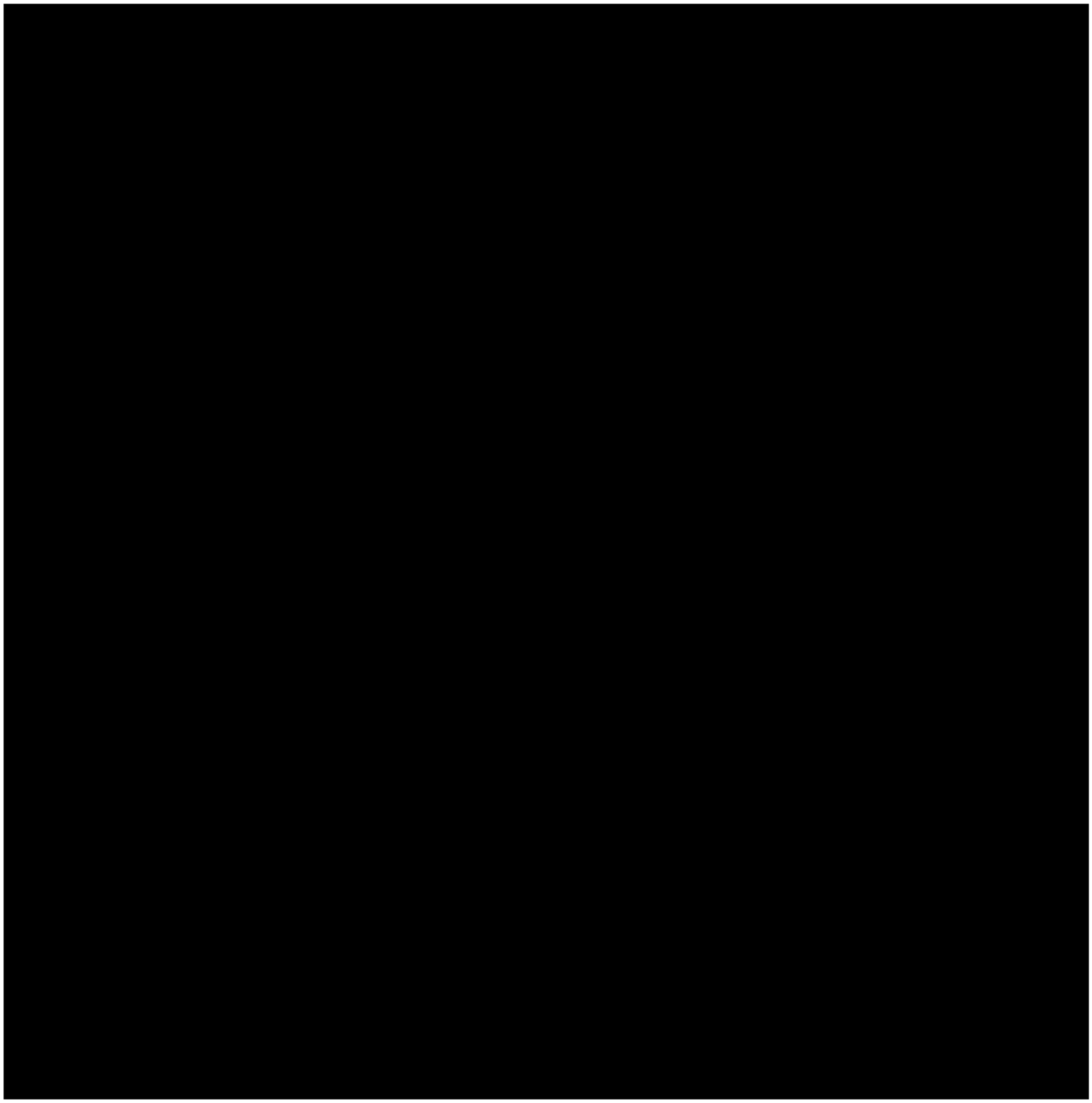
LTX-315 is a de novo designed oncolytic peptide being developed by Lytix Biopharma AS (Lytix) as a potential treatment for cancer. LTX-315 is generated from a host defense peptide. Host defense peptides are present in almost every life form and are an integrated part of the eukaryotic immune system mounting a first-line defense against pathogens. These peptides can have both a direct killing activity and immunomodulatory properties.[1,2] Several cationic host defense peptides have shown anticancer activity due to their ability to bind to negatively charged membrane components on cells via electrostatic interactions.[1,2] Extensive structure activity-relationship studies on peptides derived from the host defense peptide lactoferricin have enabled the de novo design of LTX-315, in which the features important to oncolytic activity have been optimized.

LTX-315 has been designed to enhance the cytolytic activity of lactoferricin, subsequently inducing lysis and cell death at high local concentration.[3–5] LTX-315 induces immunogenic cell death; it kills the cells in a manner that activates the immune system. A tumor-specific immune response is activated due to an effective release of potent immune-stimulants (i.e., danger signals) and a broad repertoire of tumor antigens.[3–5] In the clinical Phase 1/2 study, LTX-315 has shown clear clinical benefit with an ability to enhance systemic T-cell responses and regression (25% to 82%) of single distant nontreated lesions in 30% of the patients treated with LTX-315 monotherapy. LTX-315 has also demonstrated synergy with anti-PD-1/PD-L1 therapy in preclinical models,[6] has shown no added toxicity in combination with ipilimumab or with pembrolizumab in a previous Phase 1/2 study and shown clear signs of clinical benefit in combination with pembrolizumab (dose escalation of LTX-315).

1.2.1 KEY NONCLINICAL FINDINGS

The nonclinical pharmacology data obtained for LTX-315 support its use as a locally injected agent for the treatment of solid tumors. LTX-315 has shown potent activity against multiple cancer cell lines and pharmacodynamic studies of intratumoral injections have demonstrated prolonged local antitumor effects of complete responses (CR) and transfer of T-cell-mediated tumor protection from treated to untreated mice.

Although no-observed-adverse-effect levels (NOAELs) could not be determined in nonclinical studies due to the pharmacological action of the compound, these studies generated data that assisted in the selection of a tolerable clinical starting dose.



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1.2.2 CLINICAL EXPERIENCE

Efficacy, safety, and pharmacokinetics (PK) of LTX-315 injection in humans have been evaluated in clinical studies that included patients with tumors accessible for injection, mainly (but not restricted to) cutaneous, subcutaneous, oral, and lymph node. To date, three clinical studies (C08-315-01, N=14; C09-315-02, N=12; C12-315-03, N=65) in patients with end-stage cancer have been concluded to assess the clinical dose, the optimum combination and dosing schedule. In these studies, LTX-315 has been administered as monotherapy or in combination with GV1001, ipilimumab, or pembrolizumab. An open-label Phase 2 study investigating the safety and efficacy of LTX-315 and adoptive T-cell therapy in patients with advanced/metastatic soft tissue sarcoma is ongoing.

Overall, from the studies conducted so far, it can be concluded that LTX-315 is clinically beneficial and well tolerated. An optimal dose has been identified and, with the addition of prophylaxis (H1 and H2 receptor antagonists and leukotriene receptor antagonist) for the

[redacted]

The main AE of interest described after LTX-315 treatment was hypersensitivity reactions to LTX-315.

Fourteen patients were treated in Study C08-315-01 with once-weekly injection of LTX-315 into a single lesion, ≤6 dosing days (Days 1, 8, 15, 22, 29, and 36). In Cohorts 1 and 2, the injected volume was calculated to be 10% of the tumor volume. Concentration of LTX-315 was 10 and 20 mg/mL for Cohorts 1 and 2, respectively. The LTX-315 dose per injected tumor lesion in Cohort 1 varied from 1.7 to 4.8 mg. The LTX-315 dose per injected tumor lesion in Cohort 2 varied from 1.4 to 11.6 mg. In Cohort 3, the concentration of LTX-315 was 20 mg/mL. Doses were independent of tumor volume and were 2 mg for the first injection day then 4 mg for the next injection days.

Six out of 14 patients (42.8%) experienced hypersensitivity-like adverse reactions. In Cohort 1 (10 mg/mL; N=3), there was one unrelated serious AE (SAE) of coughing. All AEs in this cohort were moderate and transient. In Cohort 2 (20 mg/mL; N=5), two of the patients experienced SAEs of symptomatic hypotension (Grades 3 and 4, respectively) as part of the hypersensitivity reaction spectrum after the first dose in excess of 8 mg of LTX-315. One of these patients (Grade

[redacted]

In the second study (C09-315-02), the use of LTX-315 doses from 0.05 to 0.2 mg in 0.1 mL (0.5 to 2.0 mg/mL) in combination with GV1001 in patients with cancer resected with curative intent was very well tolerated and no AEs were experienced.

In the third study (C12-315-03), LTX-315 was used as monotherapy or in combination with anti-PD-1/PD-L1 therapy (pembrolizumab or ipilimumab). Arms A and B consisted of monotherapy; Arms C and D involved combination of LTX-315 with ipilimumab or pembrolizumab, respectively.

- In Arm A (N=23), lesions were treated sequentially. The first lesion was treated for 6 weeks (induction treatment; Days 1, 2, 3, 15, 22, 29, and 36); second and third lesions could be injected sequentially, in Weeks 7 to 12 and then Weeks 13 to 18, respectively. This was followed by a maintenance phase with one treatment day every second week in the last injected lesion for ≤20 weeks. Patients in Cohorts 1 to 3 received dosing twice daily, and the remaining cohorts received dosing once daily. Dose per injection ranged from 2 to 7 mg; 20 mg/mL in Cohorts 1 to 6, 10 mg/mL in Cohort 7.

- Arm B included two different treatment regimens. First regimen (N=5): concurrent treatment of multiple lesions (3 to 12 lesions), one injection (3 to 4 mg, 10 to 20 mg/mL) in each lesion on the same dosing days (Days 1, 2, 3, 8, and 22), then every 3 weeks for maintenance up to 43 weeks. Second regimen (N=11): one or more lesions were treated with a fixed dose (3 to 5 mg, 10 mg/mL) of LTX-315 per injection on Days 1, 2, 8, 9, 15, and 16. The number of injections to each lesion was calculated per lesion based on tumor volume. For practical reasons, a maximum of 12 injections per day was allowed in a minimum of a 1-hour period.
- The second dosing regimen of LTX-315 described for Arm B above was used in Arm C (N=8) and Arm D (N=18) in combination with the standard approved ipilimumab dose (3 mg/kg × 4 IV infusions) or with IV pembrolizumab 200 mg every 21 days.

Four patients (17.4%) in Arm A experienced LTX-315-related hypersensitivity SAEs that led to discontinuation of treatment.



Eighteen out of the 65 patients in this study were treated with LTX-315 with doses of 3 to 5 mg per injection in combination with pembrolizumab 200 mg every 3 weeks starting on Day 2 of Week 1. Several AEs occurred during combined treatment with LTX-315 and pembrolizumab. The majority were Grade 1 to 2 and were similar to those observed with LTX-315 alone. In the LTX-315 + pembrolizumab arm, a total of four Grade 3 AEs considered related to LTX-315 occurred in three patients: three AEs of pain at injection site (events of same type in same patient counted as one AE) and one AE of hypertension.

Based on these data, we can expect that the combined use of LTX-315 and pembrolizumab will have a safety profile that is within the range of known toxicities for either treatment alone. The addition of LTX-315 to the dosing schedule of pembrolizumab does not seem to confer additive toxicities.

Among the complete safety population (65 patients) in the C12-315-03 study, the five most reported treatment-emergent AE (TEAE) system organ class (SOC) of any relationship were: general disorders and administration site conditions (43 patients, 78.3%), vascular disorders (35 patients, 66.2%), nervous system disorders (31 patients, 47.7%), skin and subcutaneous tissue disorders (30 patients, 46.2%), and gastrointestinal disorders (28 patients, 43.0%). The three most reported SOCs for LTX-315-related events were: general disorders and administration site conditions (37 patients, 56.9%), vascular disorders (33 patients, 50.8%), and skin and subcutaneous tissue disorders (25 patients, 38.5%). Of the reported Grade 3 or higher events, related to LTX-315, the three most common SOCs were: vascular disorders (5 patients, 7.7%), immune system disorders (4 patients, 6.2%), and general disorders and administration site conditions (3 patients, 4.6%). Twenty-one out of 65 safety evaluable patients (32.3%) who received LTX-315 injections reported SAEs. Five patients (7.7%) had SAEs related to LTX-315, of whom four were in Arm A (monotherapy). After the new treatment schedule was introduced with additional treatment guidelines, no further SAEs were experienced with LTX-315 monotherapy (Arm B). Overall, patients administered the new treatment schedule included 11 patients in Arm B (monotherapy), eight patients in Arm C (LTX-315 + ipilimumab), and 18 patients in Arm D (LTX-315 + pembrolizumab).

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In a first Phase 1 dose-escalation study (C08-315-01), a positive effect of LTX-135 treatment was observed in five out of 14 patients (35.7%) when assessed by tumor necrosis. Two patients had a reduction of ≥50% in the volume of the injection lesion. Analysis of a resected tumor from a breast cancer patient demonstrated fatty tissue necrosis and the presence of tumor-infiltrating lymphocytes (TILs), indicating an effect induced by LTX-315 treatment.

In a second Phase 1, open-label, dose-escalation study (Study C09-315-02), where LTX-315 was injected in tumors as an adjuvant stimulator for an anticancer vaccine (GV1001), the use of the combined drugs demonstrated T-cell responses against GV1001 in three out of 12 patients (25%).

A third study, a Phase 1/2, open-label, multi-arm, dose-escalation study (Study C12-315-03), was conducted to assess the effects of the treatment of LTX-315 in monotherapy and in combination with anti-PD-1/PD-L1 therapy ipilimumab (anti-cytotoxic T-lymphocyte antigen-4 [CTLA-4]) and pembrolizumab (anti-PD-1). There were clear indications that LTX-315 is clinically active, with a number of patients showing marked tumor regression in both the injected and noninjected lesions.[11] The immune analysis of tissue samples clearly indicated that LTX-315 has the ability to invoke necrosis, stimulate infiltration and clonal expansion of T cells, and increase the repertoire of T cells both within injected tumors as well as in the blood. Substantial clinical benefit was seen; in the monotherapy arms, a clinical benefit rate of 44.4% was observed, with 12 out of 27 patients showing stabilization of their underlying disease. In the combination arms with ipilimumab and pembrolizumab, the CBR was 37.5% in each arm, with one and two patients achieving a partial response (PR) per the immune-related response criteria (irRC) in the ipilimumab and pembrolizumab arms, respectively.

1.2.3 Rationale for the Combination of LTX-315 and Immune Checkpoint Inhibitors

Since LTX-315 enhances T-cell infiltration, T-cell clonality, and T-cell repertoire, LTX-315 may

be ideal for combination with immune checkpoint inhibitors (ICIs). It has recently been documented that ICIs benefit patients with T-cell inflamed tumor tissue ("hot" tumors) and not patients with non-T-cell inflamed tumor microenvironment ("cold" tumors).[12,13] LTX-315 has shown the ability to induce T-cell infiltration in treated and non-treated tumors in animals models[7,8,10] and in treated lesions in cancer patients[10,11] and hence has the potential to extend the proportion of patients responding to ICIs.

Significant synergy with anti-CTLA-4 therapy and LTX-315 has been obtained in preclinical models.[6] It was found that LTX-315 rapidly reprograms the tumor microenvironment by decreasing the local abundance of immunosuppressive regulatory T cells (Treg) and myeloid-derived suppressor cells with a concomitant increase in CTLA-4 expression level. In tumors that were resistant to intratumoral or systemic CTLA-4 blockade, subsequent local injection of LTX-315 after anti-CTLA-4 treatment cured the animals or caused tumor regressions with abscopal effects.[6]

Enhanced (not synergistic) anticancer effects have also been obtained with LTX-315 combined with anti-PD-1 (Study R15-315-1) or anti-PD-L1 (Study R15-315-10) in preclinical tumor models. There are indications that the clinical efficacy of ICIs correlates with the level of mutational burden and the number of TILs. Since LTX-315 enhances infiltration of T cells as well as T-cell clonality in both tumor and blood, LTX-315 may be ideal for combining with anti-PD-1/anti-PD-L1. Regression of non-injected single lesions up to 82% was observed in the last completed study with LTX-315 monotherapy, but no objective response rate (ORR) was achieved. Combination of LTX-315 with ICI resulted in 12.5% ORR in both Arm C and Arm D, where LTX-315 was combined with ipilimumab and pembrolizumab, respectively. In Arm C, two patients with melanoma who were refractory to prior anti-PD-1 treatment achieved progression-free survival (PFS) for 170 and 360 days, respectively. The patient with 170 days was close to PR (assessed by irRC) with 49.6% reduction of the total tumor burden. One out of eight patients achieved PR as best response in Arm C. In Arm D, two out of 16 patients achieved PR, of whom one patient also had significant regression of two liver lesions, which has not been observed with pembrolizumab alone.[14] There was also a better ORR in Arm D (12.5%; two out of 16 patients with PR) compared to pembrolizumab alone.[14]

1.2.4 IMMUNE RESPONSES INDUCED IN PATIENTS BY LTX-315



In Study C12-315-03, the percentage of evaluable patients showing a substantial and relevant

increase in necrosis in the injected lesions was 42% after monotherapy (Arms A and B), 50% in Arm C, and 43% in Arm D. All evaluable patients who had tumor biopsies in Arms A and B had an increase in CD3+ TILs in the injected lesions after treatment. In Arm C, 71% of the patients had an increase in CD3+ TILs. In Arm D, CD3+ TILs were not evaluated. An increased infiltration of CD8+ T cells was found in 86% of evaluable patients in Arms A and B. In Arms C and D, 57% and 54% of the patients had an increase in CD8+ T cells, respectively.

The analysis of tumor tissue and blood samples from patients before and after treatment showed that a higher number of T-cell clones were significantly expanding in blood in 71% of the evaluated monotherapy patients after treatment compared to what is shown in healthy individuals.[15] A median of 40% of the expanding T-cell clones in blood were present in tumor after treatment compared to 15% before treatment, suggesting that the T-cell clones expanding in blood may be tumor associated. Moreover, novel T-cell clones were expanding in blood and present in tumor after treatment, indicating that LTX-315 generates de novo tumor-associated immune responses.

