cytovation

COVER PAGE

CONFIDENTIAL

CLINICAL TRIAL PROTOCOL

A first-in-human, open-label, dose escalation followed by dose expansion phase I/IIa trial to evaluate the safety, preliminary efficacy and pharmacokinetics of intratumoral CyPep-1 monotherapy and in combination with pembrolizumab in patients with advanced solid cancers.

Trial name:	CICILIA		
Trial code:	CyPep-1		
EudraCT number:	2019-003317-33 NCT number: 04260529		
Trial development phase:	I/IIa		
Investigational medicinal product:	CyPep-1 / pembrolizumab		
Indication	Advanced solid cancers		
Version:	10.0		
Date:	30Jan2023		
Document history: This version of the Clinical Trial Protocol includes: V1 (23Sep2019) up to V9.0 (4Jul2022)			
Sponsor Signatory:	[redacted], [redacted]		

Protocol prepared by: [redacted]

SIGNATURES

Sponsor's Agreement:

Title	A first-in-human, open-label, dose escalation followed by dose expansion phase I/IIa trial to evaluate the safety, preliminary efficacy and pharmacokinetics of intratumoral CyPep-1 monotherapy and in combination with pembrolizumab in patients with advanced solid cancers.
Trial Code	CyPep-1
Investigational Product	CyPep-1 / pembrolizumab
Version / Date	10.0 / 30Jan2023
Sponsor Representative	[redacted]
Sponsor Address	Solheimsgaten 11 5058 Bergen Norway

By signing below, the Sponsor approves the Protocol as outlined.

Cytovation ASA

[redacted],
Bergen, Norway

Signature and date:

DocuSigned by:
[redacted]
Signer Name: [redacted]
Signing Reason: I have reviewed this document
Signing Time: 20-Feb-2023 | 07:21 EST
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Investigator's Agreement:

Title	A first-in-human, open-label, dose escalation followed by dose expansion phase I/IIa trial to evaluate the safety, preliminary efficacy and pharmacokinetics of intratumoral CyPep-1 monotherapy and in combination with pembrolizumab in patients with advanced solid cancers.
Trial code	CyPep-1
Investigational Product	CyPep-1 / pembrolizumab
Version / Date	10.0 / 30Jan2023

I have read this protocol and agree to conduct this study in accordance with all stipulations of the protocol and in accordance with all relevant local regulations, the current International Council for Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice (GCP), and with the principles of the most recent version of the Declaration of Helsinki.

Investigator name	Signature	Date

Institution Name	Site #

City and State/Province	Country

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PATHOLOGY AND IMMUNE CELL PHENOTYPING

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TCR-CLONALITY

[redacted]

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

List of Abbreviations

ADR	*Adverse drug reaction*
AE	*Adverse event*
ALAT	*Alanine transaminase*
ANC	*Absolute neutrophil count*
APTT	*Activated partial thromboplastin time*
ART	*Anti-retroviral therapy*
ASAT	*Aspartate transaminase*
ATC	*Anatomical Therapeutic Chemical*
AUC	*Area under the curve*
BE	*Belgium*
BRAF	*B-Raf gene*
CA	*Competent Authority*
C_{avg}	*Average concentration over the dosing interval*
CD3ζ	*CD3 zeta*
CD	*Cluster of differentiation*
CL	*Clearance*
C_{max}	*Maximum plasma concentration*
C_{min}	*Minimum plasma concentration*
cm	*Centimetre*
CNS	*Central nervous system*
CR	*Complete response*
CT	*Computerized tomography*
CrCl	*Creatinine clearance*
CRA	*Clinical Research Associate*
CTCAE	*Common Terminology Criteria for Adverse Events*
CTLA-4	*Cytotoxic T-lymphocyte-associated protein 4*
CV	*Curriculum vitae*
DCF	*Data Clarification Form*
DEC	*Dose Escalation Committee*
DLT	*Dose-limiting toxicity*
ECG	*Electrocardiogram*
ECI	*Events of Clinical Interest*
ECOG	*Eastern Cooperative Oncology Group*
eCRF	*Electronic case report form*
EDC	*Electronic Data Capture*
EEA	*European Economic Area*
E-R	*Exposure-response*
EU	*European Union*
GCP	*Good Clinical Practice*
GDPR	*General Data Protection Regulation*
GFR	*Glomerular filtration rate*
GLP	*Good Laboratory Practice*

