"An open-label phase II single-centre study investigating the safety and efficacy of LTX-315 and adoptive T-cell therapy in patients with advanced/metastatic soft tissue sarcoma"

Clinical Trial Protocol

Version 3.0, 31st March 2020

NCT: 03725605

Study Protocol No.	C17-315-04
Title	An open-label phase II single-centre study investigating the safety and efficacy of LTX-315 and adoptive T-cell therapy in patients with advanced/metastatic soft tissue sarcoma
EudraCT No.	2017-002877-20
Study Phase	Phase II
Sponsor	Lytix Biopharma AS, Hoffsveien 4, NO-0275 Oslo, Norway
Date	31 March 2020
Version	Final (version 3.0)

The following Amendment(s) and Administrative change have been made to this protocol since the date of preparation:

Amendment no.	Date of Amendment
#1	05-Sep-2018
#2	31-Mar-2020

Confidentiality Statement

Medical Monitor	[redacted]
Notification of serious adverse events	[redacted]

SPONSOR SIGNATURE PAGE

Study number: C17-315-04

Study Title: An open-label phase II single-centre study investigating the safety and efficacy of LTX-315 and adoptive T-cell therapy in patients with advanced/metastatic soft tissue sarcoma

Sponsor Project Manager: [redacted]

Signature: [redacted]

Date: April 2, 2020

Sponsor : [redacted]

Signature: [redacted]

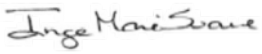
Date: April 2, 2020

PRINCIPAL INVESTIGATOR SIGNATURE PAGE

Study number: C17-315-04

Study Title: An open-label phase II single-centre study investigating the safety and efficacy of LTX-315 and adoptive T-cell therapy in patients with advanced/metastatic soft tissue sarcoma

Principal Investigator: Inge Marie Svane, Professor, MD, PhD

Signature: 

Date: April 2, 2020

CONTRACT RESEARCH ORGANISATIONS

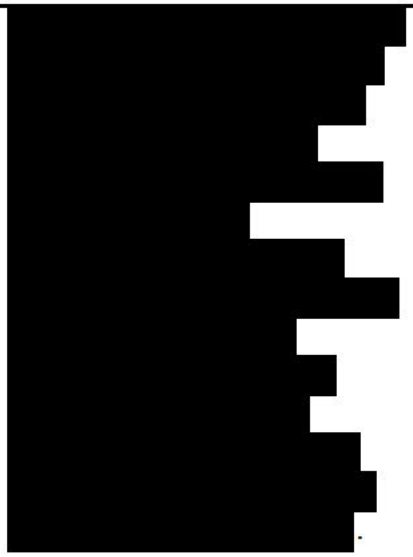
Clinical Project Manager:	[redacted]
Study Statistician:	[redacted]
Central Laboratories:	[redacted]

Summary of Key changes

Section (Number & Title)	Description of Change	Brief Rationale
Synopsis(1 Inclusion criteria) 5.2 Inclusion criteria	Add patients with stable disease.	Patients with STS progress rapidly and estimated median survival time is 10 months. By including patients with metastatic disease the patients will receive the TIL product at an earlier time point after progression. This change may also enhance the recruitment rate of patients to the study.
Synopsis(1 Inclusion criteria) 5.2 Inclusion criteria	Change of lesion size from ≥ [redacted] cm to ≥ [redacted] cm.	A lesion with longest diameter (LD) of [redacted] cm may include sufficient tumor tissue and TILs to expand T cells for ACT. This change may also enhance the recruitment rate of patients to the study.
Synopsis(1 Inclusion criteria Inclusion criteria 5.2 7.4.1.1 Biopsies 7.4.1.4 Identification of tumour-antigen specific T-cells 9.4.1.1 Objective response rate Appendix IV	Change from irRC to RECIST only.	RECIST v.1.1 is the standard way to evaluate response. irRC is developed for assessment of response upon treatment with immune checkpoint inhibitors which may result in pseudoprogression.
Synopsis (secondary endpoint 1 and 2) 3.2 Secondary endpoint	Change in CD3+ T-cell and CD3+CD8+ T cell density in non-injected tumour tissues from Baseline (Step 1, Week 1, Day 1) to EoT (Step 2, Week 7) if the patient has a feasible bystander lesion and accept it to be biopsied. Total number of CD3+CD8+ T cells and % CD3+CD8+T cells of total CD3+ T cells in final	Endpoint changes to better describe assessment of objective 2.

Section (Number & Title)	Description of Change	Brief Rationale
	TIL infusion product .	
Synopsis 7.4.1.1. Biopsies	If a non-injected lesion regress >30% and is feasible for biopsy, an optional biopsy of this lesion will allow a comparison with the TIL product and blood before and after LTX-315.	Add a biopsy of non-injected lesion if a clinical response (>30% regression) in one non-injected lesion. This will allow for analysis of whether T-cell clones generated after LTX-315 treatment has infiltrated non-injected lesions.
Synopsis 4.2 Study rationale 6.3 Treatments Administrated	LTX-315 will be administered on dosing days (i.e. Step 1, Days [redacted] for patients that were in stable disease upon enrollment in the study).	Treatment schedule will be changed from day [redacted] to day [redacted] for patients in PD at enrollment. The rationale for this is that better abscopal and immune effects have been observed in patients treated with intensive treatment in week 1 (in the C12-315-03 study). Patients in SD at enrollment may have two additional dosing days at day [redacted] and [redacted] to enable induction of a stronger immune response induced by LTX-315. see rationale for including patients with SD.
Synopsis 4.1 Study design	Step 1 (W1 to W3-5).	Patients that are in stable disease at enrollment will have an additional 2 weeks before resection compared to patients that have PD. A longer time period before resection will enable increased number of TILs for expansion of TIL product.
4.1 Study design 8.6 Source data	Treatment period (including screening): up to 26 weeks.	
Synopsis 3.2 Secondary endpoints 4.1 Overall study design 5.2 Inclusion criteria 7.3 End of treatment	24 month to 15 month follow-up.	10 months is mean for overall survival for the patient group. Thus, 15 months is a HR of 1.5 and for a single arm study this should be more than enough for drawing adequate conclusions.

Section (Number & Title)	Description of Change	Brief Rationale
and follow-up 8.3 Data analysis and reporting 8.6 Source data 9.4.1.1.Objective response rate 9.4.1.2 Clinical benefit rate 9.4.1.3 Progression free survival 9.5 Exploratory Analyses 9.6 Other Assessments		
2.5 Clinical experience	The LTX-315 injection schedule comprised either of injection of multiple lesions for [redacted] dosing days per week for weeks [redacted]	[redacted]

Section (Number & Title)	Description of Change	Brief Rationale
		
2.5.3 Clinical Experience	In this ongoing C17-314-04 study, three subjects have been exposed to LTX-315 (as of March 2020). No SAE and no AEs leading to discontinuation were reported. A total of 43 non-serious AEs were reported of which 9 were considered related to LTX-315. Eight were CTCAE grade 1 and one was grade 2 (injection site pain). The LTX-315 related AEs were: 4 injection site pain, 1 injection site redness, 1 itching, 1 diarrhoea, 1 fever and 1 stomach pain.	New safety data on combination.
6.3 Treatments administrated Table 5	Updated table only by deleting the two first rows (< [redacted] and so on)-to reduce number of changes.	LPD for lesion < [redacted] cm is not relevant to include.
Synopsis 9.7 Sample size determination	6 patients who have completed Step 1.	Number of patients changed from 15 to 6 because of low recruitment rate.

Section (Number & Title)	Description of Change	Brief Rationale
Synopsis (Incl. criteria No 11) 5.2 Inclusion criteria no 11 5.4 Recruitment and screening	Re-screening is allowed once on a case by case basis as judged by the investigator .	Allow to re-screen a patient once to increase recruitment rates.

TABLE OF CONTENTS

ABBREVIATIONS

Abbreviation	Term
ADL	Activities of Daily Living
AE	Adverse Event
ANC	Absolute Neutrophil Count
ALT	Alanine Transferase
AST	Aspartate Transferase
CBR	Clinical Benefit Rate
CCIT	Center for Cancer Immune Therapy
CR	Complete response
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Event
DCIS	Ductal Carcinoma in situ
DLT	Dose Limiting Toxicity
EBV	Epstein-Barr Virus
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EDTA	Ethylene Diamine Tetra-acetic Acid
ELISPOT	Enzyme-Linked ImmunoSpot assay
EoT	End of Treatment
ESMO	European Society for Medical Oncology
ET	Early Termination
FAS	Full Analysis Set
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HLA	Human Leukocyte Antigen
HTLV	Human T-cell Leukemia-lymphoma Virus
IB	Investigator's Brochure
ICH	International Conference of Harmonisation
IEC	Independent Ethics Committee
IFNγ	Interferon Gamma
Ig	Immunoglobulin

IL-2	Interleukin 2
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
INR	International Normalised Ratio
IRB	Institutional Review Board
IUD	Intrauterine Device
LCIS	Lobular Carcinoma in situ
LD	Longest Diameter
LPD	Longest Perpendicular Diameters
LTX-315	Potential new anticancer drug developed by Lytix Biopharma AS
MAP	Mean Arterial Pressure
MedDRA	Medical Dictionary for Regulatory Activities
MHC	Major Histocompatibility Complex
MRI	Magnetic Resonance Imaging
NCI	National Cancer Institute
NED	No Evidence of Disease
NOAEL	No-Observed-Adverse-Effect-Level
ORR	Objective Response Rate
PBMC	Peripheral Blood Mononuclear Cells
PD	Progressive Disease
PET	Positron Emission Tomography
PFS	Progression Free Survival
PR	Partial Response
PP	Per Protocol
PT	Preferred Term
PT	Prothrombin Time
RECIST	Response Evaluation Criteria In Solid Tumours
SaaS	Software as a Service
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Stable Disease
SmPC	Summary of Product Characteristics
SOC	System Organ Class
SPD	Sum of Products of Largest Perpendicular Diameters
SUSAR	Suspected Unexpected Serious Adverse Reaction

TEAE	Treatment Emergent Adverse Event
TIL	Tumour infiltrating Lymphocytes
TNFα	Tumour Necrosis Factor Alpha
WHO	World Health Organization

1 SYNOPSIS

Name of Sponsor: Lytix Biopharma AS
Name of Investigational Medicinal Products (IMPs): LTX-315 Acetate 20 mg peptide/vial Tumour infiltrating Lymphocytes (TILs) Sendoxan® 200, 500, 1000 mg/vial Fludara® 25 or 50 mg/vial Proleukin® 18 million IU/vial
Name of Active Ingredient: LTX-315 Acetate TILs Cyclophosphamide Fludarabine phosphate Interleukin 2 (IL-2)
Title of Study: An open-label phase II single-centre study investigating the safety and efficacy of LTX-315 and adoptive T-cell therapy in patients with advanced/metastatic soft tissue sarcoma
Protocol Number: C17-315-04
Investigator and Study Centre: Inge Marie Svane, Professor, MD, PhD Herlev Hospital, Center for Cancer Immune Therapy (CCIT), Herlev Ringvej 75, DK-2730 Herlev, Denmark
Phase of Development: Phase II
Primary Objectives: • To determine the ability of LTX-315 to induce T-cell infiltration prior to TIL expansion in advanced/metastatic soft tissue sarcoma • To determine the safety of LTX-315 as part of adoptive T-cell therapy in advanced/metastatic soft tissue sarcoma **Secondary Objectives:** • To determine the ability of LTX-315 as part of adoptive T-cell therapy to induce T-cell infiltration in advanced/metastatic soft tissue sarcoma • To assess the ability to expand CD8+ T-cells from tumour tissues

- To assess the anti-tumour effect of LTX-315 as part of adoptive T-cell therapy in advanced/metastatic soft tissue sarcoma

Exploratory Objectives:

- To assess tumour-antigen specificity
- To investigate and characterise immune status and nature of anti-tumour immune responses

Primary Endpoints:

- Change in total T-cell level in tumour tissues from Baseline (Step 1, Week 1, Day 1) to end of Step 1 (Step 1, Week 3)
- Adverse events (AE) related to LTX-315 or to the combination of LTX-315 and adoptive T-cell therapy from Baseline (Step 1, Week 1, Day 1) to end of treatment (EoT) (Step 2, Week 7)

Secondary Endpoints:

- Change in CD3+ T-cell and CD3+CD8+ T-cell density in non-injected tumour tissues from Baseline (Step 1, Week 1, Day 1) to EoT (Step 2, Week 7) if the patient has a feasible bystander lesion and accept it to be biopsied.
- Total number of CD3+CD8+ T cells and % CD3+CD8+T cells of total CD3+ T cells in final TIL infusion product
- The anti-tumour effect assessed by:
    - Objective Response Rate (ORR) defined as proportion of patients who have acheived immune-related complete response (irCR) or immune-related partial response (irPR) at EoT (Step 2, Week 7) and up to 15 months after EoT
    - Clinical Benefit Rate (CBR) defined as proportion of patients who have achieved irCR, irPR or immune-related stable disease (irSD) at EoT (Step 2, Week 7) and up to 15 months after EoT
    - Progression free survival (PFS) evaluated by time from Baseline until immune-related progressive disease (irPD) or death up to 15 months after EoT

Exploratory Endpoint:

- Identification of tumour-antigen specific T-cells in tumour tissue and peripheral blood mononuclear cells (PBMCs) by major histocompatibility complex (MHC) multimer–based screens, enzyme-linked immuno-spot assay (ELISPOT) and flowcytometry analysis of cytokines including interferon gamma (IFNγ) and tumour necrosis factor alpha (TNFα)
- Changes in immunological parameters from Baseline (Step 1, Week 1, Day 1) to 15 months after EoT

Study Design:

This is a phase II, open-label, single-centre study in patients with advanced/metastatic soft tissue sarcoma and tumours accessible for injection, assessing LTX-315 ability to induce increased T-cell infiltration and facilitate T-cell expansion for TIL based adoptive cell therapy in soft tissue sarcoma patients.

All patients will have at least one tumour lesion available for injection with LTX-315 and 1

measurable lesion for disease assessment by the use of modified Response Evaluation Criteria in Sold Tumours (RECIST version 1.1) An available lesion is a lesion that can be injected and is not a non-injected bystander lesion.

Course of Treatment:

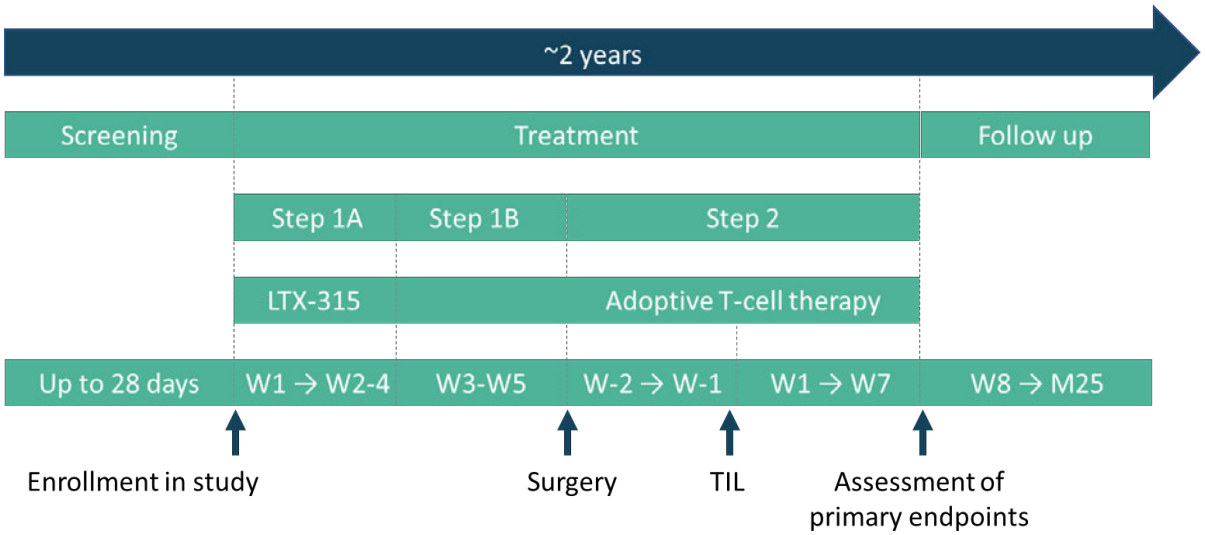
The course of treatment consists of 2 steps (Step 1 and Step 2) followed by clinical controls and evaluation-scans as follow-up.

Screening (28 days prior to Step 1): informed consent, check of eligibility and inclusion.

Step 1 (W1 to W3-5): re-check of inclusion, baseline tumour biopsy, LTX-315 injections and surgical removal of the LTX-315 injected index tumour lesion followed by production and growth of TILs in the laboratory.

Step 2 (W-2 to W-1, W1 to W7): check of eligibility for Step 2, treatment during hospitalisation with chemotherapy, TIL infusion and IL-2 administration, safety and efficacy of LTX-315/TIL treatment.

Follow-up: safety and efficacy of LTX-315/TIL treatment for a follow-up period of 15 months.



The course of treatment can vary between patients depending on the time period from start of LTX-315 treatment to surgical removal of injected index tumour lesion (Step 1), and the time period from resection to adoptive T-cell therapy (Step 2). The time period before resection will depend upon the patients condition and will vary from 3-5 weeks after start of LTX-315 treatment. In most cases (e.g. TILs undergoing massive expansion and infusion without cryopreservation of intermediate products). Patients that have confirmed PD upon enrollment in the study will receive TIL treatment approximately 4-6 weeks after surgery with a maximum of 10 weeks. Patients that have confirmed SD upon enrollment in the study will receive TIL treatment after confirmed PD during the study.

Summary of LTX-315 injection schedule and method:

The intra-tumoural injections of LTX-315 may be ultrasound-guided, based on clinical judgement. However, ultrasound guidance is not mandatory. Injection and delivery of LTX-315 should be aimed at the periphery of the lesion.

- LTX-315 will be administered on dosing day [redacted]
- The index tumour lesion (metastasis or primary tumour) for injection must have a minimum longest diameter (LD) of [redacted] cm as measured by ultrasound or calliper
- The LTX-315 dose will be [redacted] mg ([redacted] mg/ml concentration) at each injection time point. [redacted]
- On all dosing days, LTX-315 will be injected into the tumour lesion at a minimum of 5 minutes apart and only if no CTCAE (Common Terminology Criteria for Adverse Events) grade 3 or 4 or clinically significant or emergent (specifically allergy-like) AEs (considered related to LTX-315 by the Investigator) have occurred or are emerging in the observation period between injections
- The LTX-315 injection schedule can be flexible (variable number of injections per lesion per day) depending on clinical factors such as local injection site symptoms and reactive or inflammatory changes or necrosis
- LTX-315 will be injected into the periphery of the tumour close to the tumour border
- Successive injections in the same lesion should be given in an area adjacent to the previous injection site in a clockwise manner (try avoiding previously injected areas). It is important to not necrotize the tumour completely and number of injections should be judged accordingly

Summary of adoptive T-cell therapy:

- Step 1: After end of LTX-315 dosing, index tumour tissue (metastasis or primary tumour) of minimum 1 cm^3 is surgically removed from the patient. After stimulation, the expanded TILs are washed, pooled and stored until re-infused intravenously back in to the patient (Step 2)
- Step 2: 7 days of lymphodepleting chemotherapy with cyclophosphamide on Day -7 to Day -6 and fludarabine phosphate on Day -5 to Day -1 is given to the patient before TIL infusion. Following TIL infusion, low-dose IL-2 will be administered subcutaneously for up to 14 days

Rational for Drugs Used in TIL based Adoptive Cell Therapy

Activating cytokines (the signal molecules IL-2, IL-7, IL-15, IL-21 etc.) need to be available for the tumour specific T-cells to sustain an immunological response against tumour tissue. A large number of "irrelevant" T-cells will decrease the availability of these cytokines for the relevant T-cells through competition. Thus, a high number of tumour specific T-cells with a high specificity as well as a reduction of irrelevant T-cells and regulatory T-cells are needed to create an environment that facilitates the T-cell mediated anti-tumour response.