Gene expression analysis of tumors before and after LTX-315 monotherapy demonstrated that LTX-315 induces immune responses and converts "cold" tumors to "hot" tumors by inducing the expression of genes necessary for tumor regression such as perforins, granzymes, and chemokines necessary for infiltration of T cells to the tumor.

[redacted]

Furthermore, nontreated lesions from a sarcoma patient and a breast cancer patient showed regression of 66% and 54%, respectively. Treated tumors from these 2 patients exhibited an increase in CD3+ and CD8+ TILs after treatment, as well as a transition from a cold to hot tumor microenvironment as analyzed by Immunosign gene expression signature assessment and significant expansion of circulating T-cell clones after treatment above the level reported in healthy individuals.[15]

In Arm D, 15% of the evaluable population showed a significant increase in PD-L1 expression on tumor cells evaluated by immunohistochemistry in the injected lesions. Four different types of tumor microenvironment exist based on the presence or absence of TILs and PD-L1 expression.[16] Three patients with type II (CD8− PD-L1−) were converted to type IV (CD8+ PD-L1−) and two patients were converted from type II to type I (CD8+ PD-L1+). This finding supports the potential for combining LTX-315 with PD-1 blockade agents, since PD-1 blockade seems to work better in CD8+ infiltrated and PD-L1-expressing tumors.

1.3 PEMBROLIZUMAB BACKGROUND

Pembrolizumab (Keytruda) is a humanized monoclonal antibody against PD-1 and is classified as an ICI. Pembrolizumab binds and blocks the activation of PD-1 receptor, which functions as an immune checkpoint playing an important role in down-regulating the immune response. PD-1 receptors on T cells interact with its ligands, PD-L1 and programmed death Ligand 2 (PD-L2), which are expressed in antigen-presenting cells, peripheral tissues, and may be expressed by cancer or other cells in the tumor microenvironment. Pembrolizumab potentiates T-cell responses by blocking PD-1/PD-L1 and PD-L2 binding.

1.4 BENEFIT-RISK ASSESSMENT

1.4.1 BENEFITS

Patients eligible for the study will have locally advanced/metastatic disease, not be eligible for radio or surgical treatment, and have either exhausted treatment options or are not eligible for, suitable for, or willing to undergo such treatments, while continuing to progress.

LTX-315 is an oncolytic peptide that is designed to optimize the anticancer activity of the naturally occurring host defense peptide lactoferricin, which disintegrates cellular membranes, inducing lysis and cell death. LTX-315 activates the immune system by inducing immunogenic cell death through the release of potent immunostimulants and a broad repertoire of tumor antigens. Synergy with ICIs such as pembrolizumab and chemotherapy in preclinical models has been demonstrated; see Section 1.2.3.

By entering this study, the patient will have access to an experimental treatment that could potentially overcome tumor resistance to anti-PD-1/PD-L1 therapy treatment.

1.4.2 RISKS

Acute hypersensitivity symptoms are an anticipated risk of LTX-315 use.

From the studies conducted so far, it can be concluded that LTX-315 is a highly tolerated drug with manageable toxicities. The main toxicity observed was National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) Grade 1 to 2 hypersensitivity-like AEs, including symptoms and signs of flushing, paresthesia, pruritus, rash, and asymptomatic hypotension. These hypersensitivity AEs emerge quickly (seconds to minutes) and resolve quickly (typically within 60 minutes). Grade 3 and 4 hypersensitivity AEs have also been reported with LTX-315 as monotherapy or in combination with pembrolizumab (six patients total in two clinical studies: two patients with Grade 4 AEs and four patients with Grade 3 AEs; see Section 1.2.2.1 . Once ro h laxis for the revention of h ersensitivity reactions was added [redacted], no significant events were observed, and all toxicities were manageable. Prophylactic measures including antihistamines and a leukotriene receptor antagonist will be given to all patients prior to LTX-315 treatment.

Safety monitoring and risk mitigation plans for LTX-315 include:

- The dose of LTX-315 was selected based on data obtained from previous studies to ensure safe use of medication.
- Clinical monitoring, including intensive blood pressure monitoring and injection site assessments, as described below, will be conducted during all LTX-315 administrations.
- Lesions located close to airways and major blood vessels are not allowed for injection, defined as close enough to jeopardize the patient's safety, in the opinion of the Investigator.
- Injection sites will be evaluated for pain, swelling, and redness after each injection and every 5 minutes before next injection or until injection site pain resolves to zero or baseline.
- Vital signs will be checked pre-dose on the day of the first, second, fourth, and fifth

LTX-315 injections and blood pressure will be monitored just prior to injection and then approximately every 5 minutes (no later than 15 minutes between measurements) until 30 minutes after the last injection of each day.

- Dose delay/omission rules are present in the protocol for any Investigator-assessed clinically significant LTX-315 related AE.

More detailed information about the known and expected benefits and risks and reasonably expected AEs of LTX-315 may be found in the Investigator's Brochure.[17]

Immune-mediated reactions are an anticipated risk of pembrolizumab use. Such side effects will be addressed as indicated in the pembrolizumab label.

2.0

2.1 OBJECTIVES

Primary

The primary objective of this study is:

- to evaluate preliminary antitumor activity of LTX-315 in combination with pembrolizumab in the treatment of advanced melanoma in patients who have failed or are refractory to anti-PD-1/anti-PD-L1 therapy.

Secondary

The secondary objectives of this study are:

- to evaluate the safety and tolerability of LTX-315 in combination with pembrolizumab in patients with advanced melanoma who have failed or are refractory to anti-PD-1/PD-L1 therapy.
- to explore the pharmacokinetics (PK) of LTX-315 in plasma following intratumoral injection in 20 patients enrolled with advanced melanoma who have failed or are refractory to anti-PD-1/PD-L1 therapy.

Exploratory

The exploratory objectives of this study are:

- to evaluate preliminary antitumor activity of LTX-315 in combination with pembrolizumab in the treatment of advanced melanoma in patients who have failed or are refractory to anti-PD-1/PD-L1 therapy.
- to evaluate the immune response in injected and noninjected lesions.
- to perform biomarker analyses in all patients.

2.2 ENDPOINTS

The primary efficacy endpoints are:

- ORR, defined as the proportion of patients who achieved PR and/or CR per local Investigator assessment using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1.
- Clinical benefit rate (CBR), defined as the proportion of patients who respond to treatment, estimated as the proportion of patients who achieve stable disease (SD), PR, or CR per local Investigator assessment using RECIST version 1.1.
- Overall survival (OS), evaluated as time from baseline until death.

The secondary safety and tolerability endpoints are:

- incidence and severity of AEs (including physical examination findings) related to LTX-315 or to the combination of LTX-315 and pembrolizumab from baseline to end of treatment.
- incidence of clinical laboratory abnormalities based on clinical chemistry, hematology, urinalysis, and coagulation test results.

- change from baseline in vital signs measurements (pulse rate, body temperature, respiration rate).
- change from baseline in blood pressure measurements.
- change from baseline in 12-lead electrocardiogram (ECG) parameters, including QT interval corrected for heart rate using Fridericia's formula (QTcF).
- incidence of LTX-315 injection site assessment findings (injection site pain, swelling, and redness).

The secondary PK endpoints of LTX-315 in plasma in 20 patients enrolled in the study are:

- $AUC_{0-\infty}$
- C_{max}
- $t_{1/2}$

The exploratory efficacy endpoints are:

- ORR, defined as the proportion of patients who achieved PR and/or CR per local Investigator assessment using immune-related RECIST (iRECIST).
- CBR, defined as the proportion of patients who respond to treatment, estimated as the proportion of patients who achieve SD, PR, or CR per local Investigator assessment using iRECIST.
- regression of injected tumor lesion as assessed by caliper measurement or ultrasound
- PFS, evaluated as time from baseline until objective tumor progression using RECIST version 1.1 and iRECIST or death, whichever comes first, up to 90 days after end of treatment per local Investigator assessment.
- duration of response (DoR), defined as the interval between the date of the first observation of tumor response (CR or PR) and the first date that PD is objectively documented per local Investigator assessment using RECIST version 1.1 and iRECIST.
- time to response (TTR), defined as the time between the first intratumoral injection and the first observation of tumor response (CR or PR) per local Investigator assessment using RECIST version 1.1 and iRECIST.
- to evaluate biomarkers of immune response in injected and noninjected lesions at cellular and molecular levels.
- to evaluate efficacy based on specific biomarkers (such as PD-1/PD-L1 with different immunohistochemistry cut-off values) in tumor tissue, and/or additional biomarkers to be defined in the future.

3.0

3.1 OVERALL STUDY DESIGN AND PLAN DESCRIPTION

This will be a Phase 2, open-label, single-arm study in patients with advanced metastatic melanoma accessible for injection (cutaneous, subcutaneous, lymph node, or intramuscular tumors) and who have either exhausted treatment options or are not eligible for, suitable for, or willing to undergo such treatments, assessing the safety and efficacy of LTX-315 in combination with pembrolizumab. The following patients will be recruited:

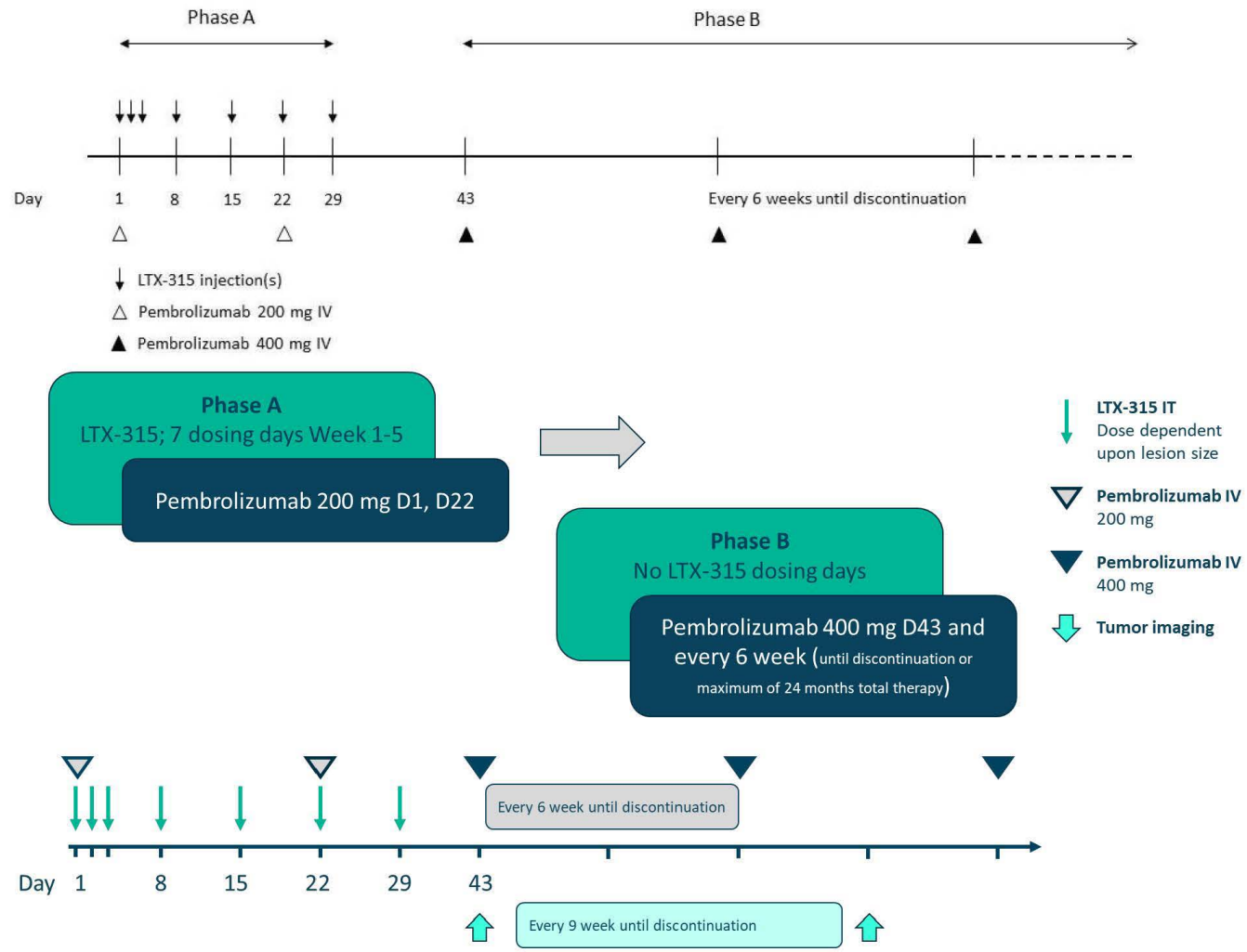
Patients with unresectable Stage III, B, C, D or Stage IVm1a, m1b metastatic melanoma, ECOG performance status of 0 or 1, who have received an approved anti-PD-1/PD-L1 therapy and have progressed on or after prior anti-PD-1 or anti-PD-L1 therapy, alone or in combination with systemic therapy.

Patients must have a minimum of two lesions. All patients will have at least one lesion with a mean longest perpendicular diameter ≤2 cm available for injection with LTX-315, and at least one additional measurable per RECIST (non-injected) lesion; the additional lesion may be located in any place and must be a measurable target lesion with a longest diameter ≥1.0 cm (or ≥1.5cm short diameter if a target lesion is a lymph node) according to RECIST version 1.1. Any lesion classed as target for RECIST should not be biopsied or injected with LTX-315.

Treatment will be administered in two phases, as depicted in Figure 1:

- **Phase A**: LTX-315 in combination with pembrolizumab (through to Day 29).
- **Phase B**: Pembrolizumab monotherapy every 6 weeks until discontinuation from the study or for a maximum of 24 months' total therapy, whichever comes first.

Patients will be discontinued from the study when they experience disease progression, unacceptable toxicity, or withdraw for another reason such as investigator's decision or withdrawal of consent, whichever comes first. In the case of disease progression, patients should remain on treatment until a confirmatory scan has been performed (4-6 weeks after initial disease progression scan), unless continued treatment is not considered acceptable by the Investigator.



Abbreviations: D = day; IT=intra tumoral, IV = intravenous.

After a screening period of up to 28 days, eligible patients will arrive at the clinical site on the first day of dosing to confirm eligibility, complete pre-dose baseline assessments, and receive the first dose of study treatment and premedication.

Phase A

On Day 1, following administration of premedication (see Section 5.1.1.4), LTX-315 will be injected into the lesion(s) and patients will receive an IV infusion of 200 mg pembrolizumab. The tumor(s) for injection will be selected based on the investigator's clinical judgment; tumors located close to airways will not be selected. Additional LTX-315 intratumoral injections will be given on Days 2, 3, 8, 15, 22, and 29, with a second dose of 200 mg pembrolizumab being given on Day 22. Multiple injections of LTX-315 may be needed per day; the number of injections per lesion per dosing day depends on lesion size (see Table 3). Patients will remain at the clinical site for ≥1 hour post the last LTX-315 injection of each dosing day, or longer at the investigator's discretion, for observation for possible acute toxicity reactions. The aim of intratumoral injection is to cause necrosis in the injected lesion(s) and thus release tumor antigens, hence aim at complete treatment of each injected lesion rather than partial treatment of many lesions. If

complete necrosis of injected lesion(s) has taken place (in the opinion of the Investigator; e.g., by visual or ultrasound-guided examination), then further injections may not be necessary; in that case, the injections shall stop earlier than Day 29. Otherwise, injections shall continue as shown in Figure 1.

Phase B

In Phase B, pembrolizumab will be administered as 400 mg IV infusion, with the first 400-mg dose given on Day 43 and further doses given every 6 weeks until discontinuation from the study or for a maximum of 24 months' total therapy, whichever comes first.

The primary efficacy evaluation will be made according to RECIST version 1.1, with exploratory efficacy assessments also performed using iRECIST ; the first on-study assessment will be done 7 weeks after the start of LTX-315 injections (on Day 43) and then repeated every 9 weeks until the patient fulfils criteria for discontinuation from the study (including disease progression). The lesion selected for injection will not be used as a target lesion for RECIST version 1.1 or iRECIST response evaluation. Any target lesion selected for RECIST 1.1 should not be biopsied. If treatment with pembrolizumab is conducted beyond disease progression (Section 5.1.2.1), efficacy evaluation frequency will be based on treating Investigator decision and institutional standard.

No other antitumor treatment is allowed during study treatment, but palliative radiotherapy or surgery may be performed; in such a case, the patient may remain in the study and be considered as evaluable if he/ or she otherwise meets the evaluability criteria (Section 4.0). If a patient requires palliative surgery during the study, the patient shall be evaluated for possible adrenal insufficiency (including blood cortisol and adrenocorticotropic hormone [ACTH] level) and any institutional standard for evaluation of adrenal function.

Pembrolizumab treatment may be continued for up to 24 months if considered clinically beneficial to the patient, in the opinion of the Investigator, and if agreed with the Sponsor (see Section 5.1.2.1).

Patients will continue with treatment on study up to 24 months, or until disease progression (confirmed by iRECIST if the Investigator considers acceptable for the patient), unacceptable toxicity, or withdrawal for another reason such as investigator's decision or withdrawal of consent. Following discontinuation from the study, patient will attend follow-up visits 30, 60, and 90 days after end of treatment (EOT) visit.

A Data Safety Committee consisting of the investigators, sponsor representatives, and Contract Research Organization (CRO) representatives will review safety and efficacy data related to patients enrolled in the study.

The Data Safety Committee shall meet when approximately half of the patients are enrolled and have completed Phase A and again following enrollment completion, or each time any of the following events occur (whichever is shorter):

- AE of Grade 4 or 5 considered by the Investigator to be related or possibly related to LTX-315 or to pembrolizumab.

3.2 Discussion of Study Design, Including the Choice of Control Groups

This open-label, single-arm study is proposed as a study of the combination of LTX-315 and the

marketed drug pembrolizumab in patients with unresectable Stage III B, C, D or Stage IVm1a, m1b metastatic melanoma who have failed anti-PD-1/PD-L1 therapy. To test the potential for LTX-315 to improve susceptibility to pembrolizumab, the following patients will be recruited: patients will have selected tumors that have an approved anti-PD-1 or anti-PD-L1 therapy and have progressed on or after prior anti-PD-1 or anti-PD-L1 therapy, alone or in combination with systemic therapy.

The study is intended to establish preliminary efficacy and the safety profile of the combination of LTX-315 and pembrolizumab based on evaluation of multiple efficacy and safety parameters.