HIV	*Human immunodeficiency virus*
HPV	*Human papillomavirus*
IB	*Investigator Brochure*
ICF	*Informed consent form*
ICH	*International Council for Harmonisation*
ICI	*Immune checkpoint inhibitor*
IEC	*Independent Evaluation Committee*
Ig	*Immunoglobulin*
IgG4	*Immunoglobulin G4*
IMP	*Investigational Medicinal Product*
IMPD	*Investigational Medicinal Product Dossier*
iRECIST	*Immune Response Evaluation Criteria in Solid Tumors*
ISF	*Investigator site file*
IT	*Intratumoral*
itRECIST	*Intratumoral Response Evaluation Criteria in Solid Tumors*
IV	*Intravenous*
ITT	*Intention to treat set*
LAG-3	*Lymphocyte activation gene-3*
LLT	*Lowest level term*
mAb	*Monoclonal antibody*
MedDRA	*Medical Dictionary for Regulatory Activities*
MRI	*Magnetic Resonance Imaging*
MTD	*Maximum tolerated dose*
NCI	*National Cancer Institute*
NL	*Netherlands*
NSCLC	*Non-small cell lung cancer*
NT-I	*Non-Target-Injected*
NT-NI	*Non-Target-Non-Injected*
ORR	*Objective response rate*
OS	*Overall survival*
PD	*Progressive disease/ Disease progression*
PD-1	*Programmed cell death protein 1*
PD-L1 programmed death ligand 1	*Programmed death ligand 1*
PFS	*Progression-free survival*
PK	*Pharmacokinetic*
PKCθ	*Protein kinase-C theta*
PR	*Partial Response*
pRBC	*Packed red blood cell*
PT-INR	*Prothrombin time – international normalized ratio*
QW	*Once weekly*
Q2W	*Every 2 weeks*
Q6W	*Every 6 weeks*
Q8W	*Every 8 weeks*
RBC	*Red blood cell*
RECIST	*Response Evaluation Criteria in Solid Tumors*
RP2D	*Recommended Phase 2 Dose*

SAE	*Serious adverse event*
SAF	*Safety analysis set*
SAP	*Statistical Analysis Plan*
SCLC	*Small cell lung cancer*
SD	*Stable Disease*
SGOT	*Serum glutamic oxaloacetic transaminase*
SGPT	*Serum glutamic pyruvic transaminase*
SITC	*Society for Immunotherapy of Cancer*
SmPC	*Summary of Product Characteristics*
SoA	*Schedule of assessments*
SoC	*Standard of Care*
SOC	*System Organ Class*
SOP	*Standard Operating Procedure*
$t_{1/2}$	*Half-life*
TCR	*T cell receptor*
T-I	*Target-Injected*
TLT	*Treatment limiting toxicity*
t_{max}	*Time to reach C_{max}*
T-NI	*Target-Non-Injected*
Treg	*Regulatory T cell*
ULN	*Upper limit of normal*
US	*Ultrasound*
VD	*Volume of distribution*
WBC	*White blood cell*
WHO	*World Health Organization*
WOCBP	*Woman of childbearing potential*
ZAP70	*Zeta-chain-associated protein kinase*

Definition of Terms

Term	***Definition***
Competent Authority (CA)	*A government body or government appointed body that has legal authority to approve or disapprove clinical studies.*
Dose Limiting Toxicity (DLT)	*DLTs are defined taking into account available toxicity and safety data for CyPep-1 and will be assessed during Phase I of the trial.*
Treatment Limiting Toxicity (TLT)	*TLTs are defined taking into account available toxicity and safety data for CyPep-1 as assessed during Phase IIa of the trial and for pembrolizumab based on IBs.*
Lost to Follow-up	*Subjects who fail to return for two consecutive scheduled visits and cannot be contacted by the trial site staff.*

PROTOCOL SYNOPSIS

Name of the Sponsor/Company: Cytovation	**Trial Code:** CyPep-1
Name of Investigational Medicinal Product: CyPep-1 / pembrolizumab	**EudraCT No.:** 2019-003317-33
Development Phase of the Trial: Phase I/IIa	**Trial under an IND:** IND No.: NA

TITLE OF THE TRIAL:

"A first-in-human, open-label dose escalation followed by dose expansion phase I/IIa trial to evaluate the safety, preliminary efficacy and pharmacokinetics of intratumoral CyPep-1 monotherapy and in combination with pembrolizumab in patients with advanced solid cancers."

NUMBER OF TRIAL CENTERS:

Approximately 14 sites in 3 countries.

NAME AND DESCRIPTION OF INVESTIGATIONAL MEDICINAL PRODUCT:

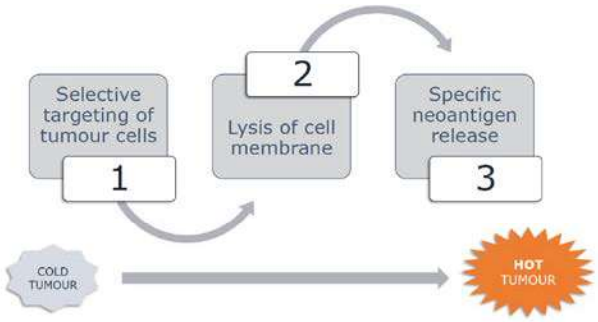
CyPep-1 is a synthetic linear 27-amino acid peptide with an acetylated amino group at the N-terminus and an amido group at the C-terminus. All optically active amino acid residues are in D-configuration with the exception of achiral glycine.