Combination chemotherapy with 2 days treatment with cyclophosphamide and 5 days with fludarabine phosphate will create such an environment. The combination has been chosen

based on earlier studies where it was shown safe and effective.

Number of Patients Planned:

6 patients who have completed Step 1.

Patient Selection:

Inclusion criteria:

1. (Histologically) Confirmed any patients with advanced or metastatic soft tissue sarcoma with stable disease or has progressed after a minimum of 1 systemic treatment of advanced/metastatic disease. Patient will have received at least 1 approved standard of care treatment for advanced/metastatic disease or be deemed unsuitable for such treatment by their treating physician
2. At least 1 index tumour lesion accessible for injection with a longest diameter (LD) of ≥ [redacted] cm which is planned for LTX-315 injection
3. At least 1 measurable (target) non-injected tumour lesion that can be used to assess response by Computed Tomography (CT)-scan (as per RECIST)
4. A life expectancy of at least 3 months
5. Willing to undergo repeat tumour biopsy and/or tumour resection procedures
6. Between 18 and 75 years of age
7. An Eastern Cooperative Oncology Group (ECOG) performance status: 0 – 1
8. Meet the following blood laboratory requirements:
    a. Absolute neutrophil count (ANC) ≥ 1.5 x 10^9/L
    b. Platelet count ≥ 75 x 10^9/L
    c. Haemoglobin ≥ 6.0 mmol/L after blood transfusion if needed
    d. Prothrombin time / International Normalised Ratio (PT/INR) within the institution's normal range
    e. Aspartate Transferase (AST) and Alanine Transferase (ALT) ≤ 2.5 x upper normal level
    f. Creatinine ≤ 1.5 x upper normal level
9. Willing and able to comply with the protocol and agree to return to the hospital for follow-up visits and examinations up to 15 months after EoT
10. Fully informed about the study and have signed the informed consent form
11. Re-screening is allowed once on a case by case basis as judged by the investigator

Exclusion criteria:

1. A history of clinical significant active systemic autoimmune disease requiring anti-inflammatory or immunosuppressive therapy within the last 3 months. Patients with history of autoimmune thyroiditis are eligible provided the patient requires only thyroid hormone replacement therapy and disease has been stable for ≥ 1 year
2. Any other malignancy active within the previous 5 years except for carcinoma in situ of the cervix, ductal carcinoma in situ (DCIS) or lobular carcinoma in situ (LCIS) of the

breast

3. Received an investigational drug within 4 weeks prior to any study drug administration, or are scheduled to receive 1 during the study participation
4. Received external radiotherapy or cytotoxic chemotherapy within 4 weeks prior to LTX-315 administration, or have not recovered from AEs (to ≤ CTCAE grade 1) due to agents administered more than 4 weeks earlier. Palliative radiotherapy to non-target and lesions planned for LTX-315 injection within 4 weeks of LTX-315 administration is allowed
5. Currently taking any agent with a known effect on the immune system. Patients are allowed to be on a stable dose of corticosteroids (up to 10 mg daily prednisolone or equivalent) for at least 2 weeks prior to LTX-315 administration
6. Any other serious illness or medical condition such as, but not limited to:
    a. Uncontrolled infection or infection requiring antibiotics
    b. Uncontrolled cardiac failure: Classification III or IV (New York Heart Association)
    c. Uncontrolled systemic and gastrointestinal inflammatory conditions
    d. Bone marrow dysplasia
7. A known history of positive tests for HIV/AIDS, syphilis, human T-cell leukemia-lymphoma virus (HTLV), active Epstein-Barr, hepatitis B or C (based on serology)
8. A history of cerebrovascular or cardiac disorders and would be at particular risk of sequelae following a short hypotensive episode
9. If of child-bearing potential, not willing to use an effective form of contraception such as hormonal birth control, intrauterine device or double barrier method from screening visit and until 3 months after last study treatment (any of the agents)
10. Breastfeeding and/or have a positive pregnancy test during screening
11. Donate sperm from LTX-315 dosing until 3 months after last study treatment (any of the agents)
12. Patients with pregnant or partner of child-bearing potential not willing to use contraception from LTX-315 administration until 3 months after last study treatment (any of the agents)
13. Expected to need any other anticancer therapy or immunotherapy to be initiated during the treatment period
14. Clinically active or unstable metastases in the central nerveous system as assessed by the treating physician
15. Any known hypersensitivity to any of the excipients in the non-IMPs including the required allergic prophylactic medications ([redacted])
16. Any known or suspected hypersensitivity to any of the IMPs

Methods and Procedures:

Biopsies of LTX-315 injected lesion:

All mandatory biopsies must include up to 3 (18 gauge) core biopsies.

Biopsies of the index tumour lesion planned for injection with LTX-315 will be done at:

- Baseline (pre-LTX-315 treatment) to assess T-cell infiltration (including CD8+)
- At the time of and as part of the excision of the injected lesion
- 6 weeks after TIL infusion if the whole tumour is not resected

A part of the resected lesion will be used for TIL production and another part for verification of diagnosis. The remaining part of the excised tumour will be stored for future research on immune responses.

If an additional lesion is present and feasible for biopsy, this will be used as a bystander lesion. The optional lesion will not be injected and will allow a comparison (by biopsy) with the injected lesion.

If a non-injected lesion regress >30% and is feasible for biopsy, an optional biopsy of this lesion will allow a comparison with the TIL product and blood before and after LTX-315 treatment.

In this scenario a patient would have 3 tumour lesions:

- One for LTX-315 injection
- A second to be followed by CT-scan for RECIST v 1.1 response assessment (measurable, non-injected lesion). Optional biopsy of this lesion if it regress >30% after treatment
- A third that is a bystander lesion (no LTX-315 injection) but available to biopsy to compare with biopsy of the injected lesion

Identification of somatic mutations in the tumour:

A PBMC sample will be collected as a source for normal cells for comparison of the DNA and RNA sequence in normal cells to the sequence in the tumour tissue. The sample will be collected at screening and will enable identification of somatic mutations in the tumour.

Immune monitoring:

Change in immune biomarkers (in both peripheral blood and tumour samples) 2 weeks after LTX-315 treatment in patients with transdermally accessible tumours will be assessed. PBMC will be isolated from blood samples at screening, before and after T-cell therapy.

In order to investigate and characterise the immune status of the patients and the nature of anti-tumour immune responses, the following immune biomarkers in biopsies and PBMCs may include but is not limited to CD3, CD4, CD8, CD11, CD14, CD19, CD27, CD56, CD68, Ki67 and Granzyme B. T-cell receptor sequencing may be performed to monitor changes in the T-cell repertoire induced by treatment.

Identification of tumour-antigen specific T-cells:

To generate a clinical effective T-cell response it is important that LTX-315 generates tumour-antigen specific T-cells. To identify sarcoma tumour-antigens in LTX-315 patients, next-generation sequencing and epitope prediction may be used. Tumour tissue DNA and RNA will be compared with data from matched normal tissue (PBMC) in order to identify tumour-unique (“somatic”) mutations. Patients will have their human leukocyte antigen (HLA) typed, and algorithms will then be used to predict which tumour-epitopes the patient’s tumour cells will express. Peptides corresponding to predicted tumour-epitopes will be synthesized and used to identify tumour-antigen specific T-cells in freshly expanded TILs as well as in PBMCs using MHC multimer–based screens. The effector functions of the tumour-antigen

specific T-cells may be analysed by ELISPOT which will reveal whether cytokines such as IFNγ and or TNFα is produced by peptide stimulation. Tumour reactive T-cells may be identified in PBMC and expanded TILs by co-culturing with autologous/ allogenic tumour cells followed by flowcytometry analysis of IFNγ and TNFα. Details are described in a separate immunology protocol.

Radiology Assessments:

CT-scans, Positron Emission Tomography (PET)-CT-scans or Magnetic Resonance Imaging (MRI) will be performed within 28 days prior to Day 1 (Step 1), W-2 prior to TIL infusion, at EoT, Week 13 after TIL infusion and thereafter every 3rd months up to 15 months.

IMPs, Doses and Modes of Administration:

LTX-315:

LTX-315 is provided in vials containing 20 mg of peptide – linear nonapeptide with a free amino group at the N-terminus and an amidated C-terminus – as a lyophilised powder for injection. Prior to use it should be reconstituted with [redacted] ml sterile saline solution 0.9% giving a concentration of [redacted] mg/ml. In total [redacted] mg LTX-315 will be administered per injection time point per dosing.

TILs:

Sarcoma TILs are T-cells isolated from tumour tissue from a patient with sarcoma. Tumour reactive T-cells will be isolated from tumour materials and TILs will be expanded ex vivo into high numbers. The specific T-cells are infused intravenously back into the patient during hospitalization on the day after the last dose of fludarabine phosphate (Step 2, Day 1). The number of T-cells in the product depends on the possible in vitro degree of expansion and is therefore variable, but will normally consist of approximately 10^{10} cells.

Cyclophosphamide:

Cyclophosphamide is given as an intravenous infusion for 2 consecutive days in a dose of 60 mg per kg body weight. The treatment takes place during hospital admission and with supplementary hydration and Mesna injections.

Fludarabine phosphate:

Fludarabine phosphate is given as an intravenous infusion for 5 consecutive days in a dose of 25 mg per m^2 body surface (starting the day after the last dose of cyclophosphamide). The treatment takes place during hospital admission.

IL-2:

A daily subcutaneous injection with IL-2 (2 MIU) will be administered from Step 2, days 1 to 14. During hospitalisation the injection will be given by a trained nurse. If the patient is well enough to be discharged within these 14 days, the patient or relatives will be taught to perform the remaining injections. The supplied amount of IL-2 injections for self-administration will be registered by use of batch number, so that the total amount of administered IL-2 can be determined.

Study Duration:

The maximum duration of study participation for each patient will be approximately 2 years, including the 4 weeks screening period, repeat of Step 1 and the 15 months follow-up after EoT.

Statistical Methods:

All analyses will be descriptive. Categorical variables will be presented with numbers and percentages, and if applicable, number of events. Continuous variables will be presented by n, mean, median, standard deviation and range (min and max) as appropriate.

2 INTRODUCTION

2.1 Therapeutic area disease background

Lytix Biopharma AS is developing a potential new anticancer drug, LTX-315 that induces long-term anticancer immune responses. LTX-315 induces immunogenic death of tumour cells leading to subsequent release of danger signals and a broad repertoire of tumour associated antigens. This breaks local tolerance and leads to recruitment of immune cells, including tumour specific CD8+ cytotoxic T-cells. Unlike exogenous targeted therapies that rely, for example, on receptor over-expression or mutation, this approach is not limited to specific tumour types.

Cancer is treated by a variety of pharmaceutical means, ranging from classical chemotherapy to targeted therapy modulating intracellular signalling cascades. In the last years various immune therapies have been studied as a means to stimulate the body's own ability to fight the cancer. Most cancers consist of a number of different malignant T-cells and since this heterogeneity varies from tumour to tumour, every patient's cancer is unique in its composition. As a consequence, it is extremely challenging to provide each patient with personalised treatment.

Local treatment of transdermally accessible tumours is generally limited to surgery and radiotherapy. However, there are a number of studies utilising tumour injection to generate an immune response as an internal mechanism for destroying the malignant tissue (29).

A novel approach of anticancer therapy is to induce the patient's own immune system which could then provide the ideal targeted therapy being specific to the patient's own cancer. This approach however, requires an adequate immune system which may be subject to local down-regulation of the immune response. Indeed, it may be in part due to this that the cancer cells persist. Removing the causes for such a down-regulation will enhance the activity of the patient's own immune system, and, in doing so, may instigate destruction of these cells. This hypothesis represents a promising approach to the personalised treatment of cancer.

2.2 Sarcoma

Sarcomas are a heterogenous group of malignant neoplasms arising from cells of mesenchymal origin. Numerous histological subtypes exist, and the tumours can occur in virtually any anatomical site. Often sarcomas are divided in soft tissue sarcomas that are the most frequent with an incidence of 4-5/100.000 per year, and bone sarcomas with an incidence of 0.8/100.000 per year (45). The overall incidence of sarcomas in Denmark is around 300 per year (8).

Advanced unresectable or metastatic stages of sarcomas, with the exception of gastrointestinal stromal tumour, respond poorly to current treatment, and prognosis is poor. Median survival for patients with metastatic soft tissue sarcoma at time of diagnosis was in a study from 2011 estimated to 10 months, and 5-year survival for this patient group was 10% (44).

2.3 Standard treatment of soft tissue sarcoma

The national guidelines for treatment of advanced/metastatic soft tissue sarcoma follow the European Society for Medical Oncology (ESMO) recommendations (18). With regard to treatment options multi-agent chemotherapy regimens are the standard of care and include:

- First line treatment has until recently been single agent doxorubicin, but will shortly be altered to doxorubicin + olaratumab. A randomised study of doxorubicin + olaratumab versus doxorubicin alone in previously untreated (anthracycline naïve) advanced/metastatic soft tissue sarcoma (46) reported a median PFS of 6.6 months with the combination versus 4.1 months with doxorubicin alone. The ORR was 18.2% with the combination versus 11.9% with doxorubicin alone
- Second or later line treatment may include:
    - High-dose ifosfamide as 2nd line treatment who received prior standard dose ifosfamide
    - Trabectidin
    - Gemcitabine + taxane and/or pazopanib

In addition to disease related factors (e.g. histological subtype) choice of treatment is guided by patient related factors such as ECOG performance status and co-morbidity.

The patient population that will be evaluated in this study have advanced/metastatic disease and will have received one (or possibly multiple) lines of treatment (as described above) for advanced/metastatic disease. They have a very poor prognosis with an estimated median survival of 10 months and a 5-year survival of 10% (44).

2.4 Drug class

2.4.1 LTX-315

LTX-315 is an oncolytic peptide, derived from lactoferricin, a member of the naturally occurring group of peptides referred to as membrane active host defence peptides (23). Membrane active host defence peptides bind to negatively charged molecules on the cell surface subsequently inducing lysis and cell death (35; 53). LTX-315 is internalized and targets intracellular organelles, particularly the mitochondria (15). This membranolytic mode of action leads to an effective release of potent immunostimulants (Damage-associated molecular patterns, DAMPs) and tumour antigens. The pharmacology studies demonstrate that LTX-315 injected directly into tumours induces necrosis and in several cases complete regression of the treated tumour.

LTX-315 is not tumour specific but is designed to generate an immune response to the tumour associated antigens, which are presented to the immune system to generate a tumour specific response.

In addition, systemic protective immune responses against tumour re-challenge have been observed in several animal models. Unlike in most phase I oncology studies, where the target tumour is independent of the route of administration and efficacy is dependent on systemic exposure, this study is designed with direct intra-tumoural injection of LTX-315.

2.4.2 LTX-315 Toxicology

A repeated-dose toxicity programme with subcutaneous injections in rats and dogs was designed to support a clinical dosing cycle of up to [redacted] dosing days per week. In both species LTX-315 was systemically well tolerated, although local necrosis, inflammation and haemorrhage were noted histologically, and hence a no-observed-adverse-effect-levels (NOAEL) could not be determined. However these pivotal studies have generated data to assist in the selection of the tolerable clinical starting dose in man. See Investigator's Brochure (IB) for more information (28).

2.4.3 LTX-315 Non-clinical safety

In the clinical study C08-315-01 a sudden, transient drop in blood pressure was observed. Based on this, non-clinical safety studies were initiated (28).

Single dose i.v. administration of LTX-315 revealed an adequate safety profile in both dog and the rat with a transient, and reversible, decrease of mean arterial pressure (MAP) in both species. The reduction in MAP results from changes in peripheral circulation and the observed increase in total vascular resistance implies this is not a direct effect on cardiac activity.

A threshold for this effect from LTX-315 on MAP occurs between [redacted] mg/kg (no effect) and [redacted] mg/kg in the rat. Acute single i.v. and sub-chronic repeated i.v. exposure with [redacted] mg/kg LTX-315 revealed this being the lowest dose for this reduction in MAP. A single dose of [redacted] mg/kg equals a dose of approximately [redacted] mg in adult humans.

Consecutive i.v. dosing at [redacted] mg/kg on the same day caused no reduction in MAP at second or third dose. However, a dose escalation revealed a dose-dependent reduction in MAP after first dose, while second and third dose revealed the occurrence of a tolerance. This may point towards a tachyphylaxis from repeated dosing with LTX-315. Sub-chronic LTX-315 i.v. dosing for [redacted] days over a period of [redacted] days, [redacted] times per day, in the rat revealed a tachyphylactic effect as judged by no changes in MAP was achieved at [redacted] dose at [redacted] mg/kg.

Studies to assess the effect of LTX-315 on histamine release have included; an in vitro study to assess direct histamine release from basophils in human peripheral blood mononuclear cells (PBMC), an in vivo study in guinea pigs to assess and visualise indirect histamine release following intradermal injection of LTX-315 using intravenously administered Evans Blue, and an in vivo study in guinea pigs to evaluate immunoglobulin (Ig) E (IgE) mediated histamine release.

The PBMC study indicated that LTX-315 may cause histamine release from basophils in a dose-dependent manner.

The first in vivo study in guinea pig (investigating cutaneous anaphylaxis) showed no evidence of a type 1 immediate hypersensitivity IgE antibody reaction, and the second study (investigating cutaneous extravasation) in guinea pigs was inconclusive most likely due to high LTX-315 concentrations used, resulting in local necrosis and disruption of vasculature. The reduction in MAP results from changes in peripheral circulation and the observed change in total vascular resistance implies this is not a direct effect on cardiac activity.

2.4.4 LTX-315 Non-clinical efficacy

The feasibility of expanding TILs from sarcoma, as well as performed functional in vitro analyses on TILs have been investigated in tumour tissue from sarcoma patients.

LTX-315 induce complete regression in several rodent sarcoma models. Cured animals showed protective immune responses towards tumour growth when re-challenged with sarcoma cells. In one of the sarcoma models it was demonstrated that LTX-315 treatment eradicated both the treated lesion and untreated distant lesions, indicating a strong and immediate systemic effect of LTX-315 therapy.

Transfer of splenic lymphocytes from cured animals inhibited tumour growth in naïve recipient animals, indicating that protective immune responses against the sarcoma cells could be adoptively transferred. Transfer of T-cell-depleted splenocytes did not protect against tumour development indicating that LTX-315 induce T-cell mediated immune responses (28).

2.4.5 TILs from sarcoma

In a preclinical study (33) the feasibility of expanding TILs from sarcomas was investigated, as well as performance of functional in vitro analyses on these. Tumour samples from 28 patients with 8 different sarcoma subtypes were obtained, and it was possible to expand a minimum of 40 million TILs from 25 of these. Reactivity analyses using ELISPOT revealed reactivity against autologous tumour cells in 11 of 22 tested tumour samples from 7 of 8 different sarcoma subtypes.

2.4.6 TILs Non-clinical efficacy

To this date TILs from 25 of 28 tumour samples have been expanded. TILs were harvested and frozen when an estimated number of $100x10^6$ to $200x10^6$ cells were reached. Mean expansion time were 32 days (16 – 61). 87,7% (36,4 – 99,1) of these cells were CD3+, and of these 66,7% (16,3 – 99,1) were CD4+, and 21,8% (0,1 – 50,6) were CD8+. Most of the expanded TILs were effector memory subtype, while a smaller fraction was the more differentiated effector T-cells.

Rapid Expansion Protocol expansion rates ranged from 630 fold to 2.300 fold, and followed expansion pattern similar to TILs from malignant melanoma.