The study will include two phases: in Phase A, LTX-315 will be administered on 7 days over a period of 5 weeks (or less if the injected tumor[s] shows complete necrosis before treatment is complete), with multiple injections potentially being given on each dosing day, depending on tumor size and number of injectable lesions. In parallel, pembrolizumab 200 mg will be infused at Days 1 and 22. Phase B will start 3 weeks after the last dose of pembrolizumab in Phase A, in accordance with the pembrolizumab prescribing information. The treatment will be continued by infusions of pembrolizumab 400 mg every 6 weeks; this treatment regimen minimizes the number of clinic visits patients are required to attend for pembrolizumab infusions.

Safety parameters will be monitored, along with clinical monitoring of site of injections and of vital signs.

Evaluation according to the RECIST version 1.1 criteria is validated for the purposes of conducting clinical trials and analysis of efficacy endpoints. Therefore, efficacy of treatment will be evaluated using RECIST version 1.1 every 9 weeks.

Immunotherapeutic agents produce antitumor effects that may manifest after an initial increase in tumor burden or the appearance of new lesions (known as pseudoprogression). Therefore, an exploratory evaluation of efficacy of treatment will be performed using iRECIST every 9 weeks. The criteria are designed to better characterize tumor responses in patients treated with immunotherapy compared with RECIST version 1.1,

Treatment will be continued until unacceptable toxicity, withdrawal of informed consent, progression of disease, or completion of 24 months total therapy. Each patient will attend End of Treatment visit at the time of treatment discontinuation, and follow-up visits approximately 30, 60, and 90 days after the end of treatment.

3.3 END OF STUDY DEFINITION

The end of the study is defined as the date of the last visit (scheduled or unscheduled) of the last patient in the study.

3.4 SELECTION OF DOSES IN THE STUDY

3.4.1 LTX-315 DOSES

LTX-315 as monotherapy has been evaluated as single doses of 2 to 7 mg per injection administered as either 10 or 20 mg/mL in 39 patients in the Phase 1/2 study C12-315-03 (see Section 1.2.2). LTX-315 injections have been administered to tumor lesions on a once-daily or twice-daily basis on planned LTX-315 injection days. Most patients have had injections to single tumor lesions (three injection days in Week 1, thereafter once weekly for 5 more weeks or six injection days in 3 weeks). Additionally, LTX-315 has been administered as monotherapy in 14

patients in the Phase 1 study C08-315-01. The patients received a weekly injection of LTX-315 into a single tumor to a maximum of six injections. In Cohorts 1 (10 mg/mL) and 2 (20 mg/mL), the injections were administered at a specified concentration with a volume calculated to be 10% of the tumor volume across cohorts; Cohort 3 received a fixed milligram dose (2 mg on first injection and 4 mg on subsequent injections, except for one patient who incorrectly received a first injection of 4 mg).

In the current study, LTX-315 will be administered at 5 mg per injection (given in a 10 mg/mL concentration) on seven dosing days in 5 weeks (Days 1, 2, 3, 8, 15, 22, and 29). The number of injections per lesion per dosing day is dependent on the size of the lesion(s) to be treated. The concentration used will be 10 mg/mL to reach a higher volume of the tumor lesion than with 20 mg/mL. The rationale for the dose and schedule is described below.

LTX-315 up to 5 mg per injection in Study C12-315-03 has been demonstrated to be a well-tolerated dose (no dose-limiting toxicities and no clinically significant dose-related AEs



In this study, LTX-315 will be administered in combination with pembrolizumab. In Study C12-315-03, the addition of LTX-315 to pembrolizumab dosing in 18 patients did not appear to increase the overall incidence or severity profile of toxicities.

3.4.2 PEMBROLIZUMAB DOSES

Two dosing regimens are defined in the pembrolizumab prescribing information: 200 mg once every 3 weeks and 400 mg once every 6 weeks. The lower dose of 200 mg will be administered in Phase A of the study to evaluate patient safety during the initial doses and because the higher dosing frequency is in line with planned visits required for study assessments. Pembrolizumab will therefore be given on Days 1 and 22 at a fixed flat dose of 200 mg per administration. Thereafter, pembrolizumab will be given at a fixed flat dose of 400 mg per administration every

6 weeks, starting on Day 43, to minimize the number of clinic visits patients are required to attend for pembrolizumab infusions.

4.0

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

4.1 INCLUSION CRITERIA

Patients must satisfy all of the following criteria at the screening visit unless otherwise stated:

1. Have one of the following confirmed histologically:
   Patients with Stage III B, C, D or Stage IV m1a, m1b unresectable metastatic melanoma who have received an approved-anti-PD-1/PD-L1 therapy and have progressed on or after prior anti-PD-1 or anti-PD-L1 therapy, alone or in combination with systemic therapy.
   For patients who have refused prior standard-of-care treatment other than anti-PD-1/PDL-1 therapy, the patient's reason for refusing standard therapy for their disease shall be clearly documented in the study electronic case report form prior to study participation.
   All patients must have received anti-PD-1 or anti-PD-L1 in addition to complying with the relevant criteria below. Melanoma patients with v-raf murine sarcoma viral oncogene homolog B1 (BRAF) mutation who are eligible and suitable for BRAF inhibitor therapy should have received specific BRAF inhibitor therapy before enrolling in this study and must have completed treatments at least 3 weeks prior to starting treatment and have no signs of rapidly progressive disease (PD). Patients who have refused BRAF inhibitor therapy are also eligible for the study.
2. The patients should have had radiologically PD after the most recent line of systemic therapy (no more than two prior lines in the metastatic setting). Adjuvant or Neoadjuvant therapy is not considered as a prior line of treatment for eligibility purposes,
   Anti-PD-1 or anti-PD-L1 does not need to be the most recent line of therapy prior to study entry.
3. Disease that is not amenable to further radiotherapy or surgery for cancer treatment.
4. Have at least one superficial, nonvisceral tumor lesion accessible for injection via cutaneous, subcutaneous, or intramuscular route. Note, lymph nodes with metastatic disease may be selected for injection if they are superficial, but not if deep-seated; visceral lesions must not be selected for injection. The lesion must not be located close to airways, defined as close enough to jeopardize the patient's safety, in the opinion of the Investigator, in the event of a local reaction to LTX-315 injection (for example, if such a reaction has the potential to interfere with swallowing or result in hemorrhaging into the airways). The size of the lesion(s) selected for injection must be ≤2 cm in the mean longest perpendicular diameter. For larger lesions or conglomerate lesions, approval from the sponsor's Medical Monitor is needed prior to enrollment.
5. At least one measurable target lesion evaluable according to RECIST version 1.1 that is not planned to be injected with LTX-315 or biopsied. The location of this noninjected tumor may be superficial, deep-seated, or visceral.
6. Have a life expectancy ≥3 months.
7. Are males or females aged 18 years or older.
8. Have an ECOG performance status of 0 or 1.

9. Resolution of all disease or prior treatment-related toxicities to Grade ≤1, with the exception of alopecia, <Grade 2 neuropathy and laboratory abnormalities (parameters below apply). If the patient underwent major surgery or received radiation therapy, they must have fully recovered from the intervention.
10. Have a left ventricular ejection fraction (LVEF) that is above the institution's lower limit of normal (by echo scan assessment).
11. Meet the following laboratory requirements:
    a. Absolute neutrophil count $\geq 1.00 \times 10^9$/L,
    b. Absolute lymphocyte count that is ≥0.5 k/μL or equivalent,
    c. Platelet count $\geq 75.0 \times 10^9$/L,
    d. Hemoglobin ≥9.0 g/dL,
    e. Prothrombin time/partial thromboplastin time ≤1.5 x upper limit of normal (ULN), if the patient is receiving oral anticoagulation international normalized ratio ≤1.5 x ULN, activated partial thromboplastin time ≤1.5 x ULN,
    f. Total bilirubin ≤1.5 x ULN (≤2 x ULN if associated with hepatobiliary metastases or Gilbert's syndrome) AND associated to the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) 2.5 x ULN,
    g. AST and ALT $\leq 2.5 \times$ ULN,
    h. Calculated creatinine clearance ≥30 mL/min using Cockcroft-Gault formula,
    i. Lactate dehydrogenase (LDH) ≤ULN,
    j. Serum albumin ≥30 g/L.
12. Are willing and able to comply with the protocol and agree to return to the clinical site for follow-up visits and examinations.
13. Are willing to undergo tumor biopsy procedures.
14. Are fully informed about the study and have signed the informed consent form (ICF).
15. Are willing to use contraceptive measures as prescribed by the protocol. Female patients of childbearing potential and their partners who are sexually active must agree to the use of two highly effective forms of contraception starting from the screening visit, throughout their participation in the study, and for at least 120 days after their last dose of pembrolizumab or at least 180 days after their last dose of LTX-315, whichever is longer. Male patients with partners who are pregnant or of childbearing potential must use a barrier method of contraception starting from the screening visit, throughout their participation in the study and for at least 120 days after their last dose of pembrolizumab or at least 180 days after their last dose of LTX-315, whichever is longer. A woman is considered of childbearing potential, that is, fertile, following menarche and until becoming postmenopausal unless permanently sterile.
16. Female patients must not be pregnant or breastfeeding. Female patients of childbearing potential must have a negative pregnancy test result during screening and at Day 1.

4.2 EXCLUSION CRITERIA

Patients will be excluded from the study if they satisfy any of the following criteria at the screening visit unless otherwise stated:

1. Patients with primary ocular melanoma or mucosal melanoma are not eligible
2. Has excessive tumor burden. The patient has more than 10 lesions available for injection, or has any injected lesion >2cm, or in the opinion of the Investigator. For larger lesions, approval from the sponsor's Medical Monitor is needed prior to

enrollment. Patients with more than 10 lesions may be deemed eligible after discussion with the sponsor's Medical Monitor, based on assessment of overall tumor burden.

3. Known bone-only or active central nervous system metastases and/or carcinomatous meningitis.
   Patients with untreated brain metastases ≤3 mm that are asymptomatic, do not have significant edema, and do not require steroids or anti-seizure medications are eligible after discussion with the sponsor's Medical Monitor. Patients with previously treated brain metastases may participate provided they are stable after treatment and without evidence of progression by imaging for at least 4 weeks prior to the first dose of study drug administration, and are not using corticosteroids for at least 7 days prior to study drug administration.
4. Have a history of cerebrovascular or cardiac disorders (e.g., Class III or IV New York Heart Association cardiac failure) and in the investigators' opinion the patient would be at particular risk of sequelae following a short hypotensive episode.
5. Have a marked baseline prolongation of QT interval corrected for heart rate using Fridericia's formula (i.e., repeated demonstration of a QTc interval >480 ms National Cancer Institute Common Terminology Criteria for Adverse Events [NCI-CTCAE] Grade 2).
6. Are currently taking immunosuppressive agents or use of systemic corticosteroids or other systemic immunosuppressive drugs within 28 days prior to study drug administration (see Appendix 4 for examples). Patients who require corticosteroids should have been on a stable dose (up to 10 mg daily prednisone or equivalent) for at least 2 weeks prior to study drug administration. Topical and inhaled corticosteroids are also permitted.
7. Have a history of systemic autoimmune disease requiring anti-inflammatory or immunosuppressive therapy within 3 months prior to Day 1, with the following exceptions:
   a. Patients with a history of autoimmune thyroiditis are eligible provided they require only thyroid hormone replacement therapy and their disease has been stable for ≥1 year.
   b. Patients with well-controlled type I diabetes (in the opinion of the Investigator) are eligible.
   c. Patients with a history of chronic disease, who have been stable for the preceding 6 months without treatment.
8. Patients who are allergic or have hypersensitivity to [redacted].
9. Have a history of severe hypersensitivity to another monoclonal antibody; are receiving immunosuppressive therapy; have a history of severe immune-related adverse reaction from treatment with a monoclonal antibody, defined as any Grade ≥3 toxicity requiring corticosteroid treatment (>10 mg/day prednisone or equivalent) for >12 weeks.
10. Have had (non-infectious) pneumonitis Grade ≥3 in the past or current pneumonitis.
11. History of interstitial lung disease.
12. Have a known hypersensitivity to pembrolizumab or LTX-315 or any of their excipients (for pembrolizumab those listed in the prescribing information).

13. Have any other serious illness or medical condition, such as, but not limited to:
    a. Uncontrolled infection or infection requiring systemic antibiotics,
    b. Uncontrolled cardiac failure: Class III or IV New York Heart Association,
    c. Uncontrolled hypertension or risk factors for uncontrolled hypertension (>160 mmHg systolic and/or >100 mmHg diastolic), despite appropriate antihypertensive medication,
    d. Uncontrolled systemic or gastrointestinal inflammatory conditions,
    e. Known bone marrow dysplasia,
    f. History of positive tests for human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome or active hepatitis B or C (based on serology); positive serology will be confirmed by a viral load test. Patients with treated HIV and a viral load test result consistent with treated HIV, as well as patients with treated hepatitis B or C with an undetectable viral load test result, are eligible,
    g. History of or current mastocytosis.
14. Have received external radiotherapy or cytotoxic chemotherapy within 4 weeks prior to Day 1 or have not recovered (to NCI-CTCAE Grade ≤1; alopecia of any grade is allowed, and peripheral neuropathy of up to Grade 2 is allowed) from adverse events (AEs) due to such agents being administered more than 4 weeks prior to Day 1.
15. Have received cancer immunotherapy within 4 weeks prior to Day 1 or have not recovered from AEs (to NCI-CTCAE Grade ≤1) due to such agents being administered more than 4 weeks prior to Day 1.
16. Have received an investigational drug within 4 weeks prior to Day 1 or are scheduled to receive one during the treatment or post-treatment periods.
17. Are expected to need any other anticancer therapy or immunotherapy to be initiated during the study period. Note: Palliative radiotherapy or surgery are allowable under certain conditions in Phase B.
18. Receipt of any BRAF and/or mitogen-activated protein kinase (MEK) inhibitor (including any investigational inhibitor) within 3 weeks before first dose of study drug and/or with signs of rapidly progressive disease.

4.3 DISEASE DIAGNOSTIC CRITERIA

Cancer diagnosis must be confirmed by histology.

4.4 DISCONTINUATION CRITERIA

4.4.1 SCREEN FAILURES

Screen failures are defined as patients who consent to participate in the clinical study but are not subsequently entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure patients to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any SAEs.

Patients who do not meet the criteria for participation in this study may be rescreened. Rescreened patients should be assigned the same patient number as for the initial screening.

4.4.2 DISCONTINUATION OF STUDY TREATMENT

Patients may withdraw from the study at any time at his/her own request or may be withdrawn at any time at the discretion of the investigator for safety, behavioral, or administrative reasons. If the patient withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent. Additionally, patients may request destruction of any samples taken and not tested, and the investigator must document this in the site study records. Refer to the Schedule of Assessments (Appendix 9) for data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed.

Additional reasons for discontinuation can include, but are not limited to:

- Death,
- Adverse event,
- Confirmed ≥20% change from screening LVEF in left ventricular ejection fraction,
- Disease progression (unless fulfilling treatment-beyond-progression criteria [see Section 5.1.2.1]). **Note: in the case of disease progression a confirmatory scan is required, and patients should remain on study until progression is confirmed per iRECIST (see Section 7.2.1.1),**
- Unacceptable toxicity, as defined in the Section 4.4.2.2 and Section 4.4.2.3,
- Sponsor determination,
- Initiation of a new systemic anticancer treatment,
- Dosing delay of >3 weeks (for any reason; unless a permitted delay under Section 6.2 to allow palliative surgery or radiotherapy to be performed),
- Patient becomes pregnant or fails to use adequate birth control (for those patients who are able to conceive),
- Intercurrent medical illness that, in the judgment of the Investigator, would make continued intervention dangerous for the patient.

Should a patient experience a post-injection reaction to LTX-315 administration, action must be taken in regard to study treatment as shown in Table 1.

Reaction	Grade	Recommended Action	Reintroduction of LTX-315 Injections	Action in Event of Repeated Reaction or Anaphylaxis
Hypersensitivity or hypersensitivity-like reactions	3 or higher	• Stop further injections on that day • Treat with: [redacted] If anaphylaxis is suspected, treat with epinephrine 0.3 to 0.5 mg i.m.	• Injections may be reintroduced on another day if reaction is fully resolved, upon agreement with sponsor's Medical Monitor and no suspicion of anaphylaxis.	• Stop injections permanently • Patient continues in study without LTX-315
Any other reaction occurring during injections or within 1 h after the last injection of any given day	3 or higher	• Stop further injections on that day • Monitor patient • Symptomatic treatment at Investigator discretion	• Injections may be reintroduced on the next scheduled dosing day if reaction is fully resolved, upon agreement with sponsor's Medical Monitor	• Follow same recommendations and discuss the possibility to continue treatment with sponsor's Medical Monitor

Abbreviations: h = hour(s); IV = intravenous.

In addition to the above, specific AEs requiring discontinuation of LTX-315 treatment are listed in the sections that follow.

Both LTX-315 and pembrolizumab should be discontinued on the occurrence of:

- Any clinically significant (Investigator-assessed) Grade 3 or 4 AE that occurs between study Days 1 and 21 whose causality cannot be clearly assigned to either LTX-315 or pembrolizumab (e.g., hypersensitivity AE).

LTX-315 must be discontinued in the event of any of the following criteria being fulfilled:

- Any LTX-315 **related**:

  Grade 4 AE.

Grade 3 hypersensitivity/anaphylaxis reaction if uncontrolled following treatment interruption.

Grade 3 hypotension AE if uncontrolled following treatment interruption.

AE assessed by the treating Investigator as warranting discontinuation.

- Serum tryptase level (at any time point) of ≥11.5 ng/mL or equivalent.

Management of adverse reactions may require withholding of a dose or permanent discontinuation of pembrolizumab. Guidelines for permanent discontinuation or withholding of doses are described in Table 2.