TILs from 6 of 10 tested tumour samples with 4 different sarcoma subtypes (undifferentiated pleomorphic sarcoma, myxofibrosarcoma, myxoid liposarcoma and osteosarcoma) demonstrated reactivity against autologous tumour cells using ELISPOT. Further assessment is ongoing.

In summary it was possible to expand TILs from approximately 90% of the included tumour samples to numbers needed for possible future clinical implementation (33). TILs were a mix of CD4+ and CD8+ with CD4+ being predominant. As of yet, TIL reactivity against autologous tumour cells from 6 of 10 tested patients have been demonstrated. Thus, in conclusion it is feasible to translate TIL based adoptive cell therapy into clinical testing in sarcoma patients.

2.5 Clinical experience

2.5.1 Clinical safety of LTX-315

Fifty-three patients in 2 Phase I studies have received LTX-315 monotherapy at varying doses and in different schedules. In addition 8 patients have been treated with LTX-315 in combination with ipilimumab in patients with malignant melanoma and in 18 patients in combination with pembrolizumab in patients with triple negative breast cancer (28).

Of the 53 patients treated with LTX-315 as monotherapy, the most common LTX-315 related Aes reported are low grade (CTCAE grade 1-2) allergy-like Aes. These include most commonly flushing (injection site or facial), parasthesia (digits of hands and feet), pruritis, rash (injection site or distant from injection site), and hypotension (asymptomatic with systolic blood pressure decrease within the normal range in most patients). These allergy-like Aes occur from 1st injection onwards, emerge quickly (seconds/minutes) and resolve quickly (seconds/minutes) with no clinical sequaelae.

The LTX-315 injection schedule comprised either of injection of multiple lesions for 2 dosing days per week for 3 weeks or injection of a single lesion 3 subsequent dosing days in week 1 followed by once weekly for 5 further weeks. Patients enrolled in the latter schedule who had more than 1 lesion for injection, had the 2nd lesion treated starting in Week 7 and were dosed as described for the 1st lesion. LTX-315 has been evaluated at doses between 2-11.6 mg per injection with the majority of patients receiving doses between 2-7 mg per injection.

The duration of LTX-315 exposure in the phase 1 monotherapy programme was therefore significantly longer (median 6-8 injection days in 9 weeks) than that proposed in this adoptive T-cell study [redacted]).

In 53 patients treated with LTX-315 at a dose of [redacted] mg (or lower) per injection for duration of treatment of a minimum of [redacted] weeks ([redacted] injection days) or longer no clinically significant LTX-315 related Aes have occurred.

Clinically significant allergic reaction or anaphylaxis LTX-315 related Aes in the clinical programme have occurred in 4 patients (4 of 53 patients) and have occurred (in 3 of 4 patients) after many weeks (≥ [redacted] weeks) of well tolerated LTX-315 treatment (no significant or CTCAE grade ≥ 3 Aes) or at doses higher than [redacted] mg per injection (1 patient).

In the 50 patients who have received LTX-315 at [redacted] mg per injection for a period of up to [redacted] weeks treatment ([redacted] injection days) no clinically significant LTX-315 related allergic reaction/anaphylaxis Aes have occurred.

All patients were pre-medicated with a [redacted]

In summary, clinically significant allergy-like Aes are expected to be unlikely with LTX-315 in the adoptive T-cell study based on:

- LTX-315 dose of [redacted] mg per injection (no significant hypotension observed at this dose)
- Short duration ([redacted] injection days in [redacted] weeks) of LTX-315 treatment (IgE mediated anaphylaxis highly unlikely)
- Comprehensive allergic prophylaxis [redacted]

No other LTX-315 organ toxicity has been reported (cardiac, renal, hepatic, and bone marrow).

The exact mechanism of these allergic Aes is currently unknown. Various allergic assessments are being conducted in the clinical program to determine the mode of action (tryptase levels, IgE/IgG assessment, skin and intradermal skin testing). LTX-315 is known to induce histamine release by inducing direct mast cell membrane destabilisation in preclinical tests (28).

2.5.2 Clinical studies with adoptive T-cell therapy

The complex method of manufacturing TILs has been established at the Center for Cancer Immune Therapy (CCIT), Herlev Hospital, as one of the few places in the world (10). CCIT and the Department of Oncology at Herlev Hospital has several years of experience in treating cancer patients with T-cell therapy and different regimens of IL-2. All patients have been treated with classic lymphodepleting chemotherapy with cyclophosphamide and fludarabine phosphate followed by TIL infusion and subsequent administration of IL-2.

In the original "T-cell regimen" described by Dudley et al (13), very high doses of IL-2 (720.000 IU/kg i.v.) where given as bolus injection every 8 hours until treatment limiting toxicity. Since this regimen is associated with a high degree of acute toxicity and since it is unknown how high a dose of IL-2 is necessary to maintain T-cell expansion, consequently CCIT has tested the treatment with low and intermediate doses of IL-2 to investigate whether clinical efficacy can be maintained while toxicity is decreased.

Initially, low dose subcutaneous IL-2 was given to 6 patients in a pilot study (17). 2 of the 6 treated patients achieved complete response. ORR was 33% and this IL-2 regimen was well tolerated.

Based on results from a Danish study on intermediate dose IL-2 monotherapy, CCIT decided to [redacted] the IL-2 dose in order to increase the response rate. Consequently, the dose of IL-2 was [redacted] dose and administered after the [redacted] regimen equal to the low-dose regimen used in Denmark as standard treatment of metastatic malignant melanoma. Additionally 25 patients have been treated after the [redacted] in IL-2 dose (3). Of the 31 treated patients, 5 achieved irCR (48 (no evidence of disease (NED)), 13 (NED) 38+, 22+ and 11+ months) and 7 patients achieved irPR (35+ (NED), 12, 18+, 25+ (NED), 11, 8 and 6+), of which 7 are having ongoing responses varying from 6-38 months (3). 13 patients had stable disease (irSD) for 4-6 months and 5 patients progressed immediately after treatment. An ORR of 39% has been observed, which is comparable to other studies administering high dose of IL-2.

The lymphodepleting chemotherapy induced, as anticipated, myelosuppression with anemia, leukopenia and thrombocytopenia and all patients received prophylactic antibiotics and blood transfusions. All patients experienced transient grade III-IV toxicities during the 3 weeks of hospitalisation but recovered quickly after the treatment (3). Markedly reduced toxicities have been observed with the low- to intermediary dose of IL-2 and the treatment has shown to be manageable at a regular Department of Oncology with limited need for intervention from an Intensive care unit.

Previous studies in melanoma carried out in the US and in Israel have described > 200 patients and > 20 patients receiving such TIL therapy, respectively (49; 36; 6; 9). In addition, TIL studies in melanoma have been initiated in Europe by groups in the Netherlands, United Kingdom and Denmark (our internal observation). In these studies as well as in research papers, the starting material of TIL for expansion has varied between TILs grown out from tumour fragments or TILs grown out from digest material. The success rate to grow out TIL from patient tumours was in the majority of reports ≥ 83% (with one exception of 75% in a small patient cohort), for either digest or fragment set-up. Clinical responses have also been observed both with TILs grown out of fragments or from digest origin, with no observed correlation between treatment efficacy and method of tumour initiation (Dudley, personal communication).

Recently, an international phase III study (NCT02278887) treating patients with metastatic malignant melanoma with TILs and a high dose bolus IL-2 regimen (600.000 IU/kg) was initiated at the Department of Oncology, Herlev Hospital. To date, toxicity has been acceptable.

Furthermore, CCIT have recently completed a pilot study (NCT02482090) in which 6 patients with ovarian cancer were treated, and a study (NCT02926053) treating patients with renal cell carcinoma is ongoing.

2.5.3 Clinical experience with LTX-315 in combination with ACT

In this ongoing C17-314-04 study, three subjects have been exposed to LTX-315 (as of March 2020). No SAE and no AEs leading to discontinuation were reported. A total of 43 non-serious AEs were reported of which 9 were considered related to LTX-315. Eight were CTCAE grade 1 and one was grade 2 (injection site pain). The LTX-315 related AEs were: 4 injection site pain, 1 injection site redness, 1 itching, 1 diarrhoea, 1 fever and 1 stomach pain.

2.5.4 Translational research

Further development of T-cell therapy with optimizing and expansion to other cancer forms has a high priority at CCIT. CCIT have already established a platform for T-cell therapy for malignant melanoma which gives a unique opportunity to study the interactions between tumour and immune system and thereby identify possible methods for optimization of T-cell therapy, as well as extension to other tumour histology.

Several studies has shown that the following T-cell characteristics are important for achieving a clinical response after T-cell therapy: long telomeres, short time spent in culture, a favourable T-cell phenotype (CD27+, CD28+), a high absolute number of T-cells and a high number of cytotoxic tumour reactive T-cells in the infusion product (11) as well as an increased persistence of T-cell in the peripheral blood after infusion (40; 10; 48).

At CCIT, the original T-cell expansion method has been modified from ”Standard TIL expansion” to ”Young TIL expansion” based on these characteristics and leading to a reduction of the length of cell manufacturing from 4-7 weeks to 2-4 weeks. A decreased amount of time in culture (Young TIL) provides the TILs with longer telomere sequences and more favourable phenotypes (CD27+, CD28+) with the ability of increased proliferation, an increased persistence in vivo and a higher anti-tumour activity, all of which are correlated to an increased clinical response (40; 10).

This optimization of TIL production has made it possible to produce clinically usable TIL infusion products from more than 90% of the patients (5; 14; 22; 26). Furthermore, during the final expansion phase of the Rapid Expansion Protocol, the use of the [redacted] (43), which optimizes the conditions of proliferation of T-cells and has made it possible to achieve a higher total number of cells as well as tumour reactive T-cells in the TIL infusion product, has been introduced. Based on the TIL production protocols developed at CCIT, TIL production methods between 3 European cancer research centres has been standardized and harmonized and a randomised, multicenter TIL based phase III study (NCT02278887) with T-cell therapy versus standard immunotherapy have been initiated with the purpose of the approval of T-cell therapy as standard treatment for patients with malignant melanoma.

3 STUDY OBJECTIVES AND ENDPOINTS

3.1 Study objectives

Primary objectives:

- To determine the ability of LTX-315 to induce T-cell infiltration prior to TIL expansion in advanced/metastatic soft tissue sarcoma
- To determine the safety of LTX-315 as part of adoptive T-cell therapy in advanced/metastatic soft tissue sarcoma

Secondary objectives:

- To determine the ability of LTX-315 as part of adoptive T-cell therapy to induce T-cell infiltration in advanced/metastatic soft tissue sarcoma
- To assess the ability to expand CD8+ T-cells from tumour tissues
- To assess the anti-tumour effect of LTX-315 as part of adoptive T-cell therapy in advanced/metastatic soft tissue sarcoma

Exploratory objectives:

- To assess tumour-antigen specificity
- To investigate and characterise immune status and nature of anti-tumour immune responses

3.2 Endpoints

Primary endpoints:

- Change in total T-cell level in tumour tissues from Baseline (Step 1, Week 1, Day 1) to end of Step 1 (Step 1, Week 3)
- Adverse events (AE) related to LTX-315 or to the combination of LTX-315 and adoptive T-cell therapy from Baseline (Step 1, Week 1, Day 1) to EoT (Step 2, Week 7)

Secondary endpoints:

- Change in CD3+ T-cell and CD3+CD8+ T cell density in non-injected tumour tissues from Baseline (Step 1, Week 1, Day 1) to EoT (Step 2, Week 7) if the patient has a feasible bystander lesion and accept it to be biopsied.
- Total number of CD3+CD8+ T cells and % CD3+CD8+T cells of total CD3+ T cells in final TIL infusion productThe anti-tumour effect assessed by:
    - Objective Response Rate (ORR) defined as proportion of patients who have I CR or PR at EoT (Step 2, Week 7) and up to 15 months after EoT
    - Clinical Benefit Rate (CBR) defined as proportion of patients who have achieved CR, PR or SD at EoT (Step 2, Week 7) and up to 15 months after EoT
    - Progression free survival (PFS) evaluated by time from Baseline until PD or death up to 15 months after EoT

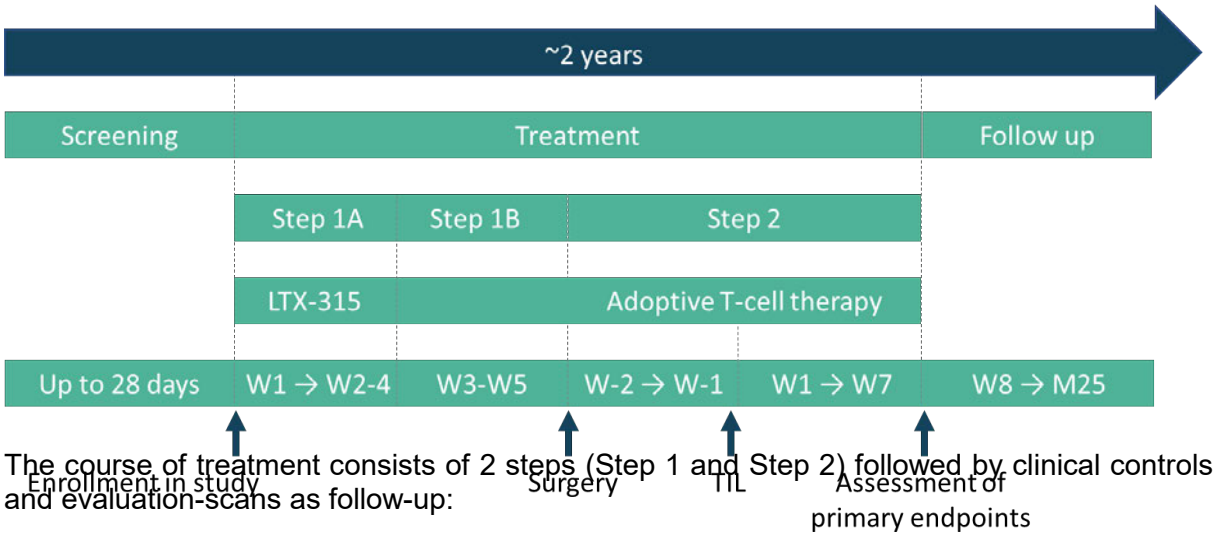
Exploratory endpoint:

- Identification of tumour-antigen specific T-cells in tumour tissue and PBMCs by MHC multimer–based screens, ELISPOT and flowcytometry analysis of cytokines including IFNγ and TNFα
- Changes in immunological parameters from Baseline (Step 1, Week 1, Day 1) to 15 months after EoT

4 STUDY DESIGN

4.1 Overall study design

Figure 1. Study design

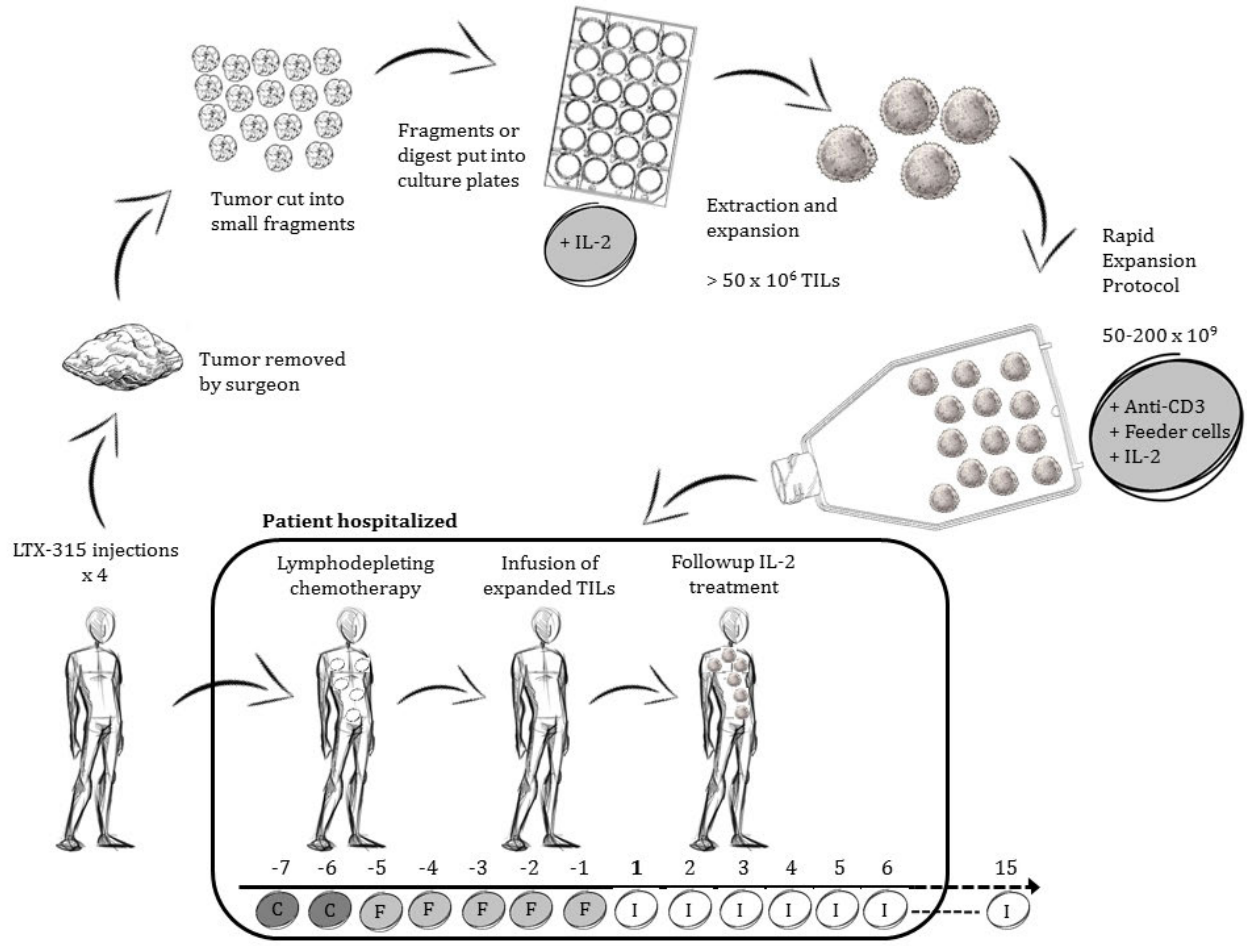


The course of treatment consists of 2 steps (Step 1 and Step 2) followed by clinical controls and evaluation-scans as follow-up:

- Screening (28 days prior to Step 1): informed consent, check of eligibility and inclusion. Identified AEs to be recorded in Medical History
- Step 1 (W1 to W3-5): re-check of inclusion, baseline tumour biopsy, LTX-315 injections and surgical removal of the LTX-315 injected index tumour lesion followed by production and growth of TILs in the laboratory
- Step 2 (W-2 to W-1, W1 to W7): check of eligibility for Step 2, treatment during hospitalisation with chemotherapy, TIL infusion and IL-2 administration, safety and efficacy of LTX-315/TIL treatment
- Follow-up: safety and efficacy of LTX-315/TIL treatment for a follow-up period of 15 months. Only AEs considered related to LTX-315 or TIL to be reported

The course of treatment can vary between patients depending on the time period from surgical removal of LTX-315 injected index tumour lesion (Step 1) to adoptive T-cell therapy treatment (Step 2). Patients with PD at enrollment will receive TIL treatment approximately 4-6 weeks after surgery with a maximum of 10 weeks. Treatment period (including screening): up to 26 weeks. Patients with SD at enrollment will receive TIL therapy after confirmed PD during the study. Follow-up period: up to 15 months.

Figure 2. Step 1 and Step 2 treatment period



C: cyclophosphamide, F: Fludarabine phosphate, I: IL-2

4.2 Study rationale

Pharmacodynamic studies of LTX-315 in animal models have demonstrated growth inhibition and complete regression following intra-tumoural injections, subsequent tumour specific protection and transfer of protection from treated to untreated animals. Abscopal effects have been obtained in some preclinical models (28). Accumulating evidence support the hypothesis that adoptive immunotherapeutic strategies may be effective as treatment in sarcoma. Early clinical observations have shown local tumour regression in sarcoma patients upon erysipelas infection and intra-tumoural injections of bacterial derived toxins (50). Also, high-dose IL-2 therapy has shown complete response rates in paediatric patients with osteosarcoma (41). Histological analyses have shown that a wide range of sarcomas express markers of inflammation by the presence of infiltrating lymphocytes and PD-L1-expression (7), and immune cell infiltration have been proven to have an independently positive prognostic impact in soft tissue sarcomas (46).

Adoptive T-cell therapy is currently an experimental intervention in soft tissue sarcoma. In a recent study, treatment with genetically engineered autologous T-cells derived from circulating blood cells, have shown significant clinical results in patients with synovial cell sarcoma and malignant melanoma (39). Overall, clinical response rates were 60% in both

melanoma and sarcoma patients, indicating that T-cell based immunotherapeutic approaches are potent in either cancer types and there is a biological rationale for further development of adoptive cell therapy strategies in sarcoma.

LTX-315, a chemically modified cationic peptide, has demonstrated (preclinical and clinical data) to convert immunogenically "cold" tumours to immunogenically "hot" tumours with significant infiltration of CD8+ T-cells comparing pre and post LTX-315 biopsies of various LTX-315 injected solid tumours. LTX-315 may therefore be a potentially valuable treatment modality in increasing the CD8+ T-cell population available for adoptive T-cell therapy of patients with advanced/metastatic soft tissue sarcoma.

In an advanced/metastatic soft tissue sarcoma population with a median survival of 10 months, who have received standard approved treatments for advanced/metastatic disease (or are deemed unsuitable for such treatments) it is therefore of interest to combine the 2 experimental modalities (LTX-315 and adoptive T-cell therapy) that may increase the potential for clinical benefit in patients with a major unmet medical need.

4.2.1 LTX-315 dose rationale

LTX-315 will be administered at [redacted] mg per injection (given in a [redacted] mg/ml concentration) on [redacted] dosing days in week [redacted] (Days [redacted]) followed by dosing once weekly for up to [redacted] weeks (Days [redacted]). The dosing at day [redacted] and [redacted] are optional dosing days and should be deemed by the treating physician.

LTX-315 doses up to [redacted] mg per injection has been demonstrated to be a safe dose (no dose limiting toxicities (DLTs) and no clinically significant dose-related AEs reported) when administered to multiple tumour lesions in a single injection day. Doses ranging from [redacted] to [redacted] mg ([redacted] mg) have been administered on each of 6 injection days (day [redacted]) in 3 weeks. No increase in the frequency or severity of LTX-315 related AEs was observed with increasing number of injections or LTX-315 total dose administered. The only LTX-315 related AEs observed were transient (emerging within seconds/minutes and resolving within minutes) low grade (CTCAE grade 1-2) allergy-like AEs (comprising most commonly of flushing, paraesthesia, pruritis, rash and asymptomatic hypotension).

Anti-tumour activity has been observed with LTX-315 at a dose of [redacted] mg per injection (regression in injected lesions, stable disease by irRC criteria and significant infiltration of CD8+ T-cells in injected tumour lesions). LTX-315 as monotherapy has been evaluated at single doses of between [redacted] mg per injection administered as either [redacted] mg or [redacted] mg/ml in 28 patients in the ongoing phase 1 study (C12-315-03). LTX-315 injections have been administered on a once daily or twice daily basis on planned LTX-315 injection days to tumour lesions. [redacted]

In 1 patient LTX-315 [redacted] mg per injection was administered to [redacted] daily (schedule above). LTX-315 doses of [redacted] mg per injection have been declared safe with no DLTs reported.

Anti-tumour activity has been observed at doses of LTX-315 > [redacted] mg including local (injected) tumour control (partial or complete regression), stable disease by irRC (CT assessment) in 8 of 16 evaluable patients and immune cell (CD8+ T-cell) infiltration in injected tumour lesions (biopsy assessment).

4.2.2 Drugs used in adoptive T-cell therapy

Activating cytokines (the signal molecules IL-2, IL-7, IL-15, IL-21 etc.) need to be available for the tumour specific T-cells to sustain a immunological response against tumour tissue. A large number of “irrelevant” T-cells will decrease the availability of these cytokines for the relevant T-cells through competition. Thus, a high number of tumour specific T-cells with a high specificity as well as a reduction of irrelevant T-cells and regulatory T-cells are needed to create an environment that facilitates the T-cell mediated anti-tumour response.

This study will use combination chemotherapy with 2 days treatment with cyclophosphamide and 5 days with fludarabine phosphate to create such an environment. This combination has been chosen based on earlier studies where it was shown safe and effective (20; 4).

4.2.2.1 Cyclophosphamide

Cyclophosphamide is an alkylating drug that works by creating covalent bindings with biologically important macromolecules. Of special interest is the creation of a binding and linkage to DNA. Cell division can be prevented if the linkage is not cancelled by the cells repair systems. The binding to important proteins in the cell can damage important cellular functions and lead to cell death. Cyclophosphamide is among others used to treat breast cancer and in the treatment of haematological diseases as myelomatos (42).

4.2.2.2 Fludarabine phosphate

Fludarabine phosphate is a pro-drug that is converted to the active triphosphate 2-fluoro-ara-ATP. It is an anti-metabolite which inhibits DNA synthesis while simultaneously reducing RNA and protein synthesis. Fludarabine phosphate is used in the treatment of haematological diseases as chronic lymphocytic leukemia among others (19).

4.2.2.3 Interleukin 2

IL-2 is physiologically produced by activated T-lymphocytes and stimulates the antigen specific and non-specific immune system through specific receptors (31). [redacted] dose IL-2 administered intravenously according to the low-dose regimen (27) is used in Denmark as standard treatment for suitable patients with metastatic malignant melanoma. Moreover, low-dose IL-2, 2 MIU is used for the treatment of metastatic kidney cancer. In this study, IL-2 could be given according to the low-dose subcutaneous regimen.

4.3 Benefit and risk assessment

4.3.1 Benefits

LTX-315 is designed to generate an immune response to the tumour associated antigens, which are presented to the immune system to generate a tumour specific response. The feasibility of expanding TILs from sarcomas was investigated in a non-clinical study. Tumour samples from 28 patients with 8 different sarcoma subtypes were obtained, and it was possible to expand a minimum of 40 million TILs from 25 of these. Reactivity analyses revealed reactivity against autologous tumour cells in 11 of 22 tested tumour samples from 7 of 8 different sarcoma subtypes. In this study, it is expected that LTX-315 will expand CD8+ T-cells from the excised index lesion thus enhancing the effect of TIL treatment in patients with soft tissue sarcoma.

Eligible patients for the study will have progressed on first line systemic treatment for locally advanced/metastatic disease, and the prognosis for these patients is poor. By entering this

protocol the patient get access to an experimental treatment that could potentially benefit disease course.

4.3.2 Risks associated with LTX-315

Side effects with LTX-315 administration are modest and predictable and include most commonly low grade (CTCAE grade 1-2) allergy-like AEs including symptoms and signs of flushing, paraesthesia, pruritis, rash and asymptomatic hypotension. These allergy-like AEs occur in approximately 55% of patients (but not necessarily after all LTX-315 injections) and emerge quickly (seconds to minutes) and resolve quickly (minutes).

Prophylactic measures are in place as antihistamines and a leukotriene antagonist will be given to all patients prior to LTX-315 treatment.

4.3.3 Risk associated with adoptive T-cell therapy

The in vitro mechanism of action of TILs has been shown to be via direct lysis of tumour cells and the production of IFNγ upon tumour stimulation (21), which could also be demonstrated in in vitro experiments in metastatic melanoma (12). Internal observation shows that also TILs obtained from sarcoma specimens have the ability to recognise autologous tumour cells. Recognition of the tumour by cytotoxic T-lymphocytes is mediated via the T-cell receptor.

In melanoma, the identity of T-cell specificities that are responsible for tumour regression upon infusion of TILs has not been clarified fully but several antigens which can be recognised by autologous TILs have been described (2). In melanoma, TILs recognising melanocyte differentiation antigens have been described, and destruction of normal melanocytes was observed in a substantial number of patients treated with TILs (37% vitiligo, 14% uveitis) and related side effects have been observed Table 1 and Table 2 summarizes the side effects which have been observed.

However, the cells to be infused in this study are represented by autologous lymphocytes which are simply activated and expanded in vitro, with the purpose to boost their natural capacity to kill tumour cells. Therefore, as in melanoma, the toxicities predicted from infusion of autologous TILs are mild and it is anticipated that the benefits outweigh the risks.

4.3.3.1 Analysis of potential side effects

Intravenous IL-2 administration in itself is known to induce side effects (30). World-wide, thousands of patients have been treated with intravenous IL-2 and its side effects have become very much manageable. Experience with low-dose subcutaneous IL-2 and intravenous intermediate dose IL-2 was obtained at CCIT, where 6 melanoma patients received low-dose subcutaneous IL-2 for 14 days (Ellebaek 2012), while subsequent patients received low-dose regimen dose IL-2 as single therapy (Adams-Graves 1997). Toxicities to low-dose subcutaneous IL-2 were fever/chills only, while decrescendo IL-2 toxicities were predictable with high fever, weight gain, rash, loss of appetite, reduced urine production and low blood pressure and manageable with standard treatments for these symptoms. All symptoms resolved completely within 48 h after the last infusion.

Other toxicities described for the National Cancer Institute (NCI) study (NCT00080353) of TIL therapy in melanoma, in conjunction with lymphodepletion and IL-2 are shown in Table 1 (toxicities within the first 35 patients were reported). CCIT's experience is reported in Table 2.

Note that only low or decrescendo IL-2 doses were infused, resulting in a reduced toxicity profile; other toxicities are similar to those observed in the NCI study. Non-haematological

and haematological toxicities were those expected from IL-2 and the non-myeloablative chemotherapy. In addition, autoimmunity was observed in the TIL therapy group directed at melanocytes (at the NCI). Even though the lymphodepleting regimen of cyclophosphamide and fludarabine phosphate was shown to be myelosuppressive, it was not myeloablative, and neutrophils and lymphocytes recovered to > 500/mm^2 in all patients within 2 to 3 weeks, respectively. More importantly, none of patients with metastatic melanoma (n=31, including 2 patients treated twice with a full regimen) treated so far at CCIT needed stem cell support to rescue marrow function.

Table 1. Toxicities observed in NIH study protocol 99-C-0158 (NCT00080353)

Time in Hospital and Non-hematological Grade 3 and 4 Toxicities Related to Lymphodepleting Chemotherapy and Cell Transfer

Attribute measured	Duration, Number or Type	Number of Patients (%)
Days in Hospital[1]	6-10	6 (17%)
	11-15	18 (51%)
	16-20	4 (11%)
	21-25	7 (20%)
pRBC Transfusions	0	2 (6%)
	1-5	18 (51%)
	6-10	13 (37%)
	11-15	2(6%)
Platelet Transfusions	0	6 (17%)
	1-5	21 (60%)
	6-10	5 (14%)
	11-15	2 (6%)
	16-20	1 (3%)
Autoimmunity	Uveitis	5 (14%)
	Vitiligo	13 (37%)
Opportunistic Infections	Herpes zoster	3 (9%)
	Pneumocystis pneumonia	2 (6%)
	EBV-B cell lymphoma	1 (3%)
	RSV pneumonia	1 (3%)
Other	Febrile neutropenia	13 (37%)
	Intubated for dyspnea	3 (9%)
	Cortical blindness	1 (3%)

[1]Measured from the day of cell administration to discharge

Table 2. Toxicities observed in TIL study/studies at CCIT (3)

Attribute measured / Toxicities	(n=24)
Days in hospital: mean (range)	21.6 (16 – 39)
pRBC transfusions: mean (range)	5.6 (1-25)
Platelet transfusions: mean (range)	5.2 (0-14)
Autoimmunity	Uveitis (n=1)
Opportunistic infections	Not observed
Toxicities due to chemotherapy (grade 3 and 4)	Neutropenia (n=18) Thrombopenia (n=18) Indefinit sepsis (n=3)
Toxicities due to TIL (all grades)	Fever, chills (n=19)
Toxicities due to intermediate dose IL-2 (all grades)	(=6)[a] Fever, chills (n=6) Dyspnea (n=2) Oliguria (n=2) Weight gain (n=4) Creatinin increase (n=1)

a)Only patients treated with intermediate doses of IL-2 (low-dose regimen) have been reported. Patients treated with low-dose IL-2 only had fever/chills as IL-2 related toxicity

In the NCI study one patient developed an Epstein-Barr virus (EBV) lymphoproliferative disease 4 months after treatment. This patient was seronegative for EBV before TIL infusion, and must have contracted an EBV infection shortly after TIL treatment. Although this patient had a 90% decrease of tumour load upon TIL treatment, he died of progressive lymphoma 8

months later. However, EBV infection of a seronegative patient is considered a very unlikely event and therefore EBV seronegativity is not an exclusion criterion in this TIL study as well as all patients will receive acyclovir or valacyclovir prophylaxis which was previously shown to reduce the incidence of lymphoproliferative diseases in immunosuppressed populations (34).

4.3.3.2 Predictability of effect

There are no biomarkers that can predict tumour regression or toxicity of the treatment. Patients will therefore be carefully monitored by physical examination, blood tests, PET-CT- and/or MRI-scans and if indicated ophthalmic examinations. Blood samples will be taken at several time points after TIL treatment to measure sarcoma cancer-reactive T-cells retrospectively.

Haematological toxicities such as low red blood cell and platelet counts are transient but may require transfusions of red blood cell or platelets, respectively. Patients will receive filgrastrim to shorten the period of neutropenia. In case of febrile neutropenia, patients will be treated with broad-spectrum antibiotics. Patients will receive antibiotic profylaxis against important opportunistic infections (P. Carinii, yeast infections albicans, herpes infections etc.). Opportunistic infections will be treated with the appropriate antiviral or antibacterial drugs. Toxicities associated with low-dose IL-2 therapy are also transient and can be easily managed using standard interventions and treatments.

4.3.4 Risks and disadvantages regarding surgery and test sampling

4.3.4.1 Risks associated with removing tumour tissue

Prior to inclusion it will be assessed whether it is possible to remove some of the patients own tumour tissue in a minor operative procedure. Surgery will mainly be performed by physicians at the Department of Orthopedic Surgery, Copenhagen University Hospital, Rigshospitalet or by physicians from other specialties if necessary. The specific procedure to be performed is to be decided by the surgeon. Also, it will be at the surgeons' discretion to decide whether the procedure can be performed in the outpatient clinic or if it requires admission.

Depending on the required surgical procedure, risk and possible adverse effects will vary, but includes bleeding and infection. The patient will be informed in more details about the risk and possible AEs both at inclusion and again prior to surgery.

The patient will not be able to participate in the study if no tumour tissue is available for removal or if removal will put the patient at a too large risk.

4.3.4.2 Risks associated with biopsies

There is a slight risk of infection and/or bleeding when performing a biopsy. Pain and bruising might also occur in the area.

4.3.4.3 Risks associated with blood tests

Pain and bruising can occur in the area.

4.3.5 Overall benefit and risk evaluation

Based on all the currently available non-clinical and clinical data for LTX-315 and adoptive T-cell therapy, as well as the design of the planned C17-315-04 study, it is considered safe to proceed with the proposed clinical study under the conditions detailed in the protocol.

5 SELECTION OF STUDY POPULATION

5.1 Population

The target population is adult male and female patients with tumour lesions accessible for injection (may include cutaneous, subcutaneous, oral or lymph node lesions) who have a confirmed diagnosis of advanced/metastatic soft tissue sarcoma who have received at least one approved standard of care treatment and/or are deemed unsuitable for such treatment(s) by their treating physician.

Patients must meet all of the inclusion criteria listed in Section 5.2 and not fulfil any of the exclusion criteria listed in Section 5.3.

5.2 Inclusion criteria

1. (Histologically) Confirmed any patients with advanced or metastatic soft tissue sarcoma with stable disease or has progressed after a minimum of 1 systemic treatment of advanced/metastatic disease. Patient will have received at least 1 approved standard of care treatment for advanced/metastatic disease or be deemed unsuitable for such treatment by their treating physician
2. At least 1 index tumour lesion accessible for injection with a longest diameter (LD) of ≥ [redacted] cm which is planned for LTX-315 injection
3. At least 1 measurable (target) non-injected tumour lesion that can be used to assess response by Computed Tomography (CT)-scan (as per RECIST 1.1)
4. A life expectancy of at least 3 months
5. Willing to undergo repeat tumour biopsy and/or tumour resection procedures
6. Between 18 and 75 years of age
7. An Eastern Cooperative Oncology Group (ECOG) performance status: 0 – 1
8. Meet the following blood laboratory requirements:
    a. Absolute neutrophil count ≥ 1.5 x 10^9/L
    b. Platelet count ≥ 75 x 10^9/L
    c. Haemoglobin ≥ 6.0 mmol/L after blood transfusion if needed
    d. Prothrombin time / International Normalised Ratio (PT/INR) within the institution's normal range
    e. Aspartate Transferase (AST) and Alanine Transferase (ALT) ≤ 2.5 x upper normal level
    f. Creatinine ≤ 1.5 x upper normal level
9. Willing and able to comply with the protocol and agree to return to the hospital for follow-up visits and examinations up to 15 months after EoT
10. Fully informed about the study and have signed the informed consent form
11. Re-screening is allowed once on a case by case basis as judged by the investigator

5.3 Exclusion criteria

1. A history of clinical significant active systemic autoimmune disease requiring anti-inflammatory or immunosuppressive therapy within the last 3 months. Patients with history of autoimmune thyroiditis are eligible provided the patient requires only thyroid hormone replacement therapy and disease has been stable for ≥ 1 year
2. Any other malignancy active within the previous 5 years except for carcinoma in situ of the cervix, ductal carcinoma in situ (DCIS) or lobular carcinoma in situ (LCIS) of the breast
3. Received an investigational drug within 4 weeks prior to any study drug administration, or are scheduled to receive 1 during the study participation
4. Received external radiotherapy or cytotoxic chemotherapy within 4 weeks prior to LTX-315 administration, or have not recovered from AEs (to ≤ CTCAE grade 1) due to agents administered more than 4 weeks earlier. Palliative radiotherapy to non-target and lesions planned for LTX-315 injection within 4 weeks of LTX-315 administration is allowed
5. Currently taking any agent with a known effect on the immune system. Patients are allowed to be on a stable dose of corticosteroids (up to 10 mg daily prednisolone or equivalent) for at least 2 weeks prior to LTX-315 administration (see Appendix I)
6. Any other serious illness or medical condition such as, but not limited to:
    a. Uncontrolled infection or infection requiring antibiotics
    b. Uncontrolled cardiac failure: Classification III or IV (New York Heart Association)
    c. Uncontrolled systemic and gastrointestinal inflammatory conditions
    d. Bone marrow dysplasia
7. A known history of positive tests for HIV/AIDS, syphilis, human T-cell leukemia-lymphoma virus (HTLV), active Epstein-Barr, hepatitis B or C (based on serology)
8. A history of cerebrovascular or cardiac disorders and would be at particular risk of sequelae following a short hypotensive episode
9. If of child-bearing potential, not willing to use an effective form of contraception such as hormonal birth control, intrauterine device or double barrier method from screening visit and until 3 months after last study treatment (any of the agents). Acceptable hormonal and non-hormonal birth control methods are listed in Appendix II.
10. Breastfeeding and/or have a positive pregnancy test during screening
11. Donate sperm from LTX-315 dosing until 3 months after last study treatment (any of the agents)
12. Patients with pregnant or partner of child-bearing potential not willing to use contraception from LTX-315 administration until 3 months after last study treatment (any of the agents). Acceptable hormonal and non-hormonal birth control methods are listed in Appendix II.
13. Expected to need any other anticancer therapy or immunotherapy to be initiated during the treatment period

14. Clinically active or unstable metastases in the central nerveous system as assessed by the treating physician
15. Any known hypersensitivity to any of the excipients in the non-IMPs including the required allergic prophylactic medications ([redacted])
16. Any known or suspected hypersensitivity to any of the IMPs

5.4 Recruitment and screening

All patients will be treated at CCIT, Herlev Hospital, Denmark. Potential patients will also be identified at hospitals in other Scandinavian countries and referred to Herlev Hospital for screening and study participation. Prior to any testing under the study protocol, including screening procedures and assessments, written informed consent will be obtained from the patient in accordance with Danish practice and applicable guidelines/regulations. If the patient is included after re-screening, a new written informed consent will be obtained from the patient.