Adverse Reaction	**Severity[a]**	**Dose Modification**
Immune-mediated pneumonitis	Grade 1 and 2	Withhold[b]
	Grades 3 or 4 or recurrent Grade 2	Permanently discontinue
Immune-mediated myocarditis	Grades 3 and 4	Permanently discontinue
Immune-mediated colitis	Grades 2 or 3	Withhold[b]
	Grade 4	Permanently discontinue
Immune-mediated hepatitis in patients	AST or ALT greater than 3 but no more than 5 × ULN or total bilirubin greater than 1.5 but no more than 3 × ULN	Withhold[b]
	In patients without liver metastases, AST or ALT greater than 5 times ULN or total bilirubin greater than 3 times ULN; In patients with liver metastasis and Grade 2 AST or ALT at baseline, with an increase in AST or ALT of 50% or more relative to baseline that persists for at least 1 week.	Permanently discontinue
Immune-mediated endocrinopathies	Grade 2 adrenal insufficiency and hypophysitis	Withhold treatment until controlled by hormone
	Grades 3 or 4 adrenal insufficiency or symptomatic hypophysitis Type 1 diabetes associated with Grade ≥ 3 hyperglycaemia (glucose > 250 mg/dL or > 13.9 mmol/L) or associated with ketoacidosis Hyperthyroidism Grade ≥ 3	For patients with Grade 3 or Grade 4 endocrinopathies that improved to Grade 2 or lower and are controlled with hormone replacement, if indicated, continuation of pembrolizumab may be considered after corticosteroid taper, if needed. Otherwise treatment should be discontinued.
	Hypothyroidism	Hypothyroidism may be managed with replacement therapy without treatment interruption

Adverse Reaction	Severity[a]	Dose Modification
Immune-mediated nephritis	Grade 2	Withhold[b]
	Grades 3 or 4	Permanently discontinue
Immune-mediated skin adverse reactions	Grade 3 or suspected SJS or TEN	Withhold until adverse reactions recover to Grades 0-1 [c]
	Grade 4 or confirmed SJS or TEN	Permanently discontinue
Other immune-mediated adverse reactions	Grades 2 or 3 based on the severity and type of reaction	Withhold[b]
	Grades 3 or 4 myocarditis Grades 3 or 4 encephalitis Grades 3 or 4 Guillain-Barré	Permanently discontinue
	Grade 4 or recurrent Grade 3	Permanently discontinue
Recurrent immune-mediated adverse reactions	Recurrent Grade 2 pneumonitis Recurrent Grades 3 or 4	Permanently discontinue
Inability to taper corticosteroid	Requirement for 10 mg per day or greater prednisone or equivalent for more than 12 weeks after last dose of pembrolizumab	Permanently discontinue
Persistent Grade 2 or 3 adverse reaction (excluding endocrinopathy)	Grades 2 or 3 adverse reactions lasting 12 weeks or longer after last dose of pembrolizumab	Permanently discontinue
Infusion-related reactions	Grades 1 or 2	Interrupt or slow the rate of infusion
	Grades 3 or 4	Permanently discontinue

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; HCC = hepatocellular carcinoma; SJS = Stevens-Johnson syndrome; TEN = toxic epidermal necrolysis; ULN = upper limit of normal.
[a] Toxicity was graded per National Cancer Institute Common Terminology Criteria for Adverse Events Version 4.0.
[b] Resume in patients with complete or partial resolution (Grades 0 to 1) after corticosteroid taper.
[c] If treatment-related toxicity does not resolve to Grades 0-1 within 12 weeks after last dose of pembrolizumab, or if corticosteroid dosing cannot be reduced to ≤ 10 mg prednisone or equivalent per day within 12 weeks, pembrolizumab should be permanently discontinued.

In addition to the immune-related reactions listed in Table 2, pembrolizumab will be permanently discontinued in the event of any of the following:

- For pembrolizumab-related Grade 4 toxicity, except for endocrinopathies that are controlled with replacement hormones.
- If any pembrolizumab-related Grade ≥3 event occurs a second time at Grade ≥3 severity in the same patient.

Patients treated with pembrolizumab will be given a copy of the patient information from the package leaflet and will be informed about the risks of pembrolizumab.

4.4.2.5.1 Heart rate correction of the QT interval and reported QTc

The QT and preceding RR value for each beat will be used for heart rate correction. QT corrected according to Fridericia's formula is defined as $QTcF = QT/RR^{1/3}$. The median QTc value from all beats in each replicate will be calculated and then the mean of all available medians (from a minimum three replicates) from a nominal time point will be used as the patient's reportable value at that time point.

If a clinically significant finding is identified (including, but not limited to, changes from baseline in QTcF) after enrollment, the Investigator or qualified designee will determine if the patient can continue in the study and if any change in patient management is needed. This review of the ECG printed at the time of collection must be documented. Any new clinically relevant finding should be reported as an AE.

A patient who meets either bulleted criterion based on the average of triplicate ECG readings will be withdrawn from the study.

- QTcF >500 ms OR uncorrected QT >600 ms.
- Change from baseline of QTcF >60 ms.

Refer to Section 7.3.4 for action to be taken in the event of pregnancy in a patient or in the partner of a male patient.

4.4.3 LOST TO FOLLOW-UP

A patient will be considered lost to follow-up if he/she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

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

- The site must attempt to contact the patient and reschedule the missed visit as soon as possible, counsel the patient on the importance of maintaining the assigned visit schedule and ascertain whether or not the patient wishes to and/or should continue in the study.
- In cases in which the patient is deemed lost to follow-up, the Investigator or designee must make every effort to regain contact with the patient (where possible, three telephone calls and, if necessary, a certified letter to the patient's last known mailing address or local equivalent methods). These contact attempts should be documented in the patient's medical record.
- Should the patient continue to be unreachable, he/she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

4.4.4 REPLACEMENT PROCEDURES

Patients who discontinue the study prior to completing five of seven injection days in Part A may be replaced at the discretion of the sponsor.

4.4.5 FOLLOW UP OF PATIENTS PREMATURELY DISCONTINUED FROM THE STUDY TREATMENT REGIMEN OR WITHDRAWN FROM STUDY

Patients who discontinue the study will be asked to complete final study procedures (End-of-Treatment/Early Termination Visit) and will attend additional Follow-up Visits approximately 30, 60, and 90 days after the End-of-Treatment/Early Termination Visit, as described in the Schedule of Assessments (Appendix 9).

4.4.6 STUDY INTERRUPTION

The study shall be interrupted (i.e., further study treatment shall be held for patients enrolled into the study, and no new patients shall be enrolled in the study) upon the occurrence of any AE of Grade 5 or two AEs of Grade 4 that are considered by the Investigator to be related or possibly related to LTX-315 and/or pembrolizumab.

In case of interruption of study, the Data Safety Committee shall perform a thorough review of the safety data and make a recommendation on further conduct of the study.

4.5 STUDY TERMINATION

The Sponsor reserves the right to close a study site or terminate the study at any time for any reason at its sole discretion. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study site closure visit has been performed. Reasons for study termination may include, but are not limited to:

- Adverse events unknown to date (i.e., not previously reported in any similar investigational study drug study with respect to their nature, severity, and/or duration).
- Increased frequency and/or severity and/or duration of known, anticipated, or previously reported AEs (this may also apply to AEs defined as baseline signs and symptoms).
- Medical or ethical reasons affecting the continued performance of the study.
- Difficulties in the recruitment of patients.
- Determination of futility, defined as $\leq$ two patients showing a PR or CR with the best response in all other patients being PD. The Sponsor has the right to continue the study if a proportion of the other patients show sustained stabilization (SD) of disease.
- Cancelation of drug development.

The Investigator may initiate study site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination. 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 institutional review board (IRB) or local health authorities, the sponsor's procedures, or Good Clinical Practice (GCP) guidelines.
- Inadequate recruitment of participants by the Investigator.

In the event for study closure for safety or lack of efficacy concerns, patients would stop dosing

immediately and should remain in the study for follow-up until they transition to an alternative treatment outside of the protocol. In the event of study closure for any other reason than safety, patients may be proposed to complete their current treatment dosing period, i.e. for LTX-315 complete Phase A. For Phase B, where patients are taking pembrolizumab alone, patients should remain in the study until their next planned dose. At this time point, patients should transition to standard of care or other treatments as indicated by their Investigator or should enter the study follow-up period

5.0

5.1 TREATMENTS ADMINISTERED

The following treatments will be administered during this study.

Study Treatment Name:	**LTX-315**	**Pembrolizumab**
Dosage Formulation:	Lyophilized powder for reconstitution	IV infusion
Unit Dose Strength(s)/ Dosage Level(s):	10 mg/mL 5 mg per injection	100 mg/4 mL (25 mg/mL) 200 mg in Phase A, 400 mg in Phase B
Route of Administration:	Intratumoral injection	30-min IV infusion
Dosing Instructions:	Multiple injections may be given per dosing day depending on tumor size as shown in Table 3. A maximum of 8 injections may be given across all lesions planned for injection per dosing day (40 mg in total). See also Section 5.1.1.	See Section 5.1.2
Packaging and Labeling:	LTX-315 will be provided in glass vials. Each vial will be labeled as required per USA and European requirement.	Pembrolizumab will be provided in vials. Each vial will be labeled as required per USA requirement. For European sites, pembrolizumab will be provided by the local pharmacy, and Lytix will reimburse the cost.
Manufacturer:	Lytix	Merck

5.1.1 MODE OF ADMINISTRATION OF LTX-315

Patients must have a minimum of two tumors; at least on superficial, nonvisceral tumor lesion accessible for injection via cutaneous, subcutaneous, or intramuscular route and at least one tumor lesion that is not planned to be injected with LTX-315 (this lesion must be evaluable according to RECIST version 1.1 and may be superficial, deep-seated, or visceral).

Sites should follow the guidance in the Manual for Administration of LTX-315 and ensure that the Injection Planning Form is submitted to the Sponsor as indicated, prior to dosing on Day 1.

The aim is to achieve complete necrosis of each injected lesion. Only viable tissue should be injected. LTX-315 will be injected directly into the selected tumor lesion(s) at Days 1, 2, 3, 8, 15, 22, and 29, as shown in Figure 1. The maximum number of injections given per dosing day is eight (5 mg each for a maximum total of 40 mg per day) equaling 56 total doses of LTX-315 in Phase A. The maximum number of injections per lesion per dosing day depends on lesion size, as shown in Table 3. A 5 minute interval between injections is recommended, where possible.

The number of injections per lesion for all LTX-315 dosing days is based only on the baseline mean LPD. The actual mean LPD will still be measured and recorded on each dosing day (and post dosing period for the duration of the study, as described in SOA) for all lesions injected. If for some reason a lesion is not injected at a required time point in Phase A, the actual mean LPD

for that lesion should still be measured and recorded. In the case of necrosis of any injected lesions, only the viable tumor tissue should be measured for the mean LPD. If the Investigator wants to inject a lesion that was not originally included on the injection planning form, approval from the sponsor's Medical Monitor is required.

The largest lesion should be injected first with the maximum number of injections allowed per Table 3. If the largest lesion requires less than 8 injections per Table 3, then it's recommended that the next largest lesion should be selected and dosed up to the allowable maximum daily dose per Table 3. The same lesion(s) should be injected on subsequent dosing days. The aim is to achieve complete necrosis of each injected lesion. Only viable tissue should be injected. If a lesion becomes necrotic during treatment, the number of injections per dosing day should be adjusted per investigator discretion.

[redacted]

The Investigator should select one or more superficial, nonvisceral tumor lesion(s) accessible for injection via cutaneous, subcutaneous, or intramuscular route. Selected tumor lesion(s) must be ≤2 cm in mean longest perpendicular diameter. For larger lesions or conglomerate lesions, approval from the sponsor's Medical Monitor is needed prior to enrollment. [redacted]

[redacted]

[redacted]

Premedication is required prior to LTX-315 injection, and shall consist of:

[redacted]

5.1.2 MODE OF ADMINISTRATION OF PEMBROLIZUMAB

Pembrolizumab will be dispensed and administered as an IV infusion.

Phase A

Pembrolizumab will be given as 200 mg IV infusion over 30 minutes on Days 1 and 22. The pembrolizumab treatment should start at least 30 minutes after both of the following two conditions have been met: 1) the last LTX-315 injection for that day is completed (safety evaluation permitting) and 2) any LTX-315-related AEs have either resolved or improved to a severity of Grade 1. Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. The pharmacists will prepare pembrolizumab according to the prescribing information.

Phase B

Pembrolizumab will be given as 400 mg IV infusion over 30 minutes every 6 weeks starting at Day 43 (3 weeks after the last dose of pembrolizumab in Phase A) for a maximum of 24 months' total therapy until disease progression, confirmed by iRECIST if the Investigator considers acceptable for the patient (unless fulfilling the treatment-beyond-progression criteria in Section 5.1.2.1), unacceptable toxicity, or another discontinuation criterion is met. Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. The pharmacists will prepare pembrolizumab according to instructions in the prescribing information.

Treatment with pembrolizumab beyond disease progression is permitted, for a maximum of 24 months' total therapy, if a patient is deemed to be receiving clinical benefit (as assessed by the Investigator) and if the following criteria are met:

- absence of clinical signs and symptoms (including worsening of laboratory abnormalities) indicating clinically important adverse reactions.
- no tumor-related decline in ECOG performance status.
- absence of rapid progressive disease or progression at critical anatomical sites (e.g., cord compression) requiring urgent alternative medical intervention.

In the case of disease progression, patients should remain on treatment until a confirmatory scan has been performed (4-6 weeks after initial disease progression scan), unless continued treatment is not considered acceptable by the Investigator.

Any further continuation of treatment beyond disease progression must be agreed with the sponsor's Medical Monitor. The patient shall sign an additional ICF if the treatment is recommended to continue after disease progression. The frequency of imaging assessment in patients receiving pembrolizumab beyond progression will be based on Investigator decision and institutional standard.

5.2 PREPARATION, STORAGE, HANDLING, AND ACCOUNTABILITY

LTX-315 will be supplied by the Sponsor (or designee), along with the batch numbers and Certificates of Analysis.

Pembrolizumab may be supplied by the Sponsor, or sourced locally by the sites, per agreement

with the Sponsor.

The Investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study treatment received and any discrepancies are reported and resolved before use of the study treatment.

All study treatments must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the investigator and authorized site staff.

Only patients enrolled in the study may receive study treatment and only authorized site staff may supply or administer study treatment. The Investigator, Institution, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records).

Further guidance and information for the final disposition of unused study treatment are provided in separate documents.

5.3 METHOD OF TREATMENT ASSIGNMENT

This is a nonrandomized study. The study has a fixed treatment sequence. Dose is determined by the number and size of lesions and not by randomization.

Patients who meet all the eligibility criteria will be assigned a patient number. Once assigned, patient numbers will not be re-used. Patients who screen fail may rescreen under the same patient number.

Patients will be identified by patient number on all study documentation. A list identifying the patients by patient number will be kept in the site master file.

5.3.1 DOSE INTERRUPTION, MODIFICATION, AND DELAY

Reductions in the dose of LTX-315 are not permitted.

If any injection of LTX-315 cannot be administered on the scheduled day, a time window of ±1 day is allowed.

Successive LTX-315 injections to different lesions may be delayed or omitted (with no discontinuation criteria being fulfilled) for any investigator-assessed clinically significant LTX-315-related AE (e.g., injection site pain, inflammation, or hypersensitivity-like symptoms such as flushing, paresthesia, or edema); see Table 1.

In addition, LTX-315 treatment may be delayed for up to 8 weeks to permit palliative radiotherapy or surgery to be performed in certain circumstances; see Section 6.2.

Pembrolizumab dose reductions are not recommended per the prescribing information.

Details of AEs requiring pembrolizumab treatment to be withheld or discontinued are presented in Section 4.4.2.4.

Pembrolizumab is associated with AEs that can differ in severity and duration from AEs caused by other therapeutic classes. Early recognition and management of AEs associated with pembrolizumab may mitigate severe toxicity. In addition to the guidelines for when to withhold and discontinue pembrolizumab in Section 4.4.2.4, any pembrolizumab-associated AEs should be managed in accordance with institutional standard practices and American Society of Clinical Oncology and National Comprehensive Cancer Network (NCCN) guidelines. In general, the following actions should be taken in the event of NCI-CTCAE v5.0 AEs:

- Grade 1: study treatment should be continued with close monitoring, with the exception of some neurologic, hematologic, and cardiac toxicities.
- Grade 2: study treatment may be suspended for most Grade 2 toxicities, with consideration of resuming when symptoms revert to Grade 1 or less. Corticosteroids may be administered (initial dose of 0.5 to 1 mg/kg/d of prednisone or equivalent).
- Grade 3: study treatment is generally suspended, and high-dose corticosteroid (prednisone 1 to 2 mg/kg/d or methylprednisolone 1 to 2 mg/kg/d) treatment should be initiated. Corticosteroids should be tapered over the course of at least 4 to 6 weeks. Some refractory cases may require infliximab or other immunosuppressive therapy.
- Grade 4: in general, permanent discontinuation of study treatment is recommended, with the exception of endocrinopathies that have been controlled by hormone replacement.

5.4 BLINDING

This is an open-label study; blinding procedures are not applicable.

5.5 TREATMENT COMPLIANCE

All study treatment administration will be performed at the study site by trained site staff.

The Investigator will record the time and dose of administrations of LTX-315 and pembrolizumab in the medical source documents. Any reasons for noncompliance will also be documented, including:

- missed visits.
- interruptions in the schedule of administration.
- nonpermitted medication (see Section 6.1).

Dosing records will be reviewed periodically on site by Clinical Research Associates to ensure study treatment is being administered consistent with the study protocol

6.0

6.1 CONCOMITANT THERAPY

No specific studies of interactions between LTX-315 and other agents have been conducted. However, the $t_{1/2}$ of LTX-315 following intratumoral injection is low (0.28 hours), so the opportunities for interaction of LTX-315 with other agents are limited.

No systemic anticancer therapy or radiotherapy is to be used during the study, with the exception of palliative radiotherapy to a nontarget lesion that does not represent disease progression. Any agent with a known effect on the immune system is prohibited during the study. Patients are allowed to be on a stable dose of corticosteroids (up to 10 mg daily prednisone or equivalent) and inhaler are allowed to receive topical corticosteroids as long as they have received them for at least 2 weeks prior to study drug administration. A list of prohibited medications can be found in Appendix 4.

Any nonprohibited medications (prescription as well as nonprescription drugs including herbal medicine and natural health remedies) necessary for the patient's welfare which are not listed among the exclusion criteria (Section 4.2) or the prohibited medications (Appendix 4) and which do not interfere with the study evaluations are permitted provided that they are carefully followed by the Investigator and documented on the applicable eCRF page.

Any concomitant medication or vaccine that the patient is receiving at the time of enrollment or receives during the study should be recorded in the eCRF along with (if possible):

- reason for use.
- dates of administration, including start and end dates.
- dosage information, including dose and frequency.