5.5 Patient withdrawal

Patients will be informed that they have the right to withdraw from the study at any time for any reason, without prejudice to their medical care. The Investigator also has the right to withdraw patients from the study.

Patients meeting any of the following criteria must be discontinued from the study:

- The patient withdraws consent. The consequence of withdrawal of all consent by a patient will be that no new information will be collected from that patient and added to the existing data or database. However, every effort will be made to follow all patients for safety.
- The Investigator can withdraw the patient due to safety or any other issues
- Initiation of a new systemic anticancer treatment
- The patient becomes pregnant or fails to use adequate birth control (for those patients who are able to conceive) in the treatment period
- Intercurrent medical illness that, in the judgement of the Investigator, would make continued intervention dangerous for the patient
- The patient is not eligible to continue to Step 2, based on judgement of the Investigator

If a patient is withdrawn prior to the scheduled EoT visit, they should complete the Early Termination (ET) visit assessments as listed in table 6.1 and 6.2, if at all possible.

The reason(s) for a patient's withdrawal from the study are to be recorded in the patient's records and in the electronic case report form (eCRF).

6 TREATMENTS

6.1 IMPs and non-IMPs

Table 3. IMP product descriptions

Product name and potency	Dosage form	Delivered by
LTX-315 Acetate 20mg peptide/vial	Lyophilised powder for injection after reconstitution	[redacted]
TILs	TIL cells for infusion	Herlev Hospital Pharmacy
Proleukin®, interleukin 2 ([redacted] MIU/vial)	Powder for solution for injection or infusion	Herlev Hospital Pharmacy
Sendoxan®, cyclophosamide (200, 500 or 1000 mg/vial)	Powder for solution for injection or infusion	Herlev Hospital Pharmacy
Fludara®, fludarabine phosphate (25 or 50 mg/vial)	Concentrate for solution for injection or infusion	Herlev Hospital Pharmacy

Table 4. Non-IMP Product descriptions

Product name and potency	Dosage form	Delivered by
[redacted]	For oral administration	Herlev Hospital Pharmacy
[redacted]	For oral administration	Herlev Hospital Pharmacy
[redacted]	For oral administration	Herlev Hospital Pharmacy
Pegfilgrastim 6 mg	Solution for s.c. injection	Herlev Hospital Pharmacy
Sulfamethizole with Trimethoprime 400/80 mg	For oral administration	Herlev Hospital Pharmacy
Aciclovir 400 mg	For oral administration	Herlev Hospital Pharmacy

6.1.1 Labelling and packaging

LTX-315 will be labelled and packaged according to EudraLex, Volume 4 – Good Manufacturing Practice (GMP) guidelines - Annex 13 Manufacture of IMPs, local and study requirements by [redacted] specialised clinical supply company. LTX-315 will be shipped to the investigational site under controlled cold chain conditions.

TILs, IL-2, cyclophosphamide and fludarabine phosphate will be labelled and packed according to Herlev Hospital pharmacy requirements.

IL-2 Injections for self-administration will be labelled by the cytostatica unit, Herlev Hospital, in accordance with Annex 13 of the GMP guidelines.

6.1.2 Storage and handling

LTX-315 must be stored according to the storage conditions specified on the label in a limited access location. The drug allocated to the study may not be used for any purpose other than the present study.

IL-2, cyclophosamide and fludarabine phosphate must be stored and handled according to the SmPCs and in a limited access location. Mixing and storage of the products is carried out according to existing standard guidelines at the Department of Oncology.

After the study is completed, all unused LTX-315 products must be destroyed or returned to the Sponsor or the Sponsor's agent upon agreement with Sponsor.

The Investigator will be responsible for the storage, dispensing, inventory, and accountability of all clinical supplies. An accurate and timely record of the inventory of all clinical supplies must be maintained. The supplies and inventory must be available for inspection by designated representatives of the Sponsor on request, and must include the information below:

- Receipt of all products from clinical supplier or Herlev Pharmacy
- The identification of the patient to whom the drug was dispensed
- The date(s), number of injections/infusions/oral administration and quantity (volume, amount) of the drug dispensed to the patient
- The product batch number

The preparation of each IMP and non-IMP must be documented on a 'Drug Preparation and Dispensing Log Form'.

A copy of the inventory record and a record of any IMP that have been destroyed must be submitted by the Investigator to the Sponsor. This form must include the information below:

- The number of administered units
- The number of unused units
- For self-administration, the number of units provided to the patient and the number of units returned by the patient. Patients are supplied with a diary to report IL-2 self-administration if discharged prior to D13.
- The number of units destroyed at the end of the study
- The date and method of destruction and the location

6.1.3 Reconstitution and dispensing

Please see the Pharmacy Manual or Summary of Product Characteristic (SmPC)s for details on reconstitution and dispensing of IMPs and non-IMPs.

The LTX-315 solution has to be used within 12 hours from the time of reconstitution.

For the production of TIL, see Section 6.3.2

6.1.4 Treatment compliance

The Investigator(s) will record the time and dose of administrations of IMPs and non-IMPs in the medical source documents. Any reasons for non-compliance will also be documented, including:

- Missed visits
- Interruptions in the schedule of administration
- Non-permitted medication (see Section 6.4)

6.2 Selection of drug doses

6.2.1 LTX-315

All patients will be treated with [redacted] mg LTX-315 ([redacted] mg/ml concentration) per injection. The maximum number of injections per day will be [redacted] (see Section 6.3.1).

6.2.2 Cyclophosphamide

Cyclophosphamide is given as an intravenous infusion for 2 consecutive days in a dose of 60 mg per kg of body weight. The treatment takes place during hospital admission and with supplementary hydration and Mesna injections.

6.2.3 Fludarabine phosphate

Fludarabine phosphate is given as an intravenous infusion for 5 consecutive days in a dose of 25 mg per m^2 body surface (starting the day after the last dose of cyclophosphamide). The treatment takes place during hospital admission.

6.2.4 TILs

The tumour specific T-cells are infused intravenously back in to the patient during hospitalisation on the day after the last dose of fludarabine phosphate (Day 1). The number of T-cells in the product depends on the possible in vitro degree of expansion and is therefore variable, but will normally consist of approximately 10^{10} cells. See the Investigational Product Medicinal Dossier (IMPD) for more information (TIL IMPD 2017).

6.2.5 Interleukin-2

A daily subcutaneous injection with IL-2 (2 MIU) will be administered from Day 1 to 14. During hospitalisation the injection will be given by a trained nurse. If the patient is well enough to be discharged within these 14 days, the patient or relatives will be taught to perform the remaining injections.

The supplied amount of IL-2 injections for self-administration will be registered by use of batch number, so that the total amount of administed IL-2 can be determined.

6.2.6 Pegfilgrastim

Is an analog of human granulocyte colony stimulatory factor. It works by stimulating the bone marrow into producing white blood cells and increasing the peripheral blood count. It is usually given to cancer patients who suffer from low blood counts following chemotherapy (32).

Pegfilgrastim is administered as a single dose of 6 mg s.c. at 2 hours after TIL infusion to help patients recover from the lymphodepleting chemotherapy.

6.2.7 Sulfamethizole with trimethoprime

To prevent opportunistic infections, sulfamethizole with trimethoprime, 400/80 mg, 1 tablet is given daily from Step 2, Day -7 and 6 months ahead.

6.2.8 Aciclovir

To prevent opportunistic infections, aciclovir, 400 mg x 2 daily on Step 2, Day 1 and 6 months ahead.

6.2.9 Chlorpheniramine

LTX-315 prophylaxis. Chlorpheniramine 4 mg or equivalent histamine H1-antagonist will be given orally 10-12 hours (self-administration) and 1 hour before the first injection each day of LTX-315 injection (or i.v. equivalent)

6.2.10 Ranitidine

LTX-315 prophylaxis. Ranitidine 150 mg (or equivalent H2-antagonist) orally 1 hour before the first injection each day of LTX-315 injection (or i.v. equivalent)

6.2.11 Montelukast

LTX-315 prophylaxis. Montelukast 10 mg orally 10-12 hours (self-administration) and 1 hour prior to the first LTX-315 injection on each planned day of administration

6.3 Treatments administered

Supportive care and resuscitation equipment should always be available when study drug is administered.

LTX-315 Prophylactic measures will be initiated in all patients with the use of [redacted] as described in Section 6.2.9, 6.2.10, and 6.2.11. Additional requirements for prophylaxis will be evaluated by the Investigator and may be instituted based on emerging safety data. Anaphylaxis treatment guideline will be described in a separate document.

For the T-cell therapy, prophylactic treatment includes fluid therapy during cyclophosphamide treatment as well as supportive treatment.

6.3.1 LTX-315 injection schedule

Detailed instructions for intra-tumoural LTX-315 injections are provided in a separate manual.

The intra-tumoural injections of LTX-315 may be ultrasound-guided, based on clinical judgement. However, ultrasound guidance is not mandatory. Injection and delivery of LTX-315 should be aimed at the [redacted]

- LTX-315 will be administered on dosing days (i.e. Step 1, Days [redacted]
- The index tumour lesion for injection must have a minimum LD of [redacted] cm as measured by ultrasound or calliper
- The LTX-315 dose will be [redacted] mg ([redacted] mg/ml concentration) at each injection time point
- The number of LTX-315 injections to a single lesion on an LTX-315 dosing day will [redacted]
  - [redacted]
  - [redacted]
- In the event of CTCAE grade 3 or 4 AEs related to LTX-315, further injections with LTX-315 must be withheld until recovery of AE or reduction to grade 1 and patient is in clinically stable condition. Further injections of LTX 315 can then be considered at Investigators discretion.

Table 5. [redacted]

[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]		

[redacted]

- The LTX-315 injection schedule can be flexible [redacted] depending on clinical factors such as local injection site symptoms and reactive or inflammatory changes or necrosis
- LTX-315 will be injected into the [redacted]
- [redacted]

[redacted]

- If no T-cells can be expanded from the first lesion after treatment with LTX-315, a second lesion can be injected with the same schedule (repeat Step 1)

N.B. At least 1cm^3 of the resected (and LTX-315 injected) tumour lesion must be reserved for identification and expansion of TIL cells.

6.3.2 Production of TILs

6.3.2.1 Acquisition of tumour tissue

A tumour biopsy will be performed after sufficient tissue material for pathological examination has been removed. This procedure will mainly be performed by physicians at the Department of Orthopedic Surgery, Copenhagen University Hospital, Rigshospitalet or by physicians from other specialties if necessary. The procedure may require admission at the surgical department. See the TIL IMPD for more details (TIL IMPD 2017).

6.3.2.2 Establishment of "Young TILs" cultures

T-cells are expanded using a recently established method for "Young TILs" (10). The tumour mass will be isolated with a scalpel, and cut into [redacted]. Fragments (typically from [redacted] fragments in total) will be placed separately in the wells of a [redacted] well/plate. A TIL culture is established from each fragment by passive migration of T-cells from tumour tissue in the IL-2 based media. IL-2 belongs to the group of homeostatic cytokines which are characterised by having a positive effect on the activation of tumour specific T-cell and thereby tumour cell killing. T-cell density is kept at about [redacted] cells/ml growth media containing the immune stimulating cytokine IL-2. Cell cultures from the different fragments are pooled to a single cell culture. T-cell expansion is performed unselected to produce a polyclonal TIL repertoire targeted against multiple epitopes to potentially achieve more effective tumour cell destruction in vivo. The establishment of "Young TIL" cultures usually takes 2-4 weeks with a rate of success more than 90%. See the TIL IMPD for more details (TIL IMPD 2017).

6.3.2.3 Rapid Expansion Protocol

When the TIL cultures are expanded to approximately 5x10^7 cells they are either frozen for later use or transferred directly for further expansion by use of the Rapid Expansion Protocol in which TIL are grown with [redacted] In this way it is possible to reach a large number of activated tumour specific T-cell with a high level of activity against tumour associated antigens and tumour in [redacted] days. Ultimately, the autologous T-cells are concentrated in a 400 ml infusion bag for intravenous infusion. See the TIL IMPD for further details (TIL IMPD 2017).

6.4 Concomitant therapy

No systemic anticancer therapy or radiotherapy is to be used during the study, with the exception of palliative radiotherapy to a non-target lesion that does not represent disease progression.

Any agent with a known effect on the immune system is prohibited during the treatment period (see Appendix I). Patients are allowed to be on a stable dose of corticosteroids (up to

10 mg daily prednisolone or equivalent). No specific studies of interactions between LTX-315 and other agents have been conducted.

Any non-prohibited medication (prescription as well as non-prescription drugs including herbal medicine and natural health remedies) necessary for the patient's wellbeing which are not listed among the exclusion criteria and which do not interfere with the study evaluations, are permitted given that they are carefully followed by the Investigator and documented on the applicable eCRF page.

All concomitant medication and therapies used within 28 days prior to day 1 (screening) and up to the EoT visit are to be recorded in the eCRF.

In the follow-up period and until study completion the following will be recorded in the eCRF:

- concomitant medication and therapies used for soft tissue sarcoma or which may alter the course of the disease
- concomitant medication and therapies used for events related to the LTX-315 and adoptive T-cell therapy

The drug name or generic name, reason for the treatment, and the start and stop dates of administration are to be noted if possible. Any changes in the dose or frequency of administration of concomitant medications should be recorded in the eCRF. Treatments initiated due to AEs that occur during the study need to be documented in the eCRF, and in such cases the medication use will be documented until end of follow-up period for the AE.

6.5 Method of assigning patients

Patients are to be registered and assigned to treatment after written informed consent has been obtained and all pre-study evaluations have been completed. Patient eligibility should be documented on the patient eligibility eCRF page and in source documents.

The study site will be required to complete a Screening Log of all screened patients, regardless of whether the patient is registered in the study. Registered patients will be assigned a unique patient number. If a patient is withdrawn and replaced, the patient identifier number will not be reused. Patient numbers will be allocated sequentially by the Electronic Data Capture (EDC) system. This study will not employ randomisation.

6.6 Blinding (not applicable)

This is an open labelled study.

6.7 Emergency unblinding procedures (not applicable)

This is an open labelled study.

7 VISIT SCHEDULE AND ASSESMENTS

7.1 Visit schedule

Table 6.1 Visit Schedule from Screening to Step 1

		Step 1 - LTX-315 treatment											
Visit (V) number	1	2	3	4	5	61	6A[1, 15]	6B[1, 15]	6C[1]	6D	6E	6F	
Time (weeks)	Screening		[redacted]		[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	ET [2]
Time (days)	-28 to Step 1	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	-	-	-	-	-
Visit window (days)			+ 1	+ 1	± 1	-	± 1	± 1	-	-	-	-	-
Informed consent	X												
Eligibility criteria	X	X											
Medical history	X												
Demography	X												
Physical examination [5]	X	X	X	X	X		X	X					X
Height and weight [6]	X												X
ECOG performance status	X	X											X
Vital signs [7]	X	X	X	X	X	X	X	X	X				X
12-lead ECG [8]	X	X											
Blood pressure monitoring [9]		X	X	X	X		X	X					
Pregnancy test [10]	X	X											
Blood for clinical safety laboratory tests [11]	X	X		X									X
Coagulation [11]	X												X
Serology	X												
Urinalysis [11]	X	X											
Full tumour assessment (by CT/MRI scan) [12]	X									X	X	X	
Immunological blood sample (for PBMC) [13]	X												
Immunological blood sample (for serum) [13]	X												

Concomitant medication/therapy	X	X	X	X	X	X	X	X	X				X
Adverse events [14]	X	X	X	X	X	X	X	X	X				X
LTX-315 Prophylactic treatment		X	X	X	X		X	X					
LTX-315 administration [15]		X	X	X	X		X	X					
LTX-315 Injected lesion biopsies [16]		X				X			X				
Untreated lesion biopsies (optional)		X											
Surgical removing of LTX-315 injected lesion						X			X				
Hospital admission							X						

1. The time period from end of Step 1 to start of Step 2 will vary between patients, with a maximum of [redacted] weeks from surgery (W3, Step 1) until admission at W-2, Step 2 for patient with PD at enrollment. Visit 6[1] is only for these patients. Patients with SD at enrollment will have optional visits at Visit 6A[1] and Visit 6B[1] and mandatory visit at Visit 6C[1]. They will enter W-2 Step 2 after confirmed PD during the study
2. Patients who withdraw from the study before EoT visit must be seen for an ET visit. The visit is performed as soon as possible after withdrawal
5. Full physical examination to be performed at screening and on D1, Step 1 prior to LTX-315 injection. Only examination of respiratory and cardiovascular systems are required at visits thereafter, if clinically indicated
6. Height only to be measured at screening
7. Includes blood pressure, heart rate and temperature
8. On Step 1, Week 1, Day 1, 12-lead ECG must be performed prior to and if indicated repeated after the LTX-315 injection(s)
9. Blood pressure is to be monitored from just prior to LTX-315 injection, at 1, 3 and 5 minutes after each injection and then every 5 minutes until 30 minutes post last dose of the day, using an automated cuff system. At Week 2, Step 1, only pre-injection readings will be recorded unless there is an AE
10. Urine or serum sample (only for women with child-bearing potential) must be repeated at Day 1,Step 1, if more than 7 days after last pregnancy test
11. Safety laboratory samples are to be collected and analysed prior to LTX-315 injection on [redacted] and [redacted] (Step 1)
12. Tumour assessments by CT-scans of chest/abdomen/pelvis (head and neck if target tumours are in this area) are to be done according to RECIST 1.1. The screening scan is to be done within 28 days prior Day 1, Step 1
13. Blood samples will be collected once during screening to achieve baseline. A window of minus 3 days applies at later visits, and only one sample is sufficient. Should consist of 100 mL heparinized blood for isolation of PBMC and 10 mL blood in dry glass for collection of serum. [redacted] Sample will be collected just prior to hospital discharge
14. AEs severity graded according to CTCAE, version 4.0
15. See intra-tumoural injection manual for details. Day [redacted] and [redacted] are optional.
16. Baseline biopsies must be taken prior to LTX-315 injection on D1, Step 1