Any treatments initiated due to AEs that occur during the study MUST be documented in the eCRF and, in such cases, the medication use will be documented until the end of the follow-up period for the AE.

The sponsor's Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

6.2 SURGICAL RESECTION AND PALLIATIVE RADIOTHERAPY DURING TREATMENT

It may be necessary to perform palliative tumor resection or palliative radiotherapy to alleviate a patient's symptoms.

If palliative surgery is required:

- in Phase A of the study (Weeks 1 through 5), treatment shall be discontinued.
- in Phase B of the study (Week 7 or later), treatment may be interrupted for the amount of time needed for surgery and recovery after surgery (but not longer than 8 weeks).
- the patient shall be evaluated for possible adrenal insufficiency (including blood cortisol and ACTH level) and any institutional standard for evaluation of adrenal function prior to palliative surgical resection.

If palliative radiotherapy is required to non-target lesions:

- in Phase A of the study (Weeks 1 through 5), treatment shall be discontinued.
- in Phase B of the study (Week 7 or later).

  pembrolizumab may be continued or interrupted (per Investigator decision) for the period of radiotherapy (but no longer than 8 weeks).

7.0

Study procedures and their timing are summarized in the Schedule of Assessments (Appendix 9). As protocol waivers or exemptions are not allowed, with the exception of immediate safety concerns, these should be discussed with the Sponsor immediately upon occurrence or awareness to determine if the patient should continue or discontinue the study treatment. Adherence to the study design requirements, including those specified in the Schedule of Assessments, is essential and required for study conduct.

7.1 SUBJECT ENROLLMENT

Sites will submit subject eligibility registration package (SERP) for sponsor's Medical Monitor (or designee) review and approval. SERP will include study-specific eligibility checklist and relevant history including redacted/anonymized source documents such as the most recent history and physical, imaging reports, and laboratory reports where available. The sponsor's Medical Monitor (or designee) will review the patient's medical information to confirm eligibility and approve patient's enrollment in the study. During normal business days, a minimum of two business days will be required for the sponsor's Medical Monitor (or designee) to review a patient for enrollment. Additional time may be required when approval is sought during a weekend or holiday.

All screening evaluations must be completed and reviewed to confirm that potential patients meet all eligibility criteria. The Investigator will maintain a screening log to record details of all patients screened and to confirm lesion eligibility or record reasons for screening failure (Section 4.4.1), as applicable. Procedures conducted as part of the patient's routine clinical management (e.g., blood count) and obtained before signing the ICF may be utilized for screening or baseline purposes provided the procedure met the protocol-specified criteria and was performed within the timeframe defined in the Schedule of Assessments.

The maximum amount of blood collected from each patient over the duration of the study, including any extra assessments that may be required, will not exceed 500 mL. Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

7.2 EFFICACY ASSESSMENTS

7.2.1 TUMOR ASSESSMENTS

Examinations of the injected lesion(s) should be done at the required time points in Phase A, Phase B, and the EOT visit per the Schedule of Assessments (Appendix 9). The examinations include both visual inspection, photography (of superficial lesions only) and measurements. Measurements of the longest perpendicular diameters should be done using calipers/ruler. Acceptable methods include physical calipers/ruler with photography for superficial lesions, or ultrasound assessment with electronic caliper measurements for all other injected lesions.

Photographs should include a size standard or caliper/ruler for scale, and be appropriately labeled for patient study ID#, study visit, date, and lesion name and ID#. The lesion photographs are required for all superficial lesions treated on study and should be saved as part of the study documentation, and provided to the Sponsor upon request.

RECIST Lesions and Tumor Data

Scans to assess systemic antitumor activity will be done for all lesions selected as either target or non-target per RECIST. Lesions will be assessed and followed according to RECIST version 1.1 (Appendix 5) and iRECIST (Appendix 6).[18–20]

Acceptable RECIST scan methods include computer tomography (CT) with contrast or magnetic resonance imaging (MRI) scans of chest/abdomen/pelvis. If any lesions on the extremities are selected as baseline RECIST lesions, the corresponding extremity should also be scanned at every scan time point. Scans are required at baseline, Day 43 and then every 9 weeks during Phase B, as outlined in Appendix 5 and Appendix 6. Additional unscheduled scans should be taken as clinically indicated in order to assess the total tumor burden of a patient.

The baseline scan is to be done within 28 days prior to first treatment. Patients with known or suspected bone or brain lesions are required to have bone and brain scans done for all scheduled or unscheduled time points during the study, starting at baseline. Bone lesions should be assessed and followed using nuclear medicine bone scans, with the exception of lytic bone lesions that should be followed by CT/MRI.

Assessment of New Lesions Post Baseline:

If a new metastatic lesion is identified on a restaging scan, the correct RECIST response of progressive disease, per RECIST 1.1, and unconfirmed progression per iRECIST should be documented. The patient should continue on treatment until disease progression is confirmed by iRECIST if the Investigators consider this is acceptable for the patient.

If a new peripheral superficial lesion is found during clinical examination, the lesion should be factored into the RECIST response as a "new lesion" at the next restaging time point starting at Day 43 and onwards.

If a new peripheral superficial lesion is found on clinical examination during the LTX-315 dosing period (prior to Day 29), that lesion may be selected as a possible candidate for injection if there are available doses on an injection day. In this situation the sponsor's Medical Monitor should be contacted to discuss and approve injection of the new lesion. Factors that will be evaluated will include the size of the lesion and number of injections, and dosing days available. In this situation the lesion would not be factored into RECIST response at later time points as it becomes an injected lesion.

Confirmation of PR and CR (per RECIST 1.1) and of PD (per iRECIST) is required. Confirmatory scans should be obtained 4-6 weeks from the initial restaging scan where the PR, CR or PD was initially identified.

In the case of disease progression per iRECIST, patients should remain on treatment until a confirmatory scan has been performed (4-6 weeks after initial disease progression scan), unless continued treatment is not considered acceptable by the Investigator.

The same diagnostic method must be used throughout the study to evaluate a lesion.

7.2.2 TUMOR BIOPSIES AND IMMUNOLOGICAL BLOOD SAMPLES

A mandatory tumor biopsy of a LTX-315-injected lesion will be performed at baseline and Day

43 (where feasible), as indicated in the Schedule of Assessments (Appendix 9). This should consist of up to three 18-gauge core biopsies from a single lesion. The same lesion should be biopsied at both required time points. The feasibility of the biopsy at Day 43 will depend on how much of the lesion remain. Samples will be used to assess immune and/or efficacy biomarkers as described in Section 7.2.2.3.

If considered safe to do so, a tumor biopsy of a suitable single noninjected lesion will be performed at baseline and Day 43, as indicated in the Schedule of Assessments (Appendix 9), where feasible. The same lesion should be biopsied at both time points. This should consist of up to three 18-gauge core biopsies. Samples will be used to assess immune and/or efficacy biomarkers as described in Section 7.2.2.3. Biopsied tumors should not be considered as target lesions for tumor assessment.

Biopsy samples of injected and noninjected lesions may be prepared for multiplex immunohistochemistry or multiplex immunofluorescence staining, RNA sequencing, whole-exome sequencing, and single-cell sequencing. Analyses may include, but are not limited to, T cells (CD3+, CD4+, CD8+, Tregs), natural killer cells, myeloid cells, dendritic cells, and relevant molecules and receptors. In addition, biomarkers of efficacy such as PD-1/PD-L1 with different immunohistochemistry cut-off values may be measured in biopsy samples.



Immunological blood samples will be collected prior to study treatment (Day 1) and during treatment (Day 15) for the measurement of immunological response parameters, as described in Appendix 9. An additional immunological blood sample will be taken concurrently with the post-dose biopsy, as described in Section 7.2.2.2.

In order to investigate and characterize the immune status of the patients' blood and the nature of antitumor immune responses that may be induced by the LTX-315 treatment, the immunological response parameters investigated may include, but are not limited to:

- Lymphocyte count and phenotype.
- T-cell clonality.

Details of sample collection and processing will be provided in a separate document.

7.3 SAFETY AND TOLERABILITY ASSESSMENTS

7.3.1 ADVERSE EVENTS

Adverse event definitions and assignment of severity and causality are detailed in Appendix 1.

Adverse events will be elicited from the patient (or, when appropriate, from a caregiver, surrogate, or the patient's legally authorized representative) by the study site staff using a nonleading question such as "How are you feeling today?" or "Have you had any health concerns since your last visit?"

The Investigator and any designees are responsible for detecting, documenting, and reporting events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study treatment or the study, or that caused the patient to discontinue the study (see Section 4.4.2).

7.3.2 DISEASE PROGRESSION AND SAFETY REPORTING

Adverse events will be followed from consent throughout phases A and B, and the 90-day FU period. If a patient starts a new anticancer therapy during the study period, then the safety data collection will stop upon initiation of that therapy. Details of the new anticancer therapy and start date will be collected in the CRF.

Progression of the malignancy under study (including signs and symptoms of progression) should not be reported as an SAE unless the outcome is fatal within the safety reporting period. Hospitalization due to signs and symptoms of disease progression should not be reported as an SAE. If the malignancy has a fatal outcome during the study or within the safety reporting period, then the event leading to death must be recorded as an AE and as an SAE with CTCAE Grade 5 (see Appendix 1).

7.3.3 REPORTING SERIOUS ADVERSE EVENTS

Reportable AEs are AEs that are associated with the use of the drug and are serious and unexpected. Reportable AEs will be reported by the study site to the Sponsor, CRO responsible for pharmacovigilance in the study, and the responsible IRB.

The sponsor or designee and CRO responsible for pharmacovigilance in the study will be notified in writing (e.g., facsimile) within 24 hours of when an AE that is potentially reportable is first recognized or reported. Subsequently, a written confirmation or summary of the AE will be sent to the Sponsor or designee within three working days of the original notification.

The IRB will be notified of any reportable AEs within the timeframe required by the IRB. The IRB serious and unexpected adverse experience submission form will be completed and submitted with a copy of the written confirmation or summary of the AE.

The responsibility for reporting SAEs will be transferred to the Sponsor 28 days after the end of the study.

7.3.4 PREGNANCY

Initial information on a pregnancy (during or after the study as defined previously) must be reported to the Sponsor and the outcome information must be provided once the outcome is known. The separate pregnancy notification worksheet must be sent to the Sponsor or its designee according to reporting procedures described in this section.

For female patients, protocol-required procedures for study discontinuation and follow-up must be performed unless contraindicated by pregnancy (e.g., X-ray studies, CT scan). Other appropriate pregnancy follow-up procedures should be considered if indicated. Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome, must be reported. Infants should be followed for a minimum of 8 weeks.

For male patients, follow-up information regarding the course of the partner's pregnancy, including perinatal and neonatal outcome, should be reported, when possible.

7.3.5 CLINICAL LABORATORY EVALUATIONS

Blood and urine samples will be collected for clinical laboratory evaluations at the times indicated in the Schedule of Assessments (Appendix 9). Clinical laboratory evaluations will be performed by a local laboratory and are listed in Appendix 2.

For all female patients of childbearing potential, a pregnancy test will be performed at the times indicated in the Schedule of Assessments.

The Investigator (or designee) will perform a clinical assessment of all clinical laboratory data.

7.3.6 VITAL SIGNS, PHYSICAL EXAMINATION, AND OTHER SAFETY EVALUATIONS

Supine blood pressure, supine pulse rate, and oral or tympanic body temperature will be assessed at the times indicated in the Schedule of Assessments in Appendix 9. Vital signs may also be performed at other times if judged to be clinically appropriate or if the ongoing review of the data suggests a more detailed assessment of vital signs is required.

All measurements will be performed singly and repeated once if outside the relevant clinical reference range.

Patients must be supine for at least 5 minutes before blood pressure and pulse rate measurements.

On LTX-315 dosing days, blood pressure should be measured at the start of the study visit with other vital signs and just prior to the first injection with LTX-315 as described in the Schedule of Assessments (Appendix 9). The lowest value of these two blood pressure measurements will be considered as the baseline measurement on that dosing day. Blood pressure will be monitored serially approximately every 5 minutes during the administration of LTX-315 and up to 30 minutes post last LTX-315 injection of the day using an automated cuff system, as described in the Schedule of Assessments (Appendix 9). Monitoring will be conducted in accordance with local practice at the clinical site.

Any patient who experiences a drop in systolic and diastolic blood pressure >20mmHg compared to the baseline measurement on the dosing day would be considered to have an adverse event of special interest and the hypotension adverse event should be recorded on the adverse event eCRF form. These events will be periodically reviewed by the Sponsor. Study treatment administration should be suspended until the subject's blood pressure returns to baseline or Grade 0 or 1. These events are not considered as SAEs or important medical events and will not be reported to regulatory authorities, unless they meet SAE reporting requirements.

Resting 12-lead ECGs will be recorded after the patient has been supine and at rest for at least 5 minutes at the times indicated in the Schedule of Assessments in Appendix 9. ECGs in triplicate will be recorded approximately 5 minutes apart.

Additional 12-lead ECGs may be performed at other times if judged to be clinically appropriate or if the ongoing review of the data suggests a more detailed assessment of ECGs is required.

The Investigator (or designee) will perform a clinical assessment of each 12-lead ECG.

A full physical examination will be performed at the time points specified in the Schedule of Assessments in Appendix 9.

Body weight measurements will be performed at the time points specified in the Schedule of Assessments in Appendix 9.

The ECOG performance status will be assessed at the time points indicated in the Schedule of Assessments in Appendix 9. The ECOG assessment criteria are presented in Appendix 7.

An echo scan will be performed at screening and at day 43.

Assessments of the maximum grade for overall injection site pain, swelling, or redness on each LTX-315 administration day will be done and data will be collected in the eCRF. Injection site assessments will be conducted during the injection period (within 1 minute of injection completion) and approximately every 5 and no later than every 15 minutes or until injection site pain, swelling or redness resolves to ≤Grade 1 or baseline.

In all patients experiencing a clinically significant Grade ≥3, LTX-315-related, hypersensitivity/anaphylaxis AE, a hypersensitivity assessment should be performed. This assessment includes:

- Serum tryptase evaluation (to be assessed pre-dose for all patients and then ideally at 30 minutes and at 4 to 6 hours after a clinically significant hypersensitivity AE) to assess the mechanism and extent of mast-cell activation. Tryptase can be elevated with IgE and non-IgE mast-cell activation and is the gold standard serum test for the diagnosis of anaphylaxis. Local collection procedures and analysis should be used for this test.
- The Investigator should organize, if possible, with the local laboratory to have a basophil activation test (optional). A small amount of blood is needed for this test and should be done within 2 to 4 weeks after the occurrence. Local laboratory procedures and analysis should be used for this test. The basophil activation test detects surface

membrane activation markers induced by the drug and provides evidence of basophil activation through IgE and non-IgE mechanisms.

7.4 ANTIDRUG ANTIBODIES TO LTX-315

Antidrug antibodies (ADAs) specific to LTX-315 will be analyzed in an immunoassay. The assay will detect ADAs to LTX-315 in patients after repeated exposure to LTX-315. A serum blood sample will be collected from patients at screening and predosing on Days 1, 22, 43 (see also Appendix 9) for analysis of ADAs to LTX-315. For collection of samples, storage, and shipment, see separate instructions.

7.5 PHARMACOKINETIC ANALYSIS

7.5.1 PHARMACOKINETIC BLOOD SAMPLE COLLECTION AND PROCESSING

Blood samples (approximately 1 × 2 mL) for analysis of LTX-315 will be collected from 20 patients by venipuncture or cannulation at the times indicated in the Schedule of Assessments in Appendix 9.

Blood samples will be collected to measure the levels of LTX-315 in plasma at the following time points: at screening and pre-dosing on Days 1, 2, 3, 8, 22, 43, post dosing at Day 3 following the last injection, post-dosing samples will be collected at the following time points; 1, 5, 10 (±2 mins), 20, 30, 40, 50, 60, 90, 120 and 150 min (±10 mins). Procedures for collection, processing, and shipping of PK blood samples will be detailed in a separate document.

If a patient is not dosed Day 3 then the serial PKs required at Day 3 should be done on the next clinic visit on Day 8.

7.5.2 ANALYTICAL METHODOLOGY

Plasma concentrations of LTX-315 will be determined using a validated analytical procedure. Specifics of the analytical method will be provided in a separate document.

8.0

8.1 DETERMINATION OF SAMPLE SIZE

A sample size of 40 patients provides approximately 99.4% power to detect a response rate of 30% for the combination treatment (LTX-315 plus pembrolizumab) assuming pembrolizumab alone would deliver a response rate of 5% or less in this population utilizing a 1-sided test with an alpha spend of 0.05. No estimation for dropouts was included due to this being a study in an advanced cancer population where patients are expected to continue on therapy until progression.

8.2 ANALYSIS POPULATIONS

The following analysis populations will be used for this study:

Safety/Intent to treat (ITT) Population: all patients who receive at least one injection of LTX-315 and/or one infusion of pembrolizumab and have at least one post-dose safety evaluation.

Efficacy Population: all patients who received at least five out of seven injection days of LTX-315 (or fewer, if injections were stopped early due to complete necrosis of the injected lesion), at least one infusion of pembrolizumab and have at least one post-dose efficacy evaluation.

Pharmacokinetic Population: all patients who received at least one injection of LTX-315 and have evaluable PK data.

If populations are identical, analyses will not be duplicated.

8.3 GENERAL CONSIDERATIONS

Descriptive statistics and table/graphic representations will be produced. Statistics may include counts, percentages, rates, means, medians, ranges and variabilities, and any statistical analysis will be exploratory. Details will be provided in a separate Statistical Analysis Plan.

Due to the small sample size planned all data will be combined and there is no intention to report data separately by region.

8.4 EFFICACY ANALYSIS

No formal statistical testing is planned. Endpoint data will be summarized by group.

8.4.1 OBJECTIVE TUMOR RESPONSE AND DERIVED ENDPOINTS

Endpoints involving RECIST version 1.1 will be used as primary source for efficacy determination; iRECIST assessments will be considered exploratory for the purposes of this study.

The objective tumor response (CR, PR, SD, PD) will be determined separately according to RECIST version 1.1 and iRECIST. Both sets of objective tumor response parameters will be listed and summarized.

The best overall tumor response (from screening until disease progression) based on RECIST version 1.1 and iRECIST will also be listed and summarized (including 80% and 90% exact confidence intervals for proportions).

The following parameters will be derived per local Investigator assessment using RECIST version 1.1 and iRECIST, and will be listed.