Table 6.2 Visit Schedule Step 2

	Step 2 – Adoptive T-cell therapy treatment																					
Visit (V) number	7	8	9	10	11	12	13	14	15	16		17	18	19	20	21	22	23	24	25	
Time (weeks)	W-2		W-1							W 1								W2	W3	W7	ET
Time (days)		[redacted]																			
Time (during day)										12	14	18									
Visit window (days)																				± 3	
Eligibility for Step 2	X	X																			
Physical examination [5]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
WeightWeight [6]		X																		X	X
ECOG performance status		X																		X	X
Vital signs [7]		X	X	X	X	X	X	X	X		X		X	X	X	X	X	X	X	X	X	X
12-lead ECG [8]		X							X													
Pregnancy test [10]		X																				
Blood for clinical safety laboratory tests [11]		X	X	X	X	X	X	X	X		X		X	X	X	X	X	X	X	X	X	X
Coagulation [11]		X	X	X	X	X	X	X	X		X		X	X	X	X	X	X	X	X	X	X
Serology																						
Urinalysis [11]		X																				
Full tumour assessment (by CT/MRI scan) [12]	X																			X	X	
Immunological blood sample (for PBMC) [13]		X															X			X		
Immunological blood sample (for serum) [13]		X		X		X		X			X			X		X				X		
Concomitant medication/therapy	X	X	X	X	X	X	X	X	X		X		X	X	X	X	X	X	X	X	X	X
Adverse events [14]	X	X	X	X	X	X	X	X	X		X		X	X	X	X	X	X	X	X	X	X
LTX-315 Injected lesion biopsies [16]																				X		
Untreated lesion biopsies (optional)																				X		
Renal function evaluation(Cr EDTA clearance) [17]	X																					
Hospital admission		X	X	X	X	X	X	X	X		X		X	X	X	X	X	X				
BAS test (for expected blood transfusion)		X																				

Sulfamethizole with trimethoprime 400/80 mg			X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X	X	
Aciclovir 400 mg x 2										X			X	X	X	X	X	X	X	X	X	
Cyclophosphamide infusion 60 mg/kg iv			X	X																		
Fludarabine phosphate 25 mg/m^2 iv					X	X	X	X	X													
TILs infusion iv										X												
Pegfilgrastime 6 mg sc											X											
IL-2 2MIU sc [3]												X	X	X	X	X	X	X	X			
End of treatment																					X	

2. Patients who withdraw from the study before EoT visit must be seen for an ET visit. The visit is performed as soon as poss ble after withdrawal
3. If the patient is well enough, he/she will be discharged from hospital after D7 and continue self-administration with IL-2 until D14
4. The[redacted], Step 2 visit is optional
5. Full physical examination to be performed at screening and on D1, Step 1 prior to LTX-315 injection. Only examination of respiratory and cardiovascular systems are required at visits thereafter, if clinically indicated
7. Includes blood pressure, heart rate and temperature
8. On Step 1, Week 1, Day 1, 12-lead ECG must be performed prior to and if indicated repeated after the LTX-315 injection(s)
10. Urine or serum sample (only for women with child-bearing potential) must be repeated at Day 1,Step 1, if more than 7 days after last pregnancy test
12. Tumour assessments by CT-scans of chest/abdomen/pelvis (head and neck if target tumours are in this area) are to be done according to RECIST 1.1.
13. Blood samples will be collected once during screening to achieve baseline. A window of minus 3 days applies at later visits, and only one sample is sufficient. Should consist of 100 mL heparinized blood for isolation of PBMC and 10 mL blood in dry glass for collection of serum. [redacted] Sample will be collected just prior to hospital discharge
14. AEs severity graded according to CTCAE, version 4.0
17. Evaluation of Cr EDTA clearance should be performed in the period after surgical removal of the LTX-315 treated lesion (Step 1) and before Step 2, Week -2, Day -8

Table 7. Visit Schedule in Follow-up Period

Visit	V26	V27	V28	V29	V30	ET
Time (weeks)	[redacted]					
Time (months)		[redacted]	[redacted]	[redacted]	[redacted]	
Adverse events [1]	X	X	X	X	X	X
Concomitant medication/therapy	X	X	X	X	X	X
Vital signs [2]	X	X	X	X	X	
Dispensing of Sulfamethizole with trimethoprime and Aciclovir	X	X				
Physical examination [3]	X	X	X	X	X	X
Weight	X	X				X
ECOG performance status	X	X	X	X	X	X
Vital signs						X
Blood for clinical safety laboratory tests [4]	X	X	X	X	X	X
Coagulation	X	X	X	X	X	X
CT/MRI scan						X
Immunological blood sample (for PBMC) [5]	X	X	X	X	X	
Immunological blood sample (for serum)[5]	X	X	X	X	X	
Full tumour assessment (by CT/MRI scan)[6]	X	X	X	X	X	
End of study						X

1. AEs severity graded according to CTCAE, version 4.0
2. Includes blood pressure, heart rate and temperature
3. Only respiratory and cardiovascular systems
4. See Section 7.5.3
5. Should consist of 100 mL heparinized blood for isolation of PBMC and 10 mL blood in dry glass for collection of serum
6. CT, MR, PET-CT or PET-MR scan can be used. A PET-CT-scan is preferred

7.2 Study procedures

7.2.1 Guidelines for supportive care and treatment

Supportive treatment is given on ordinary medical indications estimated by the Investigator responsible for the treatment. Any measures should be specified in the patient chart and flow sheet. The following is meant as guidance and other medications can be administered as it is seen fit. One exception is systemic corticosteroid which cannot be administered during LTX-315 treatment (see Appendix I).

7.2.2 Supportive treatment

T-cell therapy: During the infusion with T-cells the patient can experience shivering which can be relieved with the administration of subcutaneous Pethidine in a dose of 25 mg max x 4 if needed.

Also, light breathing difficulties can be observed with a possible decrease in saturation which can be treated with oxygen by nasal catheter.

Neutropenia: If simultaneous neutropenia and fever occurs it will be treated in accordance with Appendix III.

Diarrhea: Diarrhea will be treated immediately with the necessary supportive care and treatment, including Loperamide. Loperamide is to be withdrawn if blood or slime is observed in the faeces during the diarrhea. If this occurs, suitable diagnostic microbiological samples should be collected to exclude an infectious cause. Patients should also be instructed in the intake of plentiful amounts of fluids to prevent dehydration following the diarrhea.

Anemia: Transfusions with blood should be administered if haemaglobin < 6.0 mmol/L or if it is otherwise clinically indicated. Radiated and filtered blood may be given from Step 2, Day -7 and 6 months ahead.

Thrombocytopenia: Transfusion with platelets is indicated if platelets < 20/µl or if it is otherwise clinically indicated.

7.2.3 Local radiotherapy

Local radiotherapy can be prescribed for bone pains, wounds or if otherwise clinically indicated. Radiotherapy is preferably to be avoided within the first 3 weeks of Step 2 where the adoptive T-cell therapy treatment takes place for the interest of the patient. Radiated areas cannot be used as parameters in the assessment of treatment response. If possible, not all evaluable areas should be included in the radiated area. If this is not possible it is no longer possible to evaluate a response to treatment and the patient is withdrawn from the study.

7.3 End of treatment and follow-up

7.3.1 Normal end of treatment

The EoT visit will be performed 6 weeks after TIL infusion. If a patient is withdrawn prior to the scheduled EoT visit, they should complete the ET visit assessments as list in table 6.1 and 6.2.

All serious AEs (SAEs) and AEs considered related to study treatment that are ongoing at the EoT visit will be followed up until resolution or until clearly determined due to a patient's stable or chronic condition or intercurrent illness(s) or deemed irreversible. The reason(s) for a patient's withdrawal from the study are to be recorded in the patient's records and in the eCRF.

7.3.2 Follow-up

Patients will be followed with visits and diagnostic imaging at 12 weeks after TIL infusion (Step 2, Day 1) and every 3 months hereafter for up to 15 months.

In case of progression of the cancer, the patient will be referred back to the department in which they were followed before enrolment in the study. This department will be responsible for further treatment and follow up according to their local guidelines.

7.3.3 Standard care following study completion

After completion of the study, the patient will be treated at the Investigator's discretion or referred back to other physician according to standard practice.

7.4 Efficacy assessments

7.4.1 Local inflammatory responses

7.4.1.1 Biopsies

Biopsy and tumour resection samples will be collected and prepared for histological and immunohistochemical evaluation for verification of diagnosis.

The pathology interpretations will be done by a central laboratory.

All mandatory biopsies must include up to 3 ([redacted] gauge) core biopsies.

Biopsies of the tumour lesion planned for injection with LTX-315 will be done at:

- Baseline (pre-LTX-315 treatment) to assess T-cell infiltration (including CD8+)
- At the time of and as part of the excision of the injected lesion
- 6 weeks after TIL infusion if the whole tumour is not resected

A part of the resected lesion will be used for TIL production and another part for verification of diagnosis.

The remaining part of the excised tumour will be stored for future research on immune responses.

If an additional lesion is present and feasible for biopsy, this will be used as a bystander lesion. The optional lesion will not be injected and will allow a comparison (by biopsy) with the injected lesion.

In this scenario a patient would have 3 tumour lesions:

- One for LTX-315 injection
- A second to be followed by CT-scan for RECIST 1.1 response assessment (measurable, non-injected lesion)

- A third that is a “bystander” lesion (no LTX-315 injection) but available to biopsy to compare with biopsy of the injected lesion

If a >30% reduction in a non-injected lesion is observed by CT-scan any time after treatment, an optional biopsy can be taken from this lesion.

7.4.2 Systemic inflammatory responses

7.4.2.1 Blood samples for immunological analysis

A total of 510 ml of blood will be collected for research purposes in the period from screening until EoT (Step 2, Week 7). These blood samples are taken to assess the effect of treatment on the immune system for research purposes. The amount of blood taken during the course of the study does not exceed what the body itself is capable of producing between each test. Blood samples for research purposes will only be taken if the haemoglobin level is acceptable (≥ 6 mmol/L). Summaries of the immunological response parameters will be presented in a separate immunological report.

7.4.2.2 Identification of somatic mutations in the tumour

A PBMC sample will be collected as a source for normal cells for comparison of the DNA and RNA sequence in normal cells to the sequence in the tumour tissue. The sample will be collected at screening and will enable identification of somatic mutations in the tumour.

7.4.3 Immune monitoring

Change in immune biomarkers (in both peripheral blood and tumour samples) 2 weeks after LTX-315 treatment in patients with transdermally accessible tumours will be assessed. PBMC will be isolated from blood samples at screening, before and after T-cell therapy.

The following parameters may be monitored in biopsies and PBMCs in order to investigate and characterise the immune status of the patients and the nature of anti-tumour immune responses:

The immune biomarkers may include but is not limited to CD3, CD4, CD8, CD11, CD14, CD19, CD27, CD56, CD68, Ki67 and Granzyme B. T-cell receptor sequencing may be performed to monitor changes in the T-cell repertoire induced by treatment.

7.4.3.1 Identification of tumour-antigen specific T-cells

To generate a clinical effective T-cell response it is important that LTX-315 generates tumour-antigen specific T-cells. To identify sarcoma tumour-antigens in LTX-315 patients, next-generation sequencing and epitope prediction may be used. Tumour tissue DNA and RNA will be compared with data from matched normal tissue (PBMC) in order to identify tumour-unique (“somatic”) mutations. Patients will be HLA typed, and algorithms will then be used to predict which tumour-epitopes the patient’s tumour cells will express. Peptides corresponding to predicted tumour-epitopes will be synthesized and used to identify tumour-antigen specific T-cells in freshly expanded TILs as well as in PBMCs using MHC multimer–based screens. The effector functions of the tumour-antigen specific T-cells may be analysed by ELISPOT which will reveal whether cytokines such as IFNγ and or TNFα is produced by peptide stimulation. Tumour reactive T-cells may be identified in PBMC and expanded TILs by co-culturing with autologous/ allogenic tumour cells followed by flowcytometry analysis of IFNγ and TNFα. Details are described in a separate immunology protocol.

7.4.4 Anti-tumour effects in measurable tumours

A preliminary assessment on systemic anti-tumour activity will be done for selected measurable tumours according to RECIST 1.1 (16).

Tumour assessments by CT-scans (or MRI) of chest/abdomen/pelvis are to be done according to the hospital procedure as outlined in Appendix IV.

Additional CT-scans should be taken as clinically indicated in order to assess the total tumour burden of a patient. The baseline scan is to be done within 28 days prior to first LTX-315 treatment.

The RECIST 1.1 criteria for anti-tumour activity for measurable lesions will be used (Appendix IV).

7.4.5 Storage of biological samples for future research

Samples that are not used in the study will be transferred to a biobank at CCIT for future biomedical research for up to 15 years and is accepted by the Danish Data Protection Agency. If additional studies in other research areas are to be performed on any samples obtained during the conduct of this study/samples that are transferred to a new biobank, a request to do so will be submitted to the Scientific Ethics Committee, Capital Region of Denmark, as per the 'Act of Processing of Personal Data' § 5 and § 10, paragraph 2 and 3. After 15 years, any remaining tissue samples will be disposed of according to the local guidelines for destruction of biohazardous waste.

If a patient withdraws his/her informed consent, all biological material is to be destroyed if the patient wishes so.

7.5 Safety assessments

A full medical history of ongoing medical conditions or symptoms must be completed during the screening period (up to 28 period days prior to first LTX-315 dosing). Ongoing medical conditions will be graded according to CTCAE v. 4.0 at the time of screening. Abnormal laboratory values or vital signs at screening will be entered as a medical condition if viewed as clinical significant by the Investigator in which case a CTCAE v 4.0 grade should be assigned. AEs identified during the screening or based on screening tests, will be captured as Medical History

The patient's demographics, diagnosis and disease status/stage at screening must be recorded. The history of the cancer, including date of initial diagnosis, primary cancer site and the cancer type confirmed by histology must be recorded. Information should be collected on previous treatments for the malignant disease and concomitant non-malignant diseases. Recording of concomitant medication, see Section 6.4.

A pregnancy test (urine or serum) for women with child-bearing potential is to be done within 7 days prior to the start of LTX-315 dosing and within 7 days prior to start of chemotherapy (Step 2, Week -1, Day -7). The result is to be documented.

7.5.1 Vital signs, physical examinations and ECOG performance status

Vital signs (resting blood pressure, heart rate and body temperature) will be recorded.

Blood pressure should be monitored from prior to each LTX-315 injection, [redacted]. Blood pressure measurement should continue at a minimum of

[redacted] or as clinically indicated. The blood pressure readings pre-dose, at 1, 3 and 5 minutes will be entered in the eCRF during Week 1, Step 1. At Week 2, Step 1, only pre-injection readings will be recorded unless there is an AE.

The patient will be observed for minimum one hour post last daily LTX-315 injection or longer at the Investigator's discretion.

A complete, physical examinations required at screening and Week 1, D1 in Step 1. Thereafter, only the respiratory and cardiovascular body systems need to be examined at other visits, if clinically indicated. The physical examination findings and relevant parameters are to be documented in the medical source documents and the eCRF. Any clinically significant abnormal findings observed and recorded during the treatment period will be recorded as AE.

The patient's height (without shoes) and weight will be recorded (indoor clothing, without shoes).

ECOG performance status will be scored in accordance with the ECOG performance scale (see Appendix V).

7.5.2 Electrocardiogram (ECG)

12-lead ECG will be evaluated locally at the study site prior to LTX-315 administration. Any clinically significant abnormal findings observed and recorded during the treatment period (Step 1 and Step 2) will be recorded as AEs. The same method of assessment should be used throughout the study.

7.5.3 Local clinical safety laboratory evaluations

Blood and urine samples will be collected for clinical safety laboratory tests. The parameters to be analysed during screening, Step 1, Step 2 and T-cell follow-up are listed in Table 8.

Table 8. Clinical safety laboratory tests

Screening Visit 50 ml during screening	**Step 1** 20 ml per day	**Step 2** [2] 25 ml per day	**T-cell Follow-up** [3] 20 ml per day
Haemoglobin Thrombocytes Leukocytes Differential-count [1] Haematocrit Erythrocyte count	Haemoglobin Thrombocytes Leukocytes Differential-count [1] Haematocrit Erythrocyte count	*Haemoglobin* *Thrombocytes* *Leukocytes* *Differential-count* [1]	Haemoglobin Thrombocytes Leukocytes Differential-count [1]
Creatinine Sodium Potassium Ionized calcium Phosphate Magnesium Chloride Serum glucose	Creatinine Sodium Potassium Ionized calcium Phosphate Magnesium Chloride Serum glucose	*Creatinine* *Sodium* *Potassium* Ionized calcium Phosphate Magnesium	Creatinine Sodium Potassium Ionized calcium Phosphate Magnesium
ALAT ASAT ALP Bilirubin Albumin LDH Urea	ALAT ASAT ALP Bilirubin Albumin LDH Urea	ALAT ASAT ALP Bilirubin	ALAT ASAT ALP Bilirubin Albumin LDH
CRP	CRP	CRP	CRP
Coagulation [4]**:** APTT P-Coagulation factors II-VII-X INR	**Serology** [4]**:** HIV Hepatitis B: HBVsAg, HBVsAb, HBVcAb Hepatitis C: HCVAb HTLV: HTLV-IgG Epstein-Barr: P-EBV Syphilis: Treponema		**Urine analysis** (dipstick, pos/neg result): Protein Glucose Blood leucocytes

1. ANC to be provided or calculated
2. In Step 2, blood collected twice during the day (1. and 2. sampling). *Second blood sample analysis marked in italics*
3. Collected at EoT (Step 2, Week 7) and in Follow-up period
4. Test included in blood drawn for clinical safety laboratory tests

In case of a clinically significant abnormal laboratory value or other measurement, the Investigator should repeat the test if appropriate, to verify the result. The Investigator must comment on all clinically significant abnormal laboratory value. If the clinical significant abnormal laboratory value requires active management, it should be reported as an AE unless part of an already reported AE.

All blood samples for local laboratory evaluation will be collected by trained personnel at the study site and sent to hospital's laboratory with a study requisition form for analysis the same

day as collected. Urine samples will be collected at the study site, and will be analysed immediately by trained study personnel.

7.5.4 Pregnancy test

Urine or serum hCG test will be performed at specified timepoints.

7.5.5 Total blood volume

Blood samples will be drawn for clinical safety laboratory evaluations and immune monitoring.

From screening until EoT visit, the estimated volumes of blood to be drawn per patient are:

- Clinical Safety Laboratory: 485 ml to 685 ml dependent on day of discharge from hospital. If additional blood samples for safety are required; a total of 20-25 ml blood will be taken per day
- Immune monitoring for PBMC and serum: 510 ml

At each follow-up visit the total volume of blood drawn from each patient will be 130 mL.

The amount of blood taken during the course of the study does not exceed what the body itself is capable of producing between each test. Blood samples for research purposes will only be taken if the haemoglobin level is acceptable (≥ 6 mmol/L).

7.6 Adverse events and serious adverse events

7.6.1 Definitions

Adverse Events: any untoward medical occurrence in a subject or clinical study subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. This includes clinically significant laboratory abnormalities as judged by the Investigator.

Serious Adverse Events: A SAE is an AE which results in one of the 6 outcomes described below (International Conference of Harmonisation (ICH) serious criteria). A SAE can occur during any phase of the study and at any dose of the investigational product. This is particularly true for an AE that:

- results in death
- is immediately life-threatening
- requires patient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- is an important medical event that may have jeopardised the patient or may have required medical intervention to prevent one of the outcomes listed above

Medical and scientific judgement is to be exercised in deciding on the seriousness of a case. Important medical events may not be immediately life-threatening or result in death or hospitalisation, but may jeopardise the patient or may require intervention to prevent one of the listed outcomes in the definitions above. In such situations, or in doubtful cases, the case should be considered as serious. Drug misuse and drug overdose should be regarded as serious, even if they may not result in the above mentioned outcomes.

Hospitalisation at the Investigator's discretion is allowed and if this is for observation or social reasons this would not qualify as serious unless there is an associated AE warranting hospitalisation.

Treatment emergent AEs (TEAEs): AEs/SAEs occurring during or after administration of the IMP. AEs occurring before administration of the IMP are considered as non-treatment emergent and recorded as Medical history. Non-treatment emergent AEs/SAEs which are worsened after treatment with IMP will be added as new event and assessed as treatment emergent.