- **ORR** – proportion of patients with an objective response (PR or CR).
- **CBR** – proportion of patients who respond to treatment, estimated as the proportion of patients who achieved SD, PR, or CR.

The following parameters will be derived per local Investigator assessment using iRECIST and RECIST 1.1 will be listed and summarized:

- **PFS** – time from baseline until objective tumor progression or death, whichever comes first. If a patient goes off-treatment without evidence of progression or death, the PFS will be the calculated difference from the date of baseline assessment until the last tumor assessment date. These patients will be censored in the analysis.
- **OS** - time from baseline until death. If a patient goes off-treatment without evidence of death, the OS will be the calculated difference from the date of baseline assessment until the last tumor assessment date. These patients will be censored in the analysis.
- **DoR** – among responders, DoR is defined as the interval between the date of the first observation of tumor response (CR or PR, whichever status is recorded first) and the first date that PD is objectively documented. The DoR will be censored at the date of the last imaging data of measured lesions if no confirmation of recurrence or progression is available.
- **TTR** – time between the first intratumoral injection and the first observation of tumor response (CR or PR).

Time-to-event data, such as PFS and DoR, will be exploratory and will be summarized using the Kaplan-Meier method.

The Sponsor may expand the number of patients based on an updated statistical plan if an efficacy signal is detected (see Section 3.1).

8.4.2 Local Effects in the Injected Lesion(s)

Injected lesions will be examined by visual inspection, photography (of superficial lesions only) and measurements of mean LPD at time points in Phase A, Phase B, and EOT as per the Schedule of Assessments (Appendix 9). Visual inspection will assess site swelling and redness, as well as monitoring injection site pain during and after injections. The size of injected lesions will be measured by physical calipers/ruler for superficial lesions, or ultrasound assessment with electronic caliper measurements for all other injected lesions, and response will be assessed as change from baseline and descriptive statistics will be summarized by visit. Photographs of superficial lesions will be used for visualization of any local response that may occur post LTX-315 administration. Biomarkers of immune response will also be summarized as applicable.

8.5 Safety Analysis

Safety variables include incidence of TEAEs, clinical laboratory test results, vital signs, ECG results, physical examination findings, blood pressure monitoring findings, ECOG performance status, and LTX-315 injection site assessments. All safety analyses will be based on the safety population. No formal statistical analysis of the safety data will be performed.

Summary tables will be provided for all AEs. The incidence of AEs, AEs ≥ NCI-CTCAE

Grade 3, related AEs, SAEs, and AEs leading to discontinuation of the study treatment will be presented by Medical Dictionary for Regulatory Activities SOC and preferred term. In addition, the incidence of AEs by severity will be presented by SOC and preferred term.

The AE summary tables will include counts of patients. Therefore, if a patient experiences more than one episode of a particular AE, the patient will be counted only once for that event at the highest severity. If a patient has more than one AE that is coded to the same preferred term, the patient will be counted only once for that preferred term. Similarly, if a patient has more than one AE within a SOC, the patient will be counted only once in that SOC.

Laboratory test variables will be summarized by visit using descriptive statistics (number of patients, mean, standard deviation, minimum, maximum, and mean change from baseline). Shift tables and categorical tables by NCI-CTCAE grade between baseline and post baseline time points will be presented by laboratory test. Laboratory tests without NCI-CTCAE grading will be presented in shift tables by relationship to the normal range (low, normal, high). Laboratory tests with categorical results that cannot be analyzed by change from baseline or shift table analysis will not be included in these summaries but will be listed. Data obtained from laboratory tests not required by the protocol will not be summarized but will be listed.

Descriptive statistics of vital signs, blood pressure monitoring, and ECG results at each visit will be presented, including changes from baseline. LVEF results for will be listed and summarized.

Physical examination findings, ECOG performance status, and LTX-315 injection site assessment findings will be listed for each patient.

8.6 ANTIDRUG ANTIBODY ANALYSIS

All available ADA data will be listed and summarized by visit.

8.7 PHARMACOKINETIC ANALYSIS

The plasma PK parameters of LTX-315 will be calculated using standard noncompartmental methods for 20 patients enrolled. PK analyses will not be performed in patients enrolled into the Expansion cohort.

The primary PK parameters are $AUC_{0-\infty}$, C_{max}, and $t_{1/2}$ for LTX-315. Other PK parameters will be regarded as secondary.

Renal function will be documented at baseline and at subsequent time points for each patient enrolled in the study. The effect of renal impairment on the PK of LTX-315 may be evaluated via population PK modeling and will be reported separately.

No inferential statistical analysis will be performed on the PK parameter data.

8.8 INTERIM ANALYSIS

No interim analyses are planned for this study. As this is an open-label study, data cuts may be performed for scientific presentation prior to the end of the study. As there are no formal statistics, no statistical penalty will be associated with these data cuts.

8.9 STOPPING RULES

After 20 patients have been enrolled and have had the opportunity to show a response (i.e reached the second post-baseline scan), if less than or equal to two patients are observed with PR

or CR, and all other patients showing disease progression in the efficacy population the study will be stopped for futility. The study may be continued at the sponsor's discretion if a substantial number of patients show sustained SD. Patients enrolled in the study will be eligible to continue on study treatment if there are signs of clinical benefit or response.

9.0

The Study Monitor and/or designee (e.g. CRO monitor) will visit each site prior to enrollment of the first patient and periodically during the study. In accordance with International Conference on Harmonization (ICH) guidelines, the monitor will compare the eCRF entries with the appropriate source documents. Additional review may include but is not limited to patient ICFs, documentation of patient recruitment and follow up, AEs, SAEs, and concomitant therapy: as well as records of study drug dispensing, compliance, and accountability. A copy of the drug dispensing log must be provided to the Sponsor on request.

10.0

10.1 GCP STATEMENT

It is the responsibility of both the Sponsor and the Investigator (s) to ensure that the study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with the ICH guidelines for GCP and applicable regulatory requirements.

10.2 INFORMED CONSENT

It is the responsibility of the Investigator or designee (if acceptable by national regulations) to obtain written informed consent from each patient prior to his/her participation in the study and the aims, methods, objectives, and potential hazards of the study have been explained to the patient in language that he/she can understand. The ICF should be signed and dated by the patient and by the Investigator or authorized designee who reviewed the ICF with the patient.

The original ICF must be retained by the Investigator as part of the patients study record and a copy of the signed ICF must be given to the patient. If new safety information results in significant changes in the risk benefit assessment, the ICF must be reviewed and updated appropriately. All study patients must be informed of the new information and provide written consent they wish to continue in the study. If there is any risk for potential conflict of interest for any person administering the informed consent process, another sub-investigator, designated by the principal investigator should administer informed consent.

11.0

In accordance with 21 CFR 312.62(c) and European Union Regulation 536/2014, the Investigator must retain all essential documents, including ICFs, source documents, copies of case report forms and drug accountability records for a minimum of 25 years following the completion or discontinuation of the study. The Investigator must consult with the Sponsor before discarding or destroying any essential study documents following study completion or discontinuation.

12.0

1. Zasloff M. Antimicrobial peptides in health and disease. *N Engl J Med*. 2002;347(15):1199–200.
2. Papo N, Shai Y. Host defense peptides as new weapons in cancer treatment. *Cell Mol Life Sci CMLS*. 2005;62(7–8):784–90.
3. Eike L-M, Yang N, Rekdal Ø, Sveinbjørnsson B. The oncolytic peptide LTX-315 induces cell death and DAMP release by mitochondria distortion in human melanoma cells. *Oncotarget*. 2015;6(33):34910–23.
4. Zhou H, Forveille S, Sauvat A, Sica V, Izzo V, Durand S, et al. The oncolytic peptide LTX-315 kills cancer cells through Bax/Bak-regulated mitochondrial membrane permeabilization. *Oncotarget*. 2015;6(29):26599–614.
5. Zhou H, Forveille S, Sauvat A, Yamazaki T, Senovilla L, Ma Y, et al. The oncolytic peptide LTX-315 triggers immunogenic cell death. *Cell Death Dis*. 2016;7:e2134.
6. Yamazaki T, Pitt JM, Vétizou M, Marabelle A, Flores C, Rekdal Ø, et al. The oncolytic peptide LTX-315 overcomes resistance of cancers to immunotherapy with CTLA4 checkpoint blockade. *Cell Death Differ*. 2016;23(6):1004–15.
7. Camilio KA, Berge G, Ravuri CS, Rekdal O, Sveinbjørnsson B. Complete regression and systemic protective immune responses obtained in B16 melanomas after treatment with LTX-315. *Cancer Immunol Immunother CII*. 2014;63(6):601–13.
8. Nestvold J, Wang M-Y, Camilio KA, Zinöcker S, Tjelle TE, Lindberg A, et al. Oncolytic peptide LTX-315 induces an immune-mediated abscopal effect in a rat sarcoma model. *Oncoimmunology*. 2017;6(8):e1338236.
9. Camilio KA, Wang M-Y, Mauseth B, Waagene S, Kvalheim G, Rekdal Ø, et al. Combining the oncolytic peptide LTX-315 with doxorubicin demonstrates therapeutic potential in a triple-negative breast cancer model. *Breast Cancer Res*. 2019;21(1):9.
10. Liao H-W, Garris C, Pfirschke C, Rickelt S, Arlauckas S, Siwicki M, et al. LTX-315 sequentially promotes lymphocyte-independent and lymphocyte-dependent antitumor effects. *Cell Stress*. 2019;3(11):348–60.
11. Jebsen NL, Apelseth TO, Haugland HK, Rekdal Ø, Patel H, Gjertsen BT, et al. Enhanced T-lymphocyte infiltration in a desmoid tumor of the thoracic wall in a young woman treated with intratumoral injections of the oncolytic peptide LTX-315: a case report. J Med Case Reports [Internet]. 2019 [cited 13 Oct 2020];13. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6556957/
12. Gajewski TF. The Next Hurdle in Cancer Immunotherapy: Overcoming the Non-T-Cell-Inflamed Tumor Microenvironment. *Semin Oncol*. 2015;42(4):663–71.
13. Sharma P, Allison JP. The future of immune checkpoint therapy. *Science*. 2015;348(6230):56–61.
14. Adams S, Schmid P, Rugo HS, Winer EP, Loirat D, Awada A, et al. Pembrolizumab monotherapy for previously treated metastatic triple-negative breast cancer: cohort A of the phase II KEYNOTE-086 study. *Ann Oncol*. 2019;30(3):397–404.

15. Rytlewski J, Deng S, Xie T, Davis C, Robins H, Yusko E, et al. Model to improve specificity for identification of clinically relevant expanded T cells in peripheral blood. *PLoS ONE* [Internet]. 2019 [cited 2020 Sep 27];14(3). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6417670/

16. Teng MWL, Ngiow SF, Ribas A, Smyth MJ. Classifying Cancers Based on T-cell Infiltration and PD-L1. *Cancer Res*. 2015;75(11):2139–45.

17. Lytix Biopharma AS. LTX-315 - Investigator's Brochure (Version 13.0). 2020.

18. Seymour L, Bogaerts J, Perrone A, Ford R, Schwartz LH, Mandrekar S, et al. iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. *Lancet Oncol*. 2017;18(3):e143–52.

19. Goldmacher GV, Khilnani AD, Andtbacka RHI, Luke JJ, Hodi FS, Marabelle A, et al. Response Criteria for Intratumoral Immunotherapy in Solid Tumors: itRECIST. *J Clin Oncol*. 2020;38(23):2667–76.

20. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer*. 2009;45(2):228–47.

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Definitions

An adverse event is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. This includes the following:

- Any clinically significant worsening of a pre-existing condition, or new condition that develops after treatment initiation.
- Emergence of a new pathogen associated with a clinical event during therapy at a site other than the initial site of infection will be considered to be an AE.
- Any recurrence of a pre-existing condition.
- An AE occurring from overdose of a sponsor study treatment whether accidental or intentional (i.e., a dose higher than that prescribed by a health care professional for clinical reasons).
- An AE occurring from abuse of a sponsor study treatment (i.e., use for nonclinical reasons).
- An AE that has been associated with the discontinuation of the use of a sponsor study treatment.

Note: A procedure is not an AE, but the reason for a procedure may be an AE.

A pre-existing condition is a clinical condition (including a condition being treated) that is diagnosed before the patient signs the informed consent form and that is documented as part of the patient's medical history. All preexisting conditions and pertinent clinical history should be documented on the Medical History case report form. Medical history should be graded where applicable per the CTCAE v5.0.

The questions concerning whether the condition existed before the start of the active phase of the study and whether it has increased in severity and/or frequency will be used to determine whether an event is a treatment-emergent AE. An AE is considered to be treatment emergent if:

(1) it is not present when the active phase of the study begins and is not a chronic condition that is part of the patient's medical history, or

(2) it is present at the start of the active phase of the study or as part of the patient's medical history, but the severity or frequency increases during the active phase. The active phase of the study begins at the time of the first dose of the study treatment. The active phase of the study ends at the last Follow-up Visit.

Reporting of Adverse Events

At each visit, the investigator, or delegate, will determine whether or not any AEs have occurred. Nonleading questions such as "How are you feeling today?" or "Have you had any health concerns since your last visit?" should be used to elicit the patient to report any possible AEs.

In addition, any self-reported symptom data will be reviewed and assessed by the investigator.

If any AEs have occurred, they will be recorded in the AE section of the eCRF and in the patient's source documents. If known, the diagnosis should be recorded, in preference to listing the individual signs and symptoms.

Adverse event reporting begins from the time of informed consent and ends 90 days after the last dose of study treatment. Note: In the situation where a patient discontinues treatment on study and begins a new anticancer therapy prior to the end of the 90 day follow up period, safety data collection will end upon the initiation of the new anticancer therapy.

Disease Progression and Safety Reporting

Adverse events will be followed from consent throughout phases A and B, and the 90-day FU period. If a patient starts a new anticancer therapy during the study period, then the safety data collection will stop upon initiation of that therapy. Details of the new anticancer therapy and start date will be collected in the CRF.

Progression of the malignancy under study (including signs and symptoms of progression) should not be reported as an SAE unless the outcome is fatal within the safety reporting period. If the malignancy has a fatal outcome during the study or within the safety reporting period, then the event leading to death must be recorded as a SAE with CTCAE Grade 5 (see Section on Severity Assessment). Hospitalization due to signs and symptoms of disease progression should not be reported as an SAE.

Assessment of Severity

The investigator will assess intensity for each AE and SAE reported during the study. The assessment will be based on the investigator's clinical judgment using NCI-CTCAE Version 5.0. For AEs that cannot be graded per the NCI-CTCAE criteria the following general grading scale should be applied:

- **Grade 1/Mild**: Usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.
- **Grade 2/Moderate**: Usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the patient.
- **Grade 3/Severe**: Interrupts usual activities of daily living, significantly affects clinical status, or may require intensive therapeutic intervention.
- **Grade 4/Life threatening or disabling**: An event that poses an immediate risk of death from the reaction as it occurred.
- **Grade 5/Death**: The event resulted in death.

Relationship to Study Treatment

The investigator will make a determination of the relationship of the AE to each study treatment (LTX-315 and pembrolizumab) using a 4-category system according to the following guidelines:

- **Not related**: When the AE is definitely caused by the patient's clinical state/conditions, or any study procedures.
- **Unlikely related**: When the temporal association between the AE and the drug is such that the drug is not likely to have any reasonable association with the AE.
- **Possibly related**: When the AE follows a reasonable temporal sequence from the time of drug administration but could have been produced by the patient's clinical

state/conditions or the study procedures.

- **Related**: When the AE follows a reasonable temporal sequence from administration of the drug, abates upon discontinuation of the drug, follows a known or hypothesized cause-effect relationship, and (if appropriate) reappears when the drug is reintroduced.

Action Taken for Adverse Events

The investigator or designee will record the action taken for the AE in the eCRF. Actions taken will include:

- **Dose increased/initiate**: The medication schedule was modified by addition, either by changing the frequency, strength, or amount.
- **Dose not changed**: The medication schedule was not changed.
- **Dose reduced**: The medication schedule was modified by subtraction, either by changing the frequency, strength, or amount.
- **Drug interrupted**: The medication schedule was modified by temporarily terminating the prescribed regimen of medication.
- **Drug withdrawn**: The medication schedule was modified through termination of the prescribed regimen of medication.
- **Not applicable**.
- **Unknown**.

Follow-up of Adverse Events

All SAEs related to study treatment that are ongoing at the time of discontinuation, or that develop prior to the last Follow-up Visit, will be followed up to determine the final outcome.

All other AEs and SAEs must be followed until resolution, stabilization to a chronic state, return to baseline grade, or until the patient discontinues from the FU period of the study.

Additional site visits to follow up injection site reactions and AE/SAEs can be performed if deemed necessary by the Investigator.

Adverse Drug Reactions

All noxious and unintended responses to an investigational medicinal product (IMP; i.e., where a causal relationship between an IMP and an AE is at least a reasonable possibility) related to any dose should be considered adverse drug reactions.

For marketed medicinal products, a response to a drug which is noxious and unintended, and which occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of diseases or for modification of physiological function, is to be considered an adverse drug reaction.

An unexpected adverse drug reaction is defined as an adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure for an unapproved IMP).

Serious Adverse Events

An SAE is any AE occurring at any dose that meets 1 or more of the following criteria:

- Results in death.
- Is life threatening (see below).
- Requires patient hospitalization or prolongation of an existing hospitalization (see below).
- Results in a persistent or significant disability or incapacity (see below).
- Results in a congenital anomaly or birth defect.
- Results in an important medical event (see below).

Important medical events that may not result in death, be life threatening, or require hospitalization may be considered SAEs when, based on appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not require hospitalization, or development of drug dependency or drug abuse.

A ***life-threatening adverse event*** is any AE that places the patient at immediate risk of death from the event as it occurred. A life-threatening event does not include an event that might have caused death had it occurred in a more severe form but that did not create an immediate risk of death as it actually occurred. For example, drug-induced hepatitis that resolved without evidence of hepatic failure would not be considered life threatening, even though drug-induced hepatitis of a more severe nature can be fatal. Hospitalization is to be considered only if there was as least an overnight admission.

Hospitalization or prolongation of hospitalization is a criterion for considering an AE to be serious. In the absence of an AE, the participating investigator should not report hospitalization or prolongation of hospitalization. This is the case in the following situations:

- Hospitalization or prolongation of hospitalization is needed for a procedure required by the protocol. Day or night survey visits for biopsy or surgery required by the protocol are not considered serious.
- Hospitalization or prolongation of hospitalization is part of a routine procedure followed by the study center (e.g., stent removal after surgery). This should be recorded in the study file.
- Hospitalization for survey visits, annual physicals, or for social reasons fall in the same category.