Severity

The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations

Severity will be evaluated by Investigator using the CTCAE v. 4.0:

- Grade 1: Mild: the AE is asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2: Moderate: minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (ADL)
- Grade 3: Severe: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL.
- Grade 4: Life-threatening: the AE has life-threatening consequences OR urgent intervention indicated.
    - Note, a grade 4 event is not always life-threatening. The ICH serious criterion "life-threatening" is used for reporting purposes.

Grade 5: Death related to AE.

7.6.2 Reporting of adverse events

All AEs are to be recorded in the eCRF, however:

- AEs with onset in the screening period or based on a screening procedure, will be recoded in the Medical History form
- AEs with onset in the treatment period will be recorded on the AE form
- AEs with onset In the follow-up period, only AEs considered related to either LTX-315 or TIL treatment are to be recorded on the AE form. No other AE collection will be done.

AEs will be recorded in response to asking the patient if they have experienced any unusual events since last visit at the site.

AEs will be collected from the time the patient signs the informed consent form. All AEs must be recorded in the patient's medical record and in the eCRF, defining relationship to study medication and severity.

Conditions that existed before screening and inclusion in the study, as well as planned hospital admissions, including hospital admissions as part of the study visit schedule, or surgical procedures for a pre-existing condition, are not to be considered as AE unless there is significant worsening during the study period.

All SAEs and AEs leading to discontinuation of IMP either observed by the Investigator or patient must be reported by the Investigator and evaluated.

The Investigator should report the diagnosis, if available. If no diagnosis is available, the Investigator should record each sign and symptom as individual AEs using separate AE forms.

Signs and symptoms of the treated cancer which, according to the investigator are expected and well known both in intensity and frequency, should not be reported as AEs or SAEs. Any unexpected change in the intensity or frequency should be reported as an AE or SAE as applicable. In addition, all deaths (including death caused by progession of disease) and secondary cancers must be reported as SAE

Timelines for initial reporting of AEs:

The investigator must complete the AE form in the eCRF:

- For SAEs within 24 hours of the Investigator's first knowledge of the SAE
- For all other AEs on an ongoing basis

If the eCRF is unavailable, the concerned SAE information must be reported on a paper SAE form and sent to KLIFO by fax or e-mail within the same timelines as stated above. When the eCRF becomes available again, the Investigator must enter the information on the form into the eCRF.

7.6.3 Causality of AEs

The causality of AE/SAEs (i.e., their relationship to study treatment) will be assessed by the Investigator at the study centre for all IMPs. Assessment of causality is based on the following considerations: associative connections (time associative connections (time or place)), pharmacological explanations, previous knowledge of the drug, presence of characteristic clinical or pathological phenomena, and exclusion of other causes and/or

absence of alternative etiology. The Investigator will be asked to assess causal relationship to the study medication according to the classifications listed in Appendix VI.

Causality will be categorised as "Related" or "Not related" to the IMP:

- Related/Possible related: The AE is related to the IMP or a causal relationship is conceivable and cannot be dismissed
- Not related/Unlikely related: The event is most likely related to an aetiology other than the IMP
    - In case the AE is deemed Not related/Unlikely related, an alternative aetiology should be described e.g. concomitant disease, concomitant medication or treated disease

Sponsor will assess causality of all SAEs to determine if the SAE is in scope for expedited reporting.

7.6.4 Procedure for reporting serious adverse events

Pharmacovigilance for the study is handled by [redacted]..

SUSARs will be reported by [redacted] according to appropriate Competent Authority and Ethical Committee requirements. [redacted] will report SUSARs to Investigators according to ICH Good Clinical Practice and to local regulations. The competent authorities will be notified of all SUSARs through the Eudravigilance database and in accordance to local legislation.

Investigators must inform [redacted] of any SAE that occurs in the course of the study **within 24 hours** (i.e. immediately) of when he or she becomes aware of it. This is done by entering the SAE into the eCRF within 24 hours of awareness. In case of IT failure, the SAE paper form must be completed and sent to [redacted]

To discuss the medical aspects of the SAE Investigator should call Medical Monitor, if needed.

The SAE must be documented in the patient's medical source documents and the outcome described.

Follow-up information on SAEs must also be reported by the Investigator within the same time frames.

If a non-serious AE becomes serious, this and other relevant follow-up information must also be provided to safety group within 24 hours as described.

Withdrawals from treatment due to a new, previously unreported SAE must be notified to the Medical Monitor, along with the SAE.

In case an AE results in withdrawal of the patient, the reason must be recorded.

7.6.5 AE/SAE follow-up

All AEs that were ongoing at the EoT visit will be followed up by the Investigator until the last follow-up visit or until the AE has resolved, unless in the Investigator's opinion the condition is unlikely to resolve due to the patient's underlying disease.

For patients withdrawn, AEs must be followed up for 28 days after the last dose of IMP or until resolution, whichever comes first.

All SAEs must be followed up by the Investigator until resolved, unless in the Investigator's opinion the condition is unlikely to resolve due to the patient's underlying disease.

Additional site visits to follow-up injection site reactions and AE/SAEs can be done if deemed necessary by the Investigator. Additional post treatment follow-up visits may be scheduled by the Investigator according to clinical judgement.

Additional safety information is provided in Appendix V.

7.6.6 Pregnancy

Each pregnancy that occurs during the trial participation must be reported to sponsor within 24 hours of learning of its occurrence using the pregnancy form. The pregnancy must be followed up to determine outcome and status of mother and child. The child must be followed at least to the age of one month. Pregnancy complications and elective terminations for medical reasons must be reported as an AE or SAE. Spontaneous abortions must be reported as an SAE.

7.7 Known adverse reactions to LTX-315 and adoptive T-cell therapy

7.7.1 LTX-315

Adverse reactions with LTX-315 administration are modest and predictable and include most commonly low grade (CTCAE v. 4.0 grade 1-2) allergy-like AEs including symptoms and signs of flushing, paraesthesia, pruritis, rash and asymptomatic hypotension. These allergy-like AEs emerge quickly (seconds to minutes) and resolve quickly (minutes). For additional information please see current IB (28).

7.7.2 Chemotherapy

The adverse reactions to chemotherapy described in the following are all general adverse reactions seen when the drugs are given as the primary treatment for oncological and haematological diseases.

In these cases the treatment is often given over several series. In this study, the treatment will be given as a single dose, why a milder adverse reaction profile is expected.

7.7.3 Cyclophosphamide

The adverse reactions are myelosuppression (neutropenia, thrombocytopenia and anemia) and urotoxicity (cystitis, haematuria and haemorrhagic cystitis). Sufficient treatment with

Mesna alongside rehydration markedly reduces the frequency and severity of the urotoxicity. Other common adverse reactions are alopecia, nausea and vomiting.

Patients receiving treatment with cyclophosphamide can experience the following adverse reactions described in the current version of the summary of product characteristics (42).

7.7.4 Fludarabine phosphate

The adverse reactions are myelosuppression (neutropenia, thrombocytopenia and anemia), infection including pneumonia, coughing, fatigue, limpness, nausea, vomiting and diarrhea. Other common adverse reactions are shivering, edema, malaise, peripheral neuropathy, visual disturbances, anorexia, mucositis, stomatitis and rash. Severe opportunistic infections have occurred in patients receiving treatment with fludarabine phosphate. Deaths have been recorded as a cause of severe adverse reactions.

Patients receiving treatment with fludarabine phosphate can experience the following adverse reactions described in the current version of the summary of product characteristics (19).

7.7.5 TILs

No SAEs are expected due to TIL infusion. The patients might experience transient fever, shivering and mild dyspnoea with a few cases of an observed light decrease in oxygen saturation. There is a theoretical risk of the development of allergic reactions/anaphylactic shock. This has not yet been observed according to literature. See the IMPD for more details on previous human exposure and anticipated risks (TIL IMPD 2017).

7.7.6 Interleukin-2

Frequency and severity of adverse reactions to IL-2 has generally shown to be related to the route of administration, dose and frequency of treatment. Most adverse reactions are self-limiting and will disappear within 1-2 days after cessation of treatment. Subcutaneous IL-2 administration can cause a local inflammatory reaction with reddening and induration at the site of injection. World Health Organization (WHO) grade 1 toxicity characterised by flu-like symptoms with muscle soreness, joint pains, malaise and a slight increase in temperature with a duration of 12-18 hours has been observed when administering IL-2 locally. Experience shows that fatigue is the most common AE when administering the IL-2 dose used in this study. Detailed information on adverse reactions are described in the current version of the summary of product characteristics (37).

Any other adverse effects will be treated according to local guidelines.

7.8 Safety monitoring and precautions

7.8.1 Haematological parameters

Careful haematological monitoring of blood counts is indicated for all patients during treatment. Leukocyte count, platelet count and haemoglobin values will be controlled at fixed intervals. Measurements will be made before start of chemotherapy, IL-2 and daily during treatment until neutrophile counts is > 500/µl and leukocyte count is > 1000/µl. Chemotherapy will not be given to patients with a leukocyte count < 500/µl and/or platelet number < 50.000/ µl before the start of chemotherapy.

7.8.2 Kidney and urine infections

Any obstruction of the efferent urinary tracts, cystitis or infection will be resolved before start of treatment. Patients will be treated with Mesna and fluid therapy to decrease the frequency and severity of bladder toxicity. Treatment will be terminated if cystitis associated with micro- or macroscopic haematuria occurs during treatment with cyclophosphamide. The patients' urine will be controlled for the presence of microscopic haematuria before start of treatment with cyclophosphamide.

7.8.3 Cardiotoxicity

Cardiotoxicity is especially seen when administrating high doses of cyclophosphamide (120-240 mg/kg body weight). An electrocardiogram will be performed before the start of treatment. Patients with known heart disease will not be included in the study. Necessary investigational procedures will be performed if the patients experiences symptoms from the cardiovascular system (e.g. chest pains, shortness of breath).

7.8.4 Infertility

Women receiving chemotherapeutic treatment have a risk of affecting their fertility in the future.

7.8.5 Interactions

Cyclophosphamide inhibits cholinesterase activity which increases the effect of depolarizing muscle relaxants such as suxamethoniumchloride. This can results in prolonged apnea when anesthetized. The anesthesiologist is to be informed if the patient has received treatment with cyclophosphamide within 10 days before treatment with suxamethoniumchloride. The combination should be avoided.

The patient is to avoid eating grapefruit or drinking grapefruit juice since grapefruit contains a substance that can impair the activation of cyclophosphamide and thereby its effect.

Transfusion related graft-versus-host reactions have been observed in patients receiving treatment with fludarabine phosphate after transfusion with non-radiated/non-filtered blood. Patients requiring blood transfusion within 6 months from treatment with fludarabine phosphate is therefore to receive only irradiated or filtered blood. An agreement with the blood bank at Herlev Hospital has been made so that there will be ordered irradiated blood only for these patients for 6 months after treatment. All blood in the Capital Region is filtered.

8 DATA HANDLING AND QUALITY ASSURANCE

8.1 Data collection

The main objective is to obtain those data required by the study protocol in a complete, accurate, legible and timely fashion. EDC will be used for recording data from each patient, into an eCRF. The eCRF is based on the EDC system IBM Clinical Development, which is a Software as a Service (SaaS) application compliant with 21 CFR Part 11 regulation provided by IBM. The data in the eCRFs should be consistent with the relevant source documents. It is the responsibility of the Investigator to ensure that these eCRFs are properly completed. The Investigator will sign the eCRF screen/pages to confirm that the information on each screen is accurate and complete.

All people involved in the study will get appropriate training to handle the EDC system.

The Investigator and authorised staff at the clinical site can add data to the eCRF and must keep the eCRF current to reflect patient status during the study.

Only authorised access to the eCRF will be possible using encrypted username and password. Roles in the system are given according to functions. All tasks performed in the eCRF are logged in an audit trail.

The eCRF will contain data ranges and validation checks to maintain an ongoing quality check of data entered. All data validation will be performed as part of the eCRF system.

Medical history data and AEs will be coded according to Medical Dictionary for Regulatory Activities (MedDRA), and concomitant medication will be coded according to WHO drug dictionary.

The data will be monitored electronically and source data verification (a comparison of the data in the eCRFs with the patients' records at the hospital or practice, and other records relevant to the study) will take place at the site where all information within safety, tolerability and efficacy will be verified against the hospital patient records and other source documents.

For patients who fail to meet eligibility criteria at screening the following must be collected in the eCRF: demographics, reason for not continuing in the study, SAE and AEs.

8.2 Clinical data management

Data management will be provided by [redacted] Denmark. Clinical data management will be performed in accordance with applicable standards and data cleaning procedures and will comply with regulatory guidelines.

8.3 Data analysis and reporting

After the last patient has completed EoT (Step 2, Week 7), all data pertaining up to EoT period will be verified and locked for reporting of the results including preparation of a Clinical Study Report. The study will continue according to the protocol in the 15 months follow-up period until study completion.

An addendum to the Clinical Study Report reporting the results from the 15 months follow-up period will be prepared after the data generated during that period has been entered into the study database and the data has been verified and locked.

8.4 Archiving

The eCRFs and all medical records upon which the eCRFs are based (source data) must be kept for at least 15 years after completion of the study. Image carriers or other data carriers may be used for this purpose.

The Investigator has to keep a written or electronic patient file for every patient participating in the study.

8.5 Study monitoring

Before the initiation of the study, a representative for Sponsor will visit the investigational site to:

- Determine the adequacy of the facilities
- Discuss with the Investigator(s) (and other personnel involved with the study) their responsibilities with regard to protocol adherence, and the responsibilities of Sponsor or its representatives

During the study, a monitor will have regular contacts with the investigational site, including visits to:

- Provide information and support to the Investigator(s) and discuss findings and any other relevant issues with the Investigator and his/her personnel
- Confirm that facilities remain acceptable
- Confirm that the investigational team is adhering to the protocol, that data are being accurately recorded in the eCRFs, and that investigational product accountability checks are being performed
- Perform source data verification. Incorrect or missing entries into the eCRFs will be queried electronically and must be corrected immediately

8.6 Source Data

For all data recorded, the source document must be defined in a source document agreement or similar document at the study centre. There must only be one source defined at any time for any data element. Source data verification will require direct access to all original records for each patient (e.g. medical records).

The following should as a minimum be added to the patient's medical record:

- Date(s) of conducting the informed consent process (date of enrolment) including date of provision of information
- Eligibility criteria is met
- Patient number

- The fact that the patient is participating in a clinical study in soft tissue sarcoma including treatment with LTX-315 and adoptive T-cell therapy for up to 26 weeks and 15 months follow-up period
- Specification of the patient's cessation in the study (e.g. premature withdrawal)
- Other relevant medical information

8.7 Audit and inspection

During or after the study is completed, Sponsor representatives, regulatory authority representatives or Independent Ethics Committee (IEC) or Institutional Review Board (IRB) may wish to carry out an audit or inspection. These representatives must have the same access to study data and patient source data as the monitor.

9 STATISTICAL METHODS AND PLANNED ANALYSIS

This section presents the principal features of the statistical analysis of this study. Further details will be given in a separate statistical analysis plan (SAP).

Summary statistics of continuous variables will include: n, mean, median, standard deviation, minimum and maximum. Summary statistics of categorical variables will include number and percentage of patients, and, where applicable, the number of events in the corresponding group.

Baseline is used as reference to Step 1, Week 1, Day 1, End of Step 1 as reference to Step 2, Week 1 and EoT as reference to Step 2, Week 7 in the following.

9.1 Analysis data sets and populations

9.1.1 Full analysis set

Patients will be included in the full analysis set (FAS) if they receive at least one injection of LTX-315. The FAS is by this definition also the safety analysis set. FAS will be used for evaluation of all efficacy and safety endpoints. The analyses and presentations will be performed for the FAS population unless specified otherwise.

9.1.2 Per protocol analysis set

Patients will be included in the per protocol (PP) analysis set, if they have followed the study protocol without any major violations which may affect the assessment of the efficacy endpoints. This will be used as supportive for evaluation of all efficacy endpoints.

9.2 Randomisation (not applicable)

This study will not employ randomisation as this a one armed study.

9.3 Primary analyses

9.3.1 Primary efficacy endpoint

The primary efficacy endpoint is change in total T-cell level in tumour tissues from Baseline to end of Step 1 and will be presented by descriptive statistics using both FAS and PP analysis set.

9.3.2 Primary safety endpoint

The primary safety endpoint is AEs related to LTX-315 or to the combination of LTX-315 and adoptive T-cell therapy from Baseline to EoT.

Number of related events and number and percentage of patients reporting these events will be summarized by IMP relation, system organ class (SOC) and preferred term (PT), and by SOC and PT.

AEs will be coded using the MedDRA dictionary, latest version at time of first patient first visit.

Number of events and number and percentage of patients reporting these events will be summarized and in addition by SOC and PT for:

- TEAEs
- SAEs
- AEs leading to withdrawal
- AEs leading to death
- Severity of AEs by CTCAE grade

All data will be listed by patient.

9.4 Secondary analyses

9.4.1 Total T-cell level in tumour tissues

The change in total T-cell level in tumour tissues from Baseline to EoT will be presented by descriptive statistics using both FAS and PP analysis set.

9.4.2 Total CD8+ T-cell level

The change in the numbers of CD8+ T-cells in tumour tissues from Baseline to end of Step 1 and EoT will be presented by descriptive statistics using both FAS and PP analysis set.

9.4.3 Anti-tumour effect

The anti-tumour effect is assessed by

- Objective Response Rate (ORR)
- Clinical Benefit Rate (CBR)
- Progression free survival (PFS)

and will be presented using both FAS and PP analysis set as described below.

9.4.3.1 Objective response rate

ORR is defined as proportion of patients who according to RECIST 1.1 criteria (see Appendix IV) have acheived CR or PR. ORR at EoT and up to 15 months after EoT will be presented by descriptive statistics.

The objective tumour response (CR, PR, SD, PD) according to RECIST 1.1 criteria verified by imaging technique will be listed by patient and summarized by visit.

In addition, the best overall tumour response according to RECIST 1.1 criteria from screening until PD, death, or 15 months after EoT, whichever comes first will be listed and summarized.

9.4.3.2 Clinical benefit rate

CBR is defined as proportion of patients who according to RECISIT 1.1 criteria have achieved CR, PR or SD. CBR at EoT and up to 15 months after EoT will be presented by descriptive statistics.

9.4.3.3 Progression free survival

PFS is defined as the time in days from Baseline until PD or death. PFS will be estimated using the Kaplan-Meier method. A patient who leaves the study without evidence of progression or death will be censored at the last tumour assessment date. Progression free patients will be censored 15 months after EoT. Plots including the Kaplan-Meier curve and median survival time will be presented.

9.5 Exploratory analyses

The immunological response parameters are described in Section 7.4.3 Details will be described in a separate immunology protocol.

9.5.1 Tumour-antigen specific T-cells

Identification of tumour-antigen specific T-cells in tumour tissue and PBMCs by MHC multimer–based screens, ELISPOT and flowcytometry analysis of cytokines including IFNγ and TNFα, will be described in a separate immunological report.

9.5.2 Changes in immunologicical parameters

Changes in immunological parameters from Baseline to 15 months after EoT will be described in a separate immunological report.

9.6 Other assessments

The following assessments will be listed or presented by descriptive statistics: ECG, vital signs, blood and urine clinical safety laboratory tests, serology, BAS test, physical examination, height, weight, ECOG performance status, full tumour assessment, renal function evaluation.

The overall survival 15 months after EoT will be estimated and presented using the Kaplan-Meier method as described for PFS.

9.7 Sample size determination

This is a one armed study without sample size calculation. 6 patients completing Step 1 are planned to be enrolled in the study.

9.8 Handling of missing values

Missing data for time to event endpoints is handled by time to event methods. No imputation of missing data is planned for other endpoints.

10 REGULATORY AND ADMINISTRATIVE PROCEDURES

10.1 Ethics Committee and good clinical practice

This study has been designed in accordance with the Declaration of Helsinki (52).