In addition, a hospitalization planned before the start of the study for a pre-existing condition that has not worsened does not constitute an SAE (e.g., elective hospitalization for a total knee replacement due to a pre-existing condition of osteoarthritis of the knee that has not worsened during the study).

Disability is defined as a substantial disruption in a person's ability to conduct normal life functions (i.e., the AE resulted in a significant, persistent, or permanent change, impairment, damage, or disruption in the patient's bodily function/structure, physical activities, or quality of life).

Medical and scientific judgment should be exercised in deciding whether a case is serious in

those situations where important medical events may not be immediately life threatening or result in death, hospitalization, disability, or incapacity.

Pregnancy will not be considered an SAE/AE but is expected to be reported accordingly and followed until birth. Any complications of pregnancy that meets criteria for seriousness should be reported as a SAE (like miscarriage, still birth, any anomaly of the newborn).

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The following clinical laboratory analytes will be assessed:

Chemistry (Fasted):	**Hematology (CBC):**
Albumin	Hematocrit
ALP	Hemoglobin
ALT	MCH
AST	MCHC
BUN	MCV
Calcium	Platelet count
Chloride	Red blood cell (RBC) count
Cholesterol	White blood cell (WBC) count
Creatinine	WBC differential
GGT	(% and absolute):
Glucose	Basophils
LDH	Eosinophils
Potassium	Lymphocytes
Sodium	Monocytes
Total bilirubin	Neutrophils
Total protein	
Uric acid or urea	**For Women Only:**
	Urine pregnancy test [b]
Complete Urinalysis[c]:	Follicle-stimulating hormone [d]
Color and appearance	
pH and specific gravity	**Serology[e]:**
Bilirubin	Hepatitis B: HBsAg, HBsAb, HBcAb
Glucose	Hepatitis C antibody
Ketones	HIV-1 and HIV-2 antibodies and p24 antigen
Leukocytes	
Nitrite	**Coagulation:**
Occult blood	Activated partial thromboplastin time
Protein	International normalized ratio
Microscopic (including RBCs and WBCs)	Prothrombin time
Hypersensitivity Assessments[a]:	
Serum tryptase	
Basophil activation test (if possible)	

Abbreviations: ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = blood urea nitrogen; CBC = complete blood count; GGT = gamma glutamyl transferase; HBcAb = hepatitis B core antibody; HBsAb = hepatitis B surface antibody; HBsAg = hepatitis B surface antigen; HIV = human immunodeficiency virus; LDH = lactate dehydrogenase; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; RBC = red blood cell; WBC = white blood cell.

[a] Serum tryptase to be assessed at pre-dose for all patients. Serum tryptase and basophil activation test then to be assessed only in the event of clinically significant Grade >3 LTX-315-related hypersensitivity/anaphylaxis adverse events; see Section 7.3.6.9.

[b] Only in females of childbearing potential. Urine pregnancy test results may be confirmed with a serum pregnancy test, if required.

[c] Only performed at screening and Day 1.

[d] To confirm postmenopausal status, if appropriate, in postmenopausal females only (refer to Appendix 8).

[e] Any positive serology tests should be followed up with a viral load test.

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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 Council for International Organizations of Medical Sciences International Ethical Guidelines.
- Applicable ICH GCP Guidelines.
- Applicable laws and regulations.

The protocol, protocol amendments, ICF, Investigator's Brochure, and other relevant documents (e.g., advertisements) must be submitted to an IRB and reviewed and approved by the IRB before the study is initiated.

- Any amendments to the protocol will require IRB 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 written summaries of the status of the study to the IRB annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB.
- Notifying the IRB of serious adverse events or other significant safety findings as required by IRB procedures.
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 Code of Federal Regulations (CFR), ICH guidelines, the IRB, European regulation 536/2014 for clinical studies, and all other applicable local and national laws and regulations.

Finances and Insurance

Financing and insurance will be addressed in a separate agreement.

Informed Consent

Prior to starting participation in the study, each patient will be provided with a study specific ICF giving details of the study treatment, procedures, and potential risks of the study. Patients will be instructed that they are free to obtain further information from the investigator (or designee) and that their participation is voluntary, and they are free to withdraw from the study at any time.

Patients will be given an opportunity to ask questions about the study prior to providing consent for participation.

Patients or their legally authorized representative will be required to sign a statement of informed consent that meets the requirements of national and local regulations, ICH guidelines, and the IRB or study center, where applicable. The patient will be given a copy of the signed ICF, and the original will be maintained with the patient's records.

Patients must be reconsented to the most current version of the ICF(s) during their participation

in the study.

Data Subjects Data Protection

The Sponsor and designees affirm and uphold the principle of the patient's right to protection against invasion of privacy.

Patients will be assigned a unique identifier (data pseudonymization) and will not be identified by name in eCRFs, study databases, study-related forms, study reports, or any related publications. The key to identify patient is stored in a secure manner by each study site. Patient and Investigator personal data will be treated in compliance with all applicable laws and regulations. In particular, the sponsor has agreed to comply with the regulation established by the French data protection authority. In the event the study protocol, study report, or study data are included in a public registry, all identifiable information from individual patients or investigators will be redacted according to applicable laws and regulations.

To comply with ICH GCP and to verify compliance with this protocol, the Sponsor requires the Investigator to permit its monitor or designee's monitor, representatives from any regulatory authority (eg, Food and Drug Administration), the Sponsor's designated auditors, and the appropriate IRBs to review the patient's original medical records (source data or documents). Copies of any patient source documents that are provided to the Sponsor must have certain personally identifiable information removed (ie, patient name, address, and other identifier fields not collected on the patient's eCRF).

The collection of data from European patients in the study constitutes a processing of personal data within the meaning of Article 4 of the General Data Protection Regulation (GDPR) (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016. In particular, the sponsor has agreed to comply with the regulation established by the French data protection authority.

At an organizational level, to meet European requirements the sponsor has appointed a certified Data Protection Officer (DPO) in charge of ensuring compliance with data protection regulations. The DPO oversees the conduct of the required Data Protection Impact Assessment (DPIA) to assess and protect rights and freedoms of data subjects regarding processing operations. All data processors (e.g. sites and vendors) are bound by a data protection agreement (DPA). If personal data are transferred to a third country within the meaning of the GDPR, the DPA contains the Standard Contractual Clauses established by the European Commission. The DPO is also in charge of any data subject request and can be contacted directly by the data subject through the email provided in the information sheet.

At a technical level, personal data are stored in a secured environment, encrypted, and partitioned from other data. Access to personal data is limited to relevant staff through access policies. All person accessing data are bound by a confidential clause or professional secrecy

The patient must 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 patient. The patient must also be informed that his/her medical records may be examined by sponsor or CRO auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB members, and by inspectors from regulatory authorities.

The data retention period is determined according to applicable laws and regulations. This period

is divided in two periods. Firstly, the data storage period, when data are stored on an active basis. This period covers the conduct of the trial. Secondly at the end of the study, the archiving period, when data will be archived for legal purposes with restricted access. Finally, at the end of the archiving period, data will be deleted. When data are stored by a vendor, the DPA provides the return or deletion of data at the end of the service. The data retention period is provided to the data subject in the information sheet.

Committees Structure

A Data Safety Committee consisting of the principal investigators, Sponsor representatives, and CRO representatives will periodically review safety and efficacy data related to patients enrolled in the study.

The Data Safety Committee shall meet when approximately half of the patients are enrolled and when enrollment is completed or each time any of the following events occur (whichever is shorter):

- AE of Grade 4 or 5 considered by the Investigator to be related or possibly related to LTX-315 or to pembrolizumab.

Disclosure

All information provided regarding the study, as well as all information collected and/or documented during the course of the study, will be regarded as confidential. The Investigator (or designee) agrees not to disclose such information in any way without prior written permission from the sponsor.

Data Quality Assurance

The following data quality steps will be implemented:

- All patient data relating to the study will be recorded on eCRFs unless directly transmitted to the Sponsor or designee electronically (e.g., laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct by electronically signing the eCRF.
- The Investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.
- The Investigator must permit study-related monitoring, audits, IRB review, and regulatory agency inspections and provide direct access to source data documents.
- The Sponsor or designee is responsible for the data management of this study including quality checking of the data. Predefined, agreed risks, monitoring thresholds, quality tolerance thresholds, controls, and mitigation plans will be documented in a risk management register. Additional details of quality checking to be performed on the data may be included in a Data Management Plan.
- Sponsor assigned CRAs 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; that the safety and rights of patients are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable

regulatory requirements. On-site or remote monitoring visits will be conducted at study sites at periodic intervals

- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the investigator in accordance with 21 CFR 312.62(c) and EU Regulation 536/2014 unless national regulations, local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

Investigator Documentation Responsibilities

All individual, patient-specific study data will be entered into a 21 CFR Part 11-compliant electronic data capture (EDC) system on an eCRF in a timely fashion. All data generated from external sources (e.g., central laboratory, pharmacokinetics) and transmitted to the Sponsor or designee electronically will be integrated with the patient's eCRF data in accordance with the Data Management Plan.

An eCRF must be completed for each patient who signs an ICF and undergoes any prescreening or screening procedures, according to the eCRF completion instructions. The Sponsor, or CRO, will review the supporting source documentation against the data entered into the eCRFs to verify the accuracy of the electronic data. The Investigator will ensure that corrections are made to the eCRFs and that data queries are resolved in a timely fashion by the study staff.

The Investigator will sign and date the eCRF via the EDC system's electronic signature procedure. These signatures will indicate that the Investigator reviewed and approved the data on the eCRF, the data queries, and the site notifications.

Publications

If, on completion of the study, the data warrant publication, the Investigator may publish the results in recognized (refereed) scientific journals subject to the provisions of the clinical study agreement (CSA). Unless otherwise specified in the CSA, the following process shall occur:

The Institution and Investigator shall not publish or present data from an individual study center until the complete multicenter study has been presented in full or for 2 years after the termination of the multicenter study, whichever occurs first. Subsequent publications must refer to the multicenter findings. Thereafter, if the Investigator expects to participate in the publication of data generated from this site, the Institution and Investigator shall submit reports, abstracts, manuscripts, and/or other presentation materials to the Sponsor for review before submission for publication or presentation. The Sponsor shall have 60 days to respond with any requested revisions, including (without limitation) the deletion of confidential information. The Investigator shall act in good faith upon requested revisions, except the Investigator shall delete any confidential information from such proposed publications. The Investigator shall delay submission of such publication or presentation materials for up to an additional 90 days in order to have a patent application(s) filed.

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The following list is not exhaustive. When in doubt, contact the sponsor's Medical Monitor.

Immunosuppressive agents

Glucocorticoids,* e.g.:

- Dexamethasone
- Prednisone
- Cortisol. Calcineurin inhibitors, e.g.:
- Tacrolimus
- Cyclosporins.

Anti-proliferative and anti-metabolic drugs, e.g.:

- Sirolimus
- Everolimus
- Azathioprine
- Mycophenolate Mofentil (CellCept®)
- Glatiramer acetate
- Methotrexate.

Monoclonal Antibodies, e.g.:

- Alefacept
- Basiliximab
- Tumor necrosis factor binding proteins.

* See exceptions for steroid concomitant therapy in Section 6.1.

Immunostimulants

- Levamisole
- Thalidomide
- Interferons.

Note: Bisphosphonates or denosumab are allowed.

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Antitumor activity measured as shrinkage of the tumors is a primary endpoint in this study. The RECIST version 1.1 will be used to evaluate tumor response.[20]

See the Schedule of Assessments in Appendix 9 for the schedule for full tumor assessment by CT/ MRI. Note: Patients with known or suspected bone or brain lesions are required to have bone and brain scans done for all scheduled or unscheduled time points during the study, starting at baseline. Bone lesions should be followed using nuclear medicine bone scans, with the exception of lytic bone lesions that should be followed by CT/MRI.

Baseline Documentation of "Target" and "Nontarget" Lesions

All measurable lesions up to a maximum of five lesions total, and up to two per organ representative of all involved organs, should be identified as ***target lesions*** and recorded and measured at baseline.

Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, and be those that lend themselves to reproducible repeated measurements (either by imaging techniques or clinically). The injected lesion should not be considered a target lesion.

A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for *all target lesions* will be calculated and reported as the baseline sum. The baseline sum will be used as reference by which to characterize the objective tumor.

All other lesions (or sites of disease) should be identified as ***nontarget lesions*** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

Definition of Measurable Lesions

Lesions that can be accurately measured in at least one dimension with longest diameter ≥10 mm with CT scan. (CT scan slice thickness no greater than 5 mm), Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in **short** axis when assessed by CT scan (CT scan slice thickness no greater than 5 mm). At baseline and in follow up, only the short axis will be measured and followed.

Definition of Nonmeasurable Lesions

All other lesions, including small lesions (longest diameter <10 mm with CT scan), and lesions that qualify for, but were not selected as target lesions. Note that bone lesions should be followed as non-target lesions. The exception is lytic bone lesions which should be followed per RECIST criteria by CT/MRI. Lytic bone lesions may meet RECIST criteria to be selected as target lesion, if they meet the size criteria for target lesions.

Evaluation of Target Lesions

CR:	Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to < 10 mm

PR:	At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum
PD:	At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum recorded since the treatment started, or the appearance of one or more new lesions. In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5mm from the smallest sum.
SD:	Neither sufficient shrinkage (compared to baseline) to qualify for PR nor sufficient increase (taking as reference the smallest sum diameters while on study) to qualify for PD

Evaluation of Nontarget Lesions

Complete Response:	Disappearance of all nontarget lesions, and normalization of tumor marker level. All lymph nodes must be non-pathological in size (< 10 mm short axis)
Incomplete Response/ SD:	Persistence of one or more nontarget lesion(s) or/and maintenance of tumor marker level above the normal limits
PD:	Appearance of one or more new lesions and/or unequivocal progression of existing nontarget lesions (1)

(1) Although a clear progression of "nontarget" lesions only is exceptional, in such circumstances, the opinion of the treating physician should prevail.

*Note on assignment of stable disease for target or nontarget lesions: SD should be assigned from BL until either PR or CR occurs. Once a response other than stable disease is assigned, that response should be documented until the patient meets requirements for progressive disease

Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for PD the smallest sum recorded since the treatment started). In general, the patient's best response assignment will depend on the achievement of both measurement and confirmation criteria. Confirmation of PR and CR is required per RECIST 1.1 criteria in non-randomized trials**. Confirmation of PD is required per iRECIST**

* When required, confirmatory scans should be obtained approximately 4-6 weeks after the initial scan where PR, CR or PD is assessed. See SOA Appendix 9 for additional details

When no imaging/measurement is done at all at a particular time point, the patient is not evaluable (NE) at that time point. If only a subset of lesion measurements is made at an assessment, usually the case is also considered NE at that time point, unless a convincing argument can be made that the contribution of the individual missing lesion(s) would not change the assigned time point response.

Table 3 – Best overall response when confirmation of CR and PR required.

Overall response First time point	Overall response Subsequent time point	BEST overall response
CR	CR	CR
CR	PR	SD, PD or PR[a]
CR	SD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	PD	SD provided minimum criteria for duration met, otherwise,PD
CR	NE	SD provided minimum criteria for SD duration met, otherwise NE
PR	CR	PR
PR	PR	PR
PR	SD	SD
PR	PD	SD provided minimum criteria for duration met, otherwise, PD
PR	NE	SD provided minimum criteria for duration met, otherwise NE
NE	NE	NE

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

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having "symptomatic deterioration." Every effort should be made to document the objective progression even after discontinuation of treatment.

In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) to confirm the complete response status.

Duration of Overall Response

The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever status is recorded first) until the first date that recurrence or PD is objectively documented, taking as reference for PD the smallest measurements recorded since the treatment started.

Duration of Stable Disease

SD is measured from the start of the treatment until the criteria for disease progression are met, taking as reference the smallest measurements recorded since the treatment started.

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Response evaluations of noninjected lesions will be determined according to iRECIST.[18]

See the Schedule of Assessments in Appendix 9 for the schedule for full tumor assessment by CT/MRI.

Assessment Guidelines

Please note the following:

The same diagnostic method, including use of contrast, when applicable, must be used throughout the study to evaluate a lesion.

CT or MRI: Contrast-enhanced CT (as clinically indicated) of the brain, head and neck, chest, abdomen, and pelvis is recommended. The slice thickness is according to local procedures, but a minimum size of a measurable baseline lesion should be twice the slice thickness, with a minimum lesion size of 10 mm when the slice thickness is 5 mm. MRI is acceptable, but when used, the technical specification of the scanning sequences should be optimized for the evaluation of the type and site of disease and lesions must be measured in the same anatomic plane by use of the same imaging examinations. Whenever possible the same scanner (and preferably the same radiologist) should be used.

Guidelines for Evaluation of Disease

Measurable and nonmeasurable definitions Measurable lesion:

A non-nodal lesion that can be accurately measured in at least one dimension (longest dimension) of:

- ≥10 mm with MRI or CT when the scan slice thickness is no greater than 5 mm. If the slice thickness is greater than 5 mm, the minimum size of a measurable lesion must be at least double the slice thickness (e.g., if the slice thickness is 10 mm, a measurable lesion must be ≥20 mm).
- ≥10 mm caliper/ruler measurement by clinical exam or medical photography.
- Additionally, lymph nodes can be considered if pathologically enlarged and measurable.
- ≥15 mm in the short axis when assessed by CT or MRI (slice thickness recommended to be no more than 5 mm). At baseline and follow-up, only the short axis will be measured.

Nonmeasurable lesion:

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

Measurable disease:

The presence of at least one measurable lesion.