The study will be managed and conducted according to the study protocol, the latest international (ICH) guidelines for Good Clinical Practice (GCP) (24) and applicable regulatory requirements. A copy of these guidelines can be found in the Investigator's Site File.

10.1.1 Ethics committee and regulatory authority review

The final study protocol, including the final version of the written patient information sheet and informed consent form, must be approved or given a favourable opinion in writing by an IEC or IRB as appropriate. The Investigator must submit a copy of the written approval to the Sponsor before he or she can enrol any patient into the study.

The appropriate regulatory authority must be notified of/approve the clinical study as required.

10.2 Changes to the conduct of the study or protocol

10.2.1 Protocol amendments

Changes in the study protocol must take the form of written study protocol amendments.

These will require the approval of all signatories of the final protocol. Any amendments to the protocol which affect the patient, e.g. changes in procedures/assessments or matters relating to patient safety, require a favourable opinion approval from the IEC for the study centre concerned, prior to implementation. Changes of a purely administrative nature should be notified to the IEC, but do not require formal approval. Any amendment affecting the patient requires further informed consent from each patient before implementation.

10.2.2 Protocol deviations

Deviations from the study protocol, especially the prescription of doses not scheduled in the study protocol, other modes of administration, other indications, and longer treatment periods are not permissible (except in an emergency).

10.3 Informed consent process

The consent of the patient to participate in the study has to be given in writing prior to enrolment. The patient must be given sufficient time to consider the study before deciding whether to participate. Each patient must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical treatment or relationship with the Investigator.

The consent must be signed and personally dated by the patient and by the Investigator or a sub-Investigator designated by the Investigator to conduct the informed consent discussion. The signed and dated declaration of informed consent will remain at the investigator's site and must be safely archived by the investigators so that the forms can be retrieved at any time for monitoring, auditing and inspection purposes. A copy of the signed and dated information and consent should be provided to the patient prior to participation and a copy maintained in the patient's source documents if there is a separate archive file for the informed consent documents for the study.

After review of a patient information sheet and oral information to the patient and the provision of written informed consent, study specific screening procedures will be initiated.

10.4 Investigator's responsibilities

The investigator shall be responsible for ensuring that the study is performed in accordance with the protocol and the current revision of the Declaration of Helsinki (52), as well as with the Note for Guidance on Good Clinical Practice (24) and applicable regulatory requirements. These documents state that the informed consent of the patients is an essential precondition for participation in the clinical study.

10.4.1 Training of staff

The Investigator must maintain a record of all individuals involved in the study (medical, nursing and other staff). He or she will ensure that appropriate training relevant to the study is given to all these staff, and that they will receive any new information of relevance to the performance of this study.

10.5 Patient privacy

The Investigator must ensure that patient's confidentiality will be maintained. eCRFs or other documents submitted to the Sponsor should only identify patients by their patient number and study number. The Investigator should **keep a separate log of patient codes and names**. Documents not for submission to the Sponsor, e.g. patient's completed informed concent form, should be retained by the Investigator in strict confidence.

The Investigator is required to record primary efficacy and safety data, concomitant medication and patient progress in the patient's file/notes/medical record.

The patient's medical records will be reviewed by the study monitor and possibly by other Sponsor personnel or regulatory authorities, to verify adequate source documentation, accuracy and completeness of eCRFs. The review will be conducted with strict adherence to professional standards of confidentiality.

All patients screened for the study will be entered chronologically on the Patient Screening Log at the initial visit. An explanation for exclusion from admission to the protocol is to be provided on the Patient Screening Log.

10.6 Insurance and liability

Lytix Biopharma has implemented No Fault Compensation Insurance for Clinical Trials with Howden Insurance Brokers Inc. Liability for study induced injury will be covered according to local requirements.

10.7 Premature study termination criteria

The study centre or the whole study may be discontinued at the discretion of the Principal Investigator or the Sponsor in the event of any of the following:

- Occurrence of either SUSAR or an increase in known adverse drug reactions which impose an unacceptable safety concern
- Medical or ethical reasons affecting the continued performance of the study
- Difficulties in the recruitment of patients

- Cancellation of drug development

10.8 End of study

The end of the study is defined as the last visit of the last patient included in the study. The competent authorities and IEC/IRB will be notified about the end of the study.

10.9 Publication of results

All data generated from the conduct of this study is the sole property of Lytix Biopharma. Regulatory agencies and authorities will have unrestricted access to the data as required by applicable guidance, regulation and law. Lytix Biopharma retains the exclusive right to decide when to publish data from this study. When Lytix Biopharma decides to publish the results of this study in a scientific journal and/or at a scientific meeting then the following stipulations will apply:

- The authorship of this publication will include the Principal Investigator and her/his team, and representatives for Lytix Biopharma.

The published international guidelines for authorship (25) will be adhered to; i.e., 'All persons designated as authors should qualify for authorship. Each author should have participated sufficiently in the work to take public responsibility for the content.'

All draft publications, including abstracts or detailed summaries of any proposed presentations, must be submitted to the Sponsor at the earliest practicable time for review, but at any event not less than 30 days before submission or presentation unless otherwise set forth in the clinical trial agreement. Sponsor shall have the right to delete any confidential or proprietary information contained in any proposed presentation or abstract and may delay publication for up to 60 days for purposes of filing a patent application.

The information developed during the conduct of this clinical study is confidential and may only be disclosed as authorised in writing by Lytix Biopharma.

After the conclusion of the study, a report will be written which will include a descriptive statistical analysis and an appraisal of the results from a medical viewpoint. This report will be based on the items listed in this study protocol. Any publication of the results, either in part or in total (articles in journals or newspapers, oral presentation, etc.) by the Investigators, requires the approval of Lytix Biopharma.

Sponsor commits to communicate and make available for public disclosure, the results of the clinical study regardless of outcome. Public disclosure implies publication in scientific journals, abstract submission for scientific meetings and other types of disclosure (e.g. via cinicaltrials.gov).

10.10 Confidentiality

The investigator must agree to maintain the confidentiality of the study at all times and must not reveal information relating to the Investigator's Brochure, protocol, eCRFs or associated documents to unauthorised third parties.

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12 APPENDIX I

PROHIBITED MEDICATIONS

The following list is not exhaustive. When in doubt, contact the 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 • TNF binding proteins

* See exceptions for steroid concomitant therapy (see Section 6.4)

Immunostimulants
Levamisole Thalidomide Interferons

13 APPENDIX II

EFFECTIVE METHODS OF BIRTH CONTROL

Acceptable methods of contraception include:

- combined (oestrogen 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]
- vasectomised partner [2,3]
- sexual abstinence [4]

1. Hormonal contraception may be susceptible to interaction with the IMP, which may reduce the efficacy of the contraception method.
2. Contraception methods in this context are considered to have low user dependency.
3. Vasectomised partner provided that partner is the sole sexual partner of the WOCBP study participant and that the vasectomised 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 need to be evaluated in relation to the duration of the duration of the clinical study and the preferred usual lifestyle of the patient.

14 APPENDIX III

FEBRIL NEUTROPENI UNDER T-CELLE TERAPI

Neutropeni (granulocytopeni) neutrofiltal < 0,5 mia./l

Feber = tp > 38° C (rektalt > 38,5° C)

OBS: Begrundet mistanke eller påvist infektion + neutropeni + feber = sepsis!

Patienter der får T-celle terapi udvikler forventeligt neutropeni fra ca. dag 0 og 7 dage frem (kan variere betydeligt) og de udvikler forventeligt febrilia ligeledes dag 0 (efter T-celle infusion eller start af IL-2) indtil ca. 1 døgn efter ophør med IL-2.

Bivirkninger til IL-2 ligner det kliniske billede ved sepsis og en eventuelt samtidig infektion/sepsis kan derfor maskeres under IL-2 behandlingen. Der er derfor indikation for at indlede empirisk behandling med antibiotika hos patienter med febril neutropeni under T-celle terapi.

Fremgangsmåde

Diagnostik

1. Objektiv us
2. Dyrkning for bakterier og svamp fra
    - Blod (dyrkning fra CVK + evt. perifert)
    - Urin
    - Ved symptomer og/eller objektive fund endvidere: ekspektorat/svælgpodning, podning fra kateter, fæces for tarmpatogene og evt. clostridium difficile, podning fra sår
3. Rtg thorax (på stuen; kan udelades initialt (dag 0) hvis der ikke er mistanke om pneumoni)
4. CRP, hæmatologi, kreatinin/væsketal, levertal, DIC-prøver ved svær sepsis/septisk shock.

Behandling

Initialt

- Piperacillin+tazobactam (Tazocin) 4 g x 3 i.v.
- Ved penicillin-allergi: meropenem (Meronem) 1 g x 3 i.v.

Ved klinisk påvirket patient og/eller mistanke om infektion (septisk shock/svær sepsis) kan der suppleres med gentamycin (Hexamycin®) 5 mg/kg x 1 i.v.

OBS forsigtighed da kombinationen af gentamicin og IL-2 kan medføre øget risiko for nefrotoxicitet. Der gives max 3 doser gentamicin.

Ved nyreinsufficiens eller anden risiko for nefrotoxicitet (fx tidligere cisplatinbehandling) kan der i stedet anvendes ciprofloxacin (Ciproxin®) 400 mg x 2 i.v., ved svær nyreinsufficiens (GFR < 30 ml/min) 400 mg x 1 i.v.

Ved positivt dyrkningsfund foretages relevant ændring jævnfør svar. Gram negativ dækning bør dog som regel bibeholdes neutropeniperioden ud.

Evaluering under IL-2 behandling (dag 0-5)

1. Ved klinisk stabil patient med forventelige bivirkninger til IL-2 fortsættes antibiotisk behandling uændret indtil patienten har været afebril i minimum 2 døgn og neutrofile er > 0,5 x 10^9/l.

2. Ved klinisk forværring under IL-2 og mistanke om infektion (septisk shock/svær sepsis) foretages grundig objektiv us. og initiale undersøgelser gentages.

Vigtige overvejelser:

- Lungeinfiltrater: HRCT-thorax til diagnostik af evt. svampe-pneumoni. Endvidere evt. BAL, evt. ekspektorat til D+R og undersøgelse for Legionella (PCR) og Legionella antigen i urin (LUT), evt. mundskyl for pneumocyster. Evt. tillæg af azithromycin (Zitromax®) 500 mg x 1 i.v. alternativt ciproxin 400 mg x 2 i.v. i 7-10 dage til dækning af Legionella/mycoplasma.
- I.v. kateter-infektion: In situ med rødme ved indstikssted: Tillæg af vancomycin 1 g x 2 i.v., dosis-reduktion ved nyreinsufficiens, hvor der endvidere bør udføres regelmæssige serum vancomycin koncentrationsbestemmelser. Ved tunnel-infektion med rødme, ømhed langs tunnel og pussekretion fra indstikssted bør kateter endvidere fjernes.

Normal obj. us: skift af initiale behandling til meropenem (Meronem) 1 g x 3 i.v.

Evaluering ved fortsat febrilia >1 døgn efter ophør med IL-2

Febrilia udløst af IL-2 aftager typisk indenfor det første døgn efter afsluttet IL-2 behandling.

Ved klinisk påvirket patient, evt. med stigende tp. eller hvis tp. ikke er faldende 1-2 døgn efter ophør med IL-2 gentages grundig obj. us, initiale undersøgelser og vigtige overvejelser.

Nedenstående overvejes ved langvarig febril neutropeni efter afsluttet IL-2

- Fornyet grundig obj. us omfattende mundslimhinde (især mhp. svampekolonisering), genitalia externa og rektal inspektion.
- Gentagelse af initiale undersøgelser suppleret med aspergillus galactomannan antigen (SSI - sendes via KMA), CMV-antigen (SSI- sendes via KMA) og (ved normal rtg. af thorax) HRCT-scanning af thorax og UL, alternativt CT af lever/milt (aspergillus i lunger, dissemineret candidiasis i lever/milt). Systemisk

svampebehandling bør overvejes især ved påvirket patient med svampekolonisering af ex. mundhule.

Behandlingsophør

Antibiotika fortsættes indtil patienten har været afebril i minimum 2 døgn og neutrofile er > 0,5 x 10^9/l. Dog gives altid minimum 7 døgns behandling.

Er granulocyt-tallet stigende og patienten afebril kan behandlingen evt. ændres til peroral antibiotika, fx tabl. Penicillin 1MIE x 3 p.o. + tabl. Ciprofloxacin 500 mg x 2 peroralt. Ved penicillinallergi erstattes penicillin af Roxithromycin-dosis: 150 mg x 2 peroralt.

Tilpasning af antibiotika ved positive dyrkningsfund

Pseudomonas aeruginosa: Tazocin® 4 g x 3 i.v. i kombination med gentamycin i.v. 5 mg/kg x 1 til granulocyttal > 0,5 mia./l. Ved nyrefunktionsnedsættelse erstattes gentamycin af ciprofloxacin 400 mg x 2 i.v., se ovenfor.

Koagulase negative stafylokokker (overvej dog evt. forurening, ex. ved påvisning i 1 af 5 bloddyrkningskolber eller i ekspektorat hos patient uden pneumoni-symptomer): Vancomycin (Vancomycin®) 1 g x 2 i.v.

Clostridium difficile: Vancomycin® 125 mg x 4 p.o. i 10-14 dage (min. 10 dage). Ved påvirket patient (sepsis/ileus) Vancomycin® 500 mg x 4 kombineret med metronidazol (Metronidazol®) 500 mg x 3 i.v., samt evt. rektal indhældning af 500 mg vancomycin i 100 ml saltvandsopløsning hver 4.-12. time.

ESBL: gram negativ bakterie med extended spectrum beta-laktamase: Meropenem (Meronem®) 1 g x 3 i.v. Ved tidligere dokumenteret ESBL bærerskab bør patienten behandles initialt med Meropenem ved feber såfremt der ikke er andet oplagt fokus hvor ESBL producerende stamme er usandsynlig.

Stafylokokkus aureus: Dicloxacillin (Diclocil®) 1 g x 4 i.v. Patient bør vurderes for evt. endocarditis.

15 APPENDIX IV

TUMOUR ASSESSMENT CRITERIA BY RECIST

Anti-tumour activity measured as shrinkage of the tumours is a secondary endpoint in this study. The Response Evaluation Criteria in Solid Tumors (RECIST) will be used to evaluate tumour response. (51).

Baseline documentation of "Target" and "Non-Target" lesions

- All measurable lesions up to a maximum of five lesions per organ and 10 lesions in total, 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) and their suitability for accurate repeated measurements (either by imaging techniques or clinically).
- A sum of the longest diameter (LD) for *all target lesions* will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor.
- All other lesions (or sites of disease) should be identified as ***non-target 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. 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 recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Definition of Non-measurable lesions

- All other lesions, including small lesions (longest diameter <10 mm with CT scan),

Evaluation of target lesions

* Complete Response (CR):	Disappearance of all target lesions
* Partial Response (PR):	At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD

* Progressive Disease (PD):	At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions
* Stable Disease (SD):	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started

Evaluation of non-target lesions

* Complete Response (CR):	Disappearance of all non-target lesions and normalization of tumor marker level
* Incomplete Response/ Stable Disease (SD):	Persistence of one or more non-target lesion(s) or/and maintenance of tumor marker level above the normal limits
* Progressive Disease (PD):	Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions (1)

(1) Although a clear progression of “non target” lesions only is exceptional, in such circumstances, the opinion of the treating physician should prevail.

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 measurements recorded since the treatment started). In general, the patient's best response assignment will depend on the achievement of both measurement and confirmation criteria

Target lesions	Non-Target lesions	New Lesions	Overall response
CR	CR	No	CR
CR	Incomplete response/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

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.

16 APPENDIX V

ECOG PERFORMANCE STATUS

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

17 APPENDIX VI

FURTHER GUIDANCE ON THE DEFINITION OF A SERIOUS ADVERSE EVENT, CAUSALITY AND OUTCOME

Life-threatening

'Life-threatening' means that the subject was at immediate risk of death from the AE as it occurred or it is suspected that use or continued use of the product would result in the subject's death. 'Life-threatening' does not mean that had an AE occurred in a more severe form it might have caused death (i.e. hepatitis that resolved without hepatic failure).

Hospitalisation

Out-subject treatment in an emergency room is not in itself a SAE, although the reasons for it may be (e.g. bronchospasm, laryngeal oedema). Hospital admissions and/or surgical operations planned before or during a study are not considered AEs if the illness or disease existed before the subject was enrolled in the study, provided that it did not deteriorate in an unexpected way during the study.

Important medical event or medical intervention

Medical and scientific judgement should be exercised in deciding whether a case is serious in a situation where important medical events may not be immediately life-threatening or result in death, hospitalisation, disability or incapacity, but may jeopardise the subject or may require medical intervention to prevent one or more outcomes listed in the definition of serious. These should usually be considered as serious. Examples of such events are:

- Angio-oedema not severe enough to require intubation but requiring iv. hydrocortisone treatment
- Hepatotoxicity caused by paracetamol (acetaminophen) overdose requiring treatment with N-acetylcysteine
- Intensive treatment in an emergency room or at home for allergic bronchospasm
- Blood dyscrasias (e.g. neutropenia or anemia requiring blood transfusion, etc) or convulsions that do not result in hospitalisation
- Development of drug dependency or drug abuse

Causality

Not related: There is probably no relationship to the study medication or there is a reasonable alternative explanation. AEs assessed as "Unlikely related" is categorised as not related.

Related: Anything that does not fall into the "not related" category. Any event for which a causal relationship cannot be excluded should be considered as related.

The following factors should be considered when deciding if there is a "reasonable possibility" that an AE may have been caused by the investigational product:

- **Time course of events and exposure to suspect drug**. Has the patient actually received the suspect drug? Did the AE occur in a reasonable temporal relationship to the administration of suspect drug?
- **Consistency with known drug profile**. Was the AE consistent with the previous knowledge of the suspect drug (pharmacology and toxicology) or drugs of the same pharmacological class? OR could the AE be anticipated from its pharmacological properties?
- **Dechallenge experience.** Did the AE resolve or improve on stopping or reducing the dose of the suspect drug?
- **No alternative cause.** The AE cannot be reasonably explained by another aetiology, such as the underlying disease, other drugs, other host or environmental factors.
- **Re-challenge experience.** Did the AE reoccur if the suspected drug was reintroduced after having been stopped?
- **Laboratory tests.** Has a specific laboratory investigation confirmed the relationship?

A “reasonable possibility” could be considered to exist for an AE where one or more of these factors exist.

In contrast, there would not be a “reasonable possibility” of causality if none of the above criteria apply or where there is evidence of exposure and a reasonable time course but any dechallenge (if performed) is negative or ambiguous or there is another more likely cause of the AE.

In difficult cases, other factors could be considered such as:

- Is this a recognised feature of overdose of the drug?
- Is there a known mechanism?

Ambiguous cases should be considered as being a “reasonable possibility” of a causal relationship unless further evidence becomes available to refute this.

Outcome

The outcome of the event will be described in terms of:

Recovered/resolved: ‘SAE/AE stop date’ should be provided

Recovering/resolving: Can be used in cases where subject is known to be clearly recovering from an event. The event is however not resolved yet. A follow-up is required. ‘AE stop date’ should be left blank.

Not recovered/not resolved: Event is ongoing. A follow-up is required. ‘AE stop date’ should be left blank.

Recovered with sequelae: Used only with persistent incapacity/lifelong condition. ‘SAE/AE stop date’ should be entered.

Resolved with sequelae: E.g. like blindness after diabetes mellitus. ‘SAE stop date’ should be provided.

Fatal: SAE stop date’ (date of death) should be provided only for the events leading to death

Unknown/unknown to Investigator: E.g. subject is lost to follow-up