Nonmeasurable only disease:

The presence of only nonmeasurable lesions. Documentation of Target and Nontarget Lesions

- The injected lesions shall not be used as target lesions.
- Lymph nodes that have a short axis of <10 mm are considered nonpathological and should not be recorded or followed.
- Pathological lymph nodes with <15 mm but ≥10 mm short axis are considered nonmeasurable.
- Pathological lymph nodes with ≥15 mm short axis are considered measurable and can be selected as target lesion; however, lymph nodes should not be selected as target lesions when other suitable target lesions are available.
- Measurable lesions up to a maximum of two lesions per organ and five lesions in total, representative of all involved organs, should be identified as target lesions, and recorded and measured at baseline. These lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). Note: Cystic lesions thought to represent cystic metastases should not be selected as target lesions when other suitable target lesions are available. Note: Measurable lesions that have been previously irradiated and have not been shown to be progressing following irradiation should not be considered as target lesions.
- All other lesions (or sites of disease) should be identified as nontarget and should also be recorded at baseline. Nontarget lesions will be grouped by organ. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

At each subsequent full tumor assessment by CT/MRI (Appendix 9), the changes in iRECIST measurement of the selected target tumor(s) will be noted in source data and in the eCRF.

Response Criteria

Evaluation of target lesions:

Definitions for assessment of response for target lesion(s) are as follows:

- Immune Complete Response (iCR): Disappearance of all target lesions. Any pathological lymph nodes must be <10 mm in the short axis.
- Immune Partial Response (iPR): At least a 30% decrease in the sum of the diameters of target lesions, taking as a reference the baseline sum of the diameters (e.g., percent change from baseline).
- Immune Stable Disease (iSD): Neither sufficient shrinkage to qualify for iPR nor sufficient increase to qualify for progressive disease (confirmed or unconfirmed).
- Immune Confirmed/Unconfirmed Progressive Disease (iCPD/iUPD): At least a 20% increase in the sum of the diameters of target lesions, taking as a reference the smallest sum of diameters recorded since the treatment started (e.g., percent change from nadir, where nadir is defined as the smallest sum of diameters recorded since treatment start).

In addition, the sum must have an absolute increase from nadir of 5 mm.

- Not Applicable (NA): No target lesions at baseline.
- Not evaluable (NE): Cannot be classified by 1 of the 5 preceding definitions.

Note:

- If lymph nodes are documented as target lesions, the short axis is added into the sum of the diameters (e.g., sum of diameters is the sum of the longest diameters for non-nodal lesions and the short axis for nodal lesions). When lymph nodes decrease to nonpathological size (short axis <10 mm) they should still have a measurement reported in order not to overstate progression.
- If, at a given assessment time point, all target lesions identified at baseline are not assessed, sum of the diameters cannot be calculated for purposes of assessing iCR, iPR, or iSD, or for use as the nadir for future assessments. However, the sum of the diameters of the assessed lesions and the percent change from nadir should be calculated to ensure that progression has not been documented. If an assessment of iPD cannot be made, the response assessment should be NE.
- All lesions (nodal and non-nodal) should have their measurements recorded even when very small (e.g., 2 mm). If lesions are present but too small to measure, 5 mm should be recorded and should contribute to the sum of the diameters, unless it is likely that the lesion has disappeared, in which case 0 mm should be reported.
- If a lesion disappears and reappears at a subsequent time point, it should continue to be measured. The response at the time when the lesion reappears will depend upon the status of the other lesions. For example, if the disease had reached an iCR status then iCPD/iUPD would be documented at the time of reappearance. However, if the response status was iPR or iSD, the diameter of the reappearing lesion should be added to the remaining diameters and response determined based on percent change from baseline and percent change from nadir.

Evaluation of nontarget lesions:

Definitions for assessment of response for nontarget lesions are as follows:

- iCR: The disappearance of all nontarget lesions. All lymph nodes identified as a site of disease at baseline must be nonpathological (e.g., <10 mm short axis).
- Non-iCR/Non-iCPD/Non-iUPD: The persistence of one or more nontarget lesion(s) or lymph nodes identified as a site of disease at baseline ≥10 mm short axis.
- iCPD/iUPD: Unequivocal progression of existing nontarget lesions.
- NA: No nontarget lesions at baseline.
- NE: Cannot be classified by one of the 4 preceding definitions.

Note:

- In the presence of measurable disease, progression on the basis of solely nontarget disease requires substantial worsening such that even in the presence of iSD or iPR in target disease, the overall tumor burden has increased sufficiently to merit

discontinuation of therapy.

- Sites of nontarget lesions, which are not assessed at a particular time point based on the assessment schedule, should be excluded from the response determination (e.g., nontarget response does not have to be "Not Evaluable").

Confirming progression – iRECIST:

Unlike RECIST 1.1, iRECIST requires the confirmation of progression and uses the terms iUPD and iCPD. Confirmatory scans should be performed at least approximately 4 – 6 weeks, after iUPD.

iCPD is confirmed if further increase in tumor burden, compared to the last assessment, is seen as evidenced by one or more of the following:

- Continued increase in tumor burden (from iUPD) where RECIST 1.1 definitions of progression had been met (from nadir) in target, nontarget disease, or new lesions

  Progression in target disease worsens with an increase of at least 5 mm in the absolute value of the sum.

  Continued unequivocal progression in nontarget disease with an increase in tumor burden.

  Increase in size of previously identified new lesion(s) (an increase of at least 5 mm in the absolute value of the sum of those considered to be target new lesions) or additional new lesions.

- RECIST 1.1 criteria are met in lesion types (target or nontarget or new lesions) where progression was not previously identified, including the appearance of additional new lesions.

If iUPD is not confirmed at the next assessment, then the appropriate response will be assigned (iUPD if the criteria are still met, but no worsening, iSD, iPR, or iCR if those criteria are met compared to baseline). As can be seen in the table below, the prior documentation of iUPD does not preclude assigning iCR, iPR, or iSD in subsequent time point assessments or as best overall response providing that iCPD is not documented at the next assessment after iUPD.

New lesions:

New malignancies denoting disease progression must be unequivocal. Lesions identified in follow-up in an anatomical location not scanned at baseline are considered new lesions. Any equivocal new lesions should continue to be followed. Treatment can continue at the discretion of the investigator until the next scheduled assessment. If at the next assessment the new lesion is considered to be unequivocal, progression should be documented.

New lesions may either meet the criteria of NLT or NLNT to drive iUPD (or iCPD). However, the measurements of target lesions should not be included in the sum of measures of original target lesions identified at baseline. Rather, these measurements will be collected on a separate table in the eCRF.

Progressive disease is confirmed in the New Lesion category if the next imaging assessment, conducted at least 4 weeks (but not more than 8 weeks) after iUPD confirms further progression from iUPD with either an increase of at least 5 mm in the absolute value of the sum of NLT or an

increase (but not necessarily unequivocal increase) in the size of NLNT lesions or the appearance of additional new lesions.

Evaluation of iRECIST Overall Response

The table below presents the overall response at an individual time point for all possible combinations of tumor responses in target and nontarget lesions, with or without the appearance of new lesions for subjects with measurable disease at baseline.

Target Lesions	**Nontarget Lesions[a]**	**New Lesions[a]**	**Time point Response**	
			No Prior	**Prior iUPD[c]**
iCR	iCR	No	iCR	iCR
iCR	Non-iCR/ Non-iUPD	No	iPR	iPR
iPR	Non-iCR/ Non-iUPD	No	iPR	iPR
iSD	Non-iCR/ Non-iUPD	No	iSD	iSD
iUPD with no change OR decrease from last time point	iUPD with no change OR decrease from last time point	Yes	NA	NLs confirm iCPD if NLs were previously identified and increase in size (≥5 mm in SOM for NLT or any increase for NLNT) or number. If no change in NLs (size or number) from last time point, remains iUPD
iSD	iUPD	No	iUPD	Remains iUPD unless iCPD confirmed based in further increase in size of NT disease (need not meet RECIST 1.1 criteria for unequivocal PD)
iUPD	Non-iCR/ Non-iUPD	No	iUPD	Remains iUPD unless iCPD confirmed based on: further increase in SOM of at least 5 mm, otherwise remains iUPD
iUPD	iUPD	No	iUPD	Remains iUPD unless iCPD confirmed based on further increase in: previously identified T lesion iUPD SOM ≥5 mm and/or NT lesion iUPD (prior assessment - need not be unequivocal PD)
iUPD	iUPD	Yes	iUPD	Remains iUPD unless iCPD confirmed based on further increase in: previously identified T lesion iUPD ≥5 mm and/or previously identified NT lesion iUPD (need not be unequivocal) and/or size or number of new lesions previously identified

Target Lesions	Nontarget Lesions[a]	New Lesions[a]	Time point Response	
			No Prior	Prior iUPD[c]
Non-iUPD/PD	Non-iUPD/PD	Yes	iUPD	Remains iUPD unless iCPD confirmed based on increase in size or number of new lesions previously identified

Abbreviations: iCPD = immune confirmed progressive disease; iCR = immune complete response; iPR = immune partial response; iSD = immune stable disease; iUPD = immune unconfirmed progressive disease; NL = new lesion; NLNT = new lesion-nontarget; NLT = new lesion-target; PD = progressive disease; RECIST = Response Evaluation Criteria in Solid Tumors; SOM = sum of measures; T = target.

[a] Using RECIST 1.1 principles. If no pseudoprogression occurs, RECIST 1.1 and iRECIST categories for CR, PR, and SD would be the same.

[b] In any lesion category.

[c] Previously identified in assessment immediately prior to this time point.

Note:

- Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having "symptomatic deterioration." Objective response status is determined by evaluations of disease burden.
- In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of iCR depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) to confirm the iCR.

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

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Acceptable methods of contraception include:

- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal)[1]
- progestogen-only hormonal contraception associated with inhibition of ovulation[1] (oral, injectable, implantable[2])
- intrauterine device (IUD)[2]
- intrauterine hormone-releasing system (IUS)[2]
- bilateral tubal occlusion[2]
- vasectomized partner[2,3]
- sexual abstinence[4]

[1] Hormonal contraception may be susceptible to interaction with the study treatment, which may reduce the efficacy of the contraception method.

[2] Contraception methods in this context are considered to have low user dependency.

[3] Vasectomized partner provided that partner is the sole sexual partner of the woman of childbearing potential trial participant and that the vasectomized partner has received medical assessment of the surgical success.

[4] Sexual abstinence is considered a high effective method if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred usual lifestyle of the patient.

DEFINITIONS

Woman of childbearing potential (WOCBP)

A woman is considered fertile following menarche and until becoming postmenopausal unless permanently sterile (see below).

Women in the following categories are not considered WOCBP

1. Premenarchal.
2. Premenopausal female with one of the following:
   - Documented hysterectomy,
   - Documented bilateral oophorectomy,
   - Documented bilateral salpingectomy.
3. Postmenopausal female:
   - A postmenopausal state is defined by amenorrhea for at least 12 months without an alternative medical cause. A high follicle-stimulating hormone (FSH) level within the postmenopausal range may be used to confirm a postmenopausal state in females who are not using hormonal contraception or hormone replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, a single FSH

measurement is insufficient.

- Females on HRT and whose menopausal status is in doubt will be required to use one of the nonhormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status prior to study enrollment.

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		Screen	Treatment											Follow- up Visits (30, 60, and 90 [±3] days after EOT)
			Phase A								Phase B		EOT /ET[s]	
	Week		1			2	3	4	5	7	Every 6 weeks (±7 days) from Day 43 until discontinuation[a]	Every 9 weeks (±7 days) from Day 43 until discontinuation		
Assessment	Day	-28 to -1	1 (+1)	2 (±1)	3 (±1)	8 (±1)	15 (±1)	22 (±1)	29 (±1)	43 (±1)				
Informed consent		X												
Inclusion/exclusion		X												
Demographics		X												
Medical and surgical		X												
Height, BMI		X												
Echo[b]		X								X				
HIV and hepatitis B/C screening		X												
Serum or urine pregnancy test[c]		X	X					X			X			X
Follicle-stimulating hormone[d]		X												
Efficacy														
CT/MRI[e]		X								X		X	X	
Tumor examination of injected lesions[f]		X	P	P	P	P	P	P	P	P	X		X	
Biopsy of injected lesion		X								X				
Biopsy of noninjected lesion (if safe)		X								X				

		Screen	Treatment											
			Phase A							Phase B			EOT /ET[s]	Follow- up Visits (30, 60, and 90 [±3] days after EOT)
	Week		1			2	3	4	5	7	Every 6 weeks (±7 days) from Day 43 until discontinuation[a]	Every 9 weeks (±7 days) from Day 43 until discontinuation		
Assessment	Day	-28 to -1	1 (+1)	2 (±1)	3 (±1)	8 (±1)	15 (±1)	22 (±1)	29 (±1)	43 (±1)				
Immunological blood sample			P				X			X[g]				
Safety														
Adverse events[h]		X	X	X	X	X	X	X	X	X	X		X	X
Prior/concomitant medications		X	X	X	X	X	X	X	X	X	X		X	X
Clinical laboratory assessments[i]		X	X			X			X		X		X	X
Blood pressure monitoring[j]			X	X	X	X	X	X	X					X
Vital signs[k]		X	X	X	X	X	X	X	X	X	X		X	X
12-lead ECG[l]		X	X[m]	X[m]	X[m]	X[m]	X	X	X	X			X	
Physical exam		X	P	P	P	P	P	P	P	P	P		X	X
Body weight		X	P						P	P	P		X	X
ECOG performance status		X	X			X							X	X
LTX-315 injection site assessment[n]			X	X	X	X	X	X	X					
Other														
Premedication[o]			X	X	X	X	X	X	X					
LTX-315 administration			X	X	X	X	X	X	X					
Pembrolizumab administration			X[p]					X[p]		X[a]	X[a]			

		Screen	Treatment										EOT /ET[s]	Follow- up Visits (30, 60, and 90 [±3] days after EOT)
			Phase A								Phase B			
	Week		1			2	3	4	5	7	Every 6 weeks (±7 days) from Day 43 until discontinuation[a]	Every 9 weeks (±7 days) from Day 43 until discontinuation		
Assessment	Day	-28 to -1	1 (+1)	2 (±1)	3 (±1)	8 (±1)	15 (±1)	22 (±1)	29 (±1)	43 (±1)				
PK sampling[q]		X	P	P	X	P		P		P				
ADA sampling[r]		X	P					P		P				

Abbreviations: ADA = antidrug antibody; BMI = body mass index; CT = computed tomography; ECG = electrocardiogram; Echo = echocardiogram; ECOG = Eastern Cooperative Oncology Group; EOT = end of treatment; ET = early termination; HIV = human immunodeficiency virus; MRI = magnetic resonance imaging; P = pre-dose; PK = pharmacokinetic.

[a] Pembrolizumab 400 mg will be administered every 6 weeks (±7 days) for a maximum of 24 months' total therapy until disease progression, consent withdrawal, or unacceptable toxicity occurrence, starting Day 43. If agreed with sponsor's Medical Monitor, treatment may be continued after progression if there is evidence of clinical benefit.

[b] Echo scans will be performed at screening and at Day 43, additional scans may be scheduled at the discretion of the investigator.

[c] Female patients of childbearing potential. Urine pregnancy test is to be performed in the first instance, with a serum pregnancy test used if needed to confirm the result.

[d] To confirm postmenopausal status, if appropriate, in postmenopausal females only (refer to Appendix 8).

[e] Tumor assessment to be done during screening, Day 43, and repeated every 9 weeks post D43 until the patient fulfils criteria for discontinuation from the study (including disease progression). The screening tumor assessment is to be done within 28 days prior to the first treatment. A±7-day window is allowed for all other tumor assessments. RECIST tumor assessment will be performed using CT/ MRI. Any patient with suspected brain metastases should have a CT/MRI of the brain. Any patient with stable brain metastases should ensure their CT/MRIs include brain monitoring. Any patient with suspected bone lesions should have a bone scan. Patients with bone lesions at baseline should have bone scans done in addition to CT/MRI at every restaging scan. **Confirmation of PR and CR (per RECIST 1.1) and of PD per iRECIST) is required.** Confirmatory scans must be obtained approximately 4-6 weeks from the initial restaging scan where the PR, CR or PD was initially assessed. After confirmatory scans, the subject should revert back to their original scan schedule. If the last scan was within 6 weeks of EOT, the scan should not be repeated. Patients who progress per RECIST should remain on treatment until progression is confirmed per scans. If progression is not confirmed then patients will remain on treatment until confirmed disease progression occurs.

[f] Examinations of the injected lesion(s), to include visual inspection of lesion, measurement of lesion for mean LPD using photography of superficial lesions including a size standard or caliper/ruler for scale, or ultrasound assessment with electronic calipers for non superficial lesions. Procedures (including measurement of mean LPD) should be completed at every visit from screening through D43, and then at every other pembrolizumab administration visit in Phase B (every 12 weeks in phase B starting on the second pembrolizumab visit at Day 127.

[g] Immunological blood sample to be taken concurrently with the biopsy.

[h] safety data collection starts upon consent through the 90-day FU period. If a patient discontinues from study treatment and initiates a new anti-cancer therapy prior to completion of the 90-day FU period, then safety data collection ends upon initiation of that therapy.

[i] Clinical chemistry, hematology, and coagulation (see Appendix 2 for details). Urinalysis only at screening and Day 1.

[j] Blood pressure to be monitored just prior to injection and then at approximately every 5 minutes (no later than 15 minutes between measurements) until 30 minutes after the last injection of each day, using an automated cuff system.

[k] Vital signs include supine blood pressure, supine pulse rate, oral temperature, and respiratory rate and will be performed at the start of the study visit, where applicable.

[l] ECGs shall be performed in triplicate before the start of LTX-315 injections. ECGs are not required on dosing days in Phase A where LTX-315 administration does not occur

ECGs are also required prior to pembrolizumab administration.
[m] ECGs shall also be performed in triplicate at least 30 minutes after last LTX-315 injection and prior to pembrolizumab infusion.
[n] Injection site pain will be evaluated after the first injection and, assessed at approximately every 5 minute intervals (no later than 15 minutes between assessments) until pain resolves to ≤Grade 1 or baseline.

[redacted]

[p] Pembrolizumab 200 mg will be administered on Days 1 and 22.
[q] PK sampling time points on Day 3 will be as follows: Pre-dose and relative to the last injection 1, 5, 10 (±2 mins), 20, 30, 40, 50, 60-, 90-, 120-, and 150-minutes post-injection (±10 mins). If a patient misses LTX-315 dosing for any reason on Day 3 then the serial PKs required at Day 3 should be done on the next clinic visit on Day 8. Note: PK sampling is only indicated for the first 25 patients on study. PK analysis is not applicable to patients enrolled under protocol version 6, or any subsequent protocol amendments
[r] ADA samples should be collected prior to LTX-315 dosing on Days 1, 22 and prior to pembrolizumab infusion at Day 43.
[s]. EOT/ET visit should be performed at the time of study treatment discontinuation.