CyPep-1 is formulated in a clear and colorless aqueous solution (5 mg/mL).

TRIAL BACKGROUND:

CyPep-1 has been shown to selectively target tumor cell membranes based on their altered molecular composition, which in turn leads to lysis of tumor cells by removal of the cell membrane.

Figure 1. Mode of action of CyPep-1.



This mode of action of CyPep-1 induces tumor cell death resulting in the release of tumor antigens, and potentially induces a tumor-specific immune response by in-situ immunization (Figure 1).

Preclinical toxicology studies have shown a favorable safety profile and potent anti-tumor activity of CyPep-1 in several tumor models. CyPep-1 was shown to modulate the tumor microenvironment by increasing the presence of CD8+ T-cells and by potentiating the effects of immune checkpoint inhibitors (ICIs; please see Investigator Brochure). As such, we hypothesize that intratumoral (IT) injection with CyPep-1 leads to transformation of immunological “cold” and ICI treatment-resistant tumors into “hot” and immunological active tumors that can be successfully treated with immune-modulating agents.

TRIAL RATIONALE:

Treatment with ICIs has been shown to result in long lasting anti-tumor responses in selective patients with different tumor indications. However, only a subset of these patients obtains durable remission. Studies have indicated the presence of intratumoral CD8+ T-cells to be a positive predictive factor for response to ICI therapy. Thus, treatment strategies that aim at recruiting tumor antagonizing cellular components of the immune system hold great promise and are currently a main focus in oncology research.

Current evidence revealed an association between tumor mutational burden and tumor immunogenicity. In order to proliferate, antigenic tumors develop mechanisms that enable them to evade immune-mediated destruction through a process termed immunoediting. However, while mutational load represents a predictive factor for ICI response, its predictive power seems modest; rather, ICI efficacy may be associated with the presence of specific neoantigen-generating mutations. This observation suggests that a qualitative neoantigen-directed immunotherapy holds great potential. New therapeutic modalities in this category should ideally meet two critical criteria: 1) exposing tumor specific antigens, and 2) inducing an inflamed tumor microenvironment. Furthermore, the capacity of such therapeutic modalities may result in an extended patient population of responders and potentially increase the overall efficacy of ICIs.

Conceptually, clinical proof-of-concept of this strategy has been established by lytic viruses, and preliminary evidence indicates that oncolytic treatment can induce systemic anti-tumoral responses. We envision that CyPep-1, due to its unique antigen-exposing properties, could be a potent and less toxic oncolytic therapy option. In addition, CyPep-1 offers attractive pharmacological properties such as long shelf life, easy scalability and negligible batch-to-batch variations. As such, CyPep-1 may represent a unique “tumor-agnostic” compound, boosting the effect of established immunotherapy across multiple tumor types. Lastly, preclinical studies showing that CyPep-1 can synergize with anti-PD-1 antibody treatment in terms of decreased tumor volumes and prolonged survival highlight the possible clinical utility of CyPep-1 in the combination setting with ICIs

This Phase I/IIa trial is designed to assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of CyPep-1 when administered directly into malignant tumors, in monotherapy and in combination with anti-PD-1 antibody pembrolizumab. Additionally, we will monitor antitumor effects on injected lesions and distant non-injected deposits. As part of the exploratory analysis, it is planned to determine local and systemic immunological effects after CyPep-1 administration, alone and in combination with anti-PD-1.

TRIAL POPULATION:

Phase I (dose escalation) and Phase IIa CICILIA:

Histologically or cytologically confirmed locally advanced (unresectable) or metastatic tumors (solid tumors or lymphoma) with an accessible tumor lesion for intratumoral injection of CyPep-1 that meet one of the following criteria:

a. Relapsed following or progressed through standard therapy.

b. Have a disease for which no standard effective therapy exists.

NUMBER OF SUBJECTS:

The sample size is determined by clinical rather than statistical considerations.

The number of subjects in Phase I (dose escalation) will depend on the number of dose levels tested before a Dose Limiting Toxicity (DLT) is observed and the Maximum Tolerated Dose (MTD) is determined. It is anticipated that 12 subjects will be included in this phase of the trial.

It is planned that 9 subjects will be enrolled in Phase IIa (dose expansion of CyPep-1 monotherapy, Arm A) of the trial. The number of subjects may be expanded to 18 for a total of 24 subjects evaluable at RP2D, in case in the first 12 subjects at RP2D, a responder per iRECIST is observed or 2 subjects with stable disease per iRECIST are observed.

For Arm B, CyPep-1 at RP2D plus pembrolizumab, a total of 15 subjects is planned to be enrolled.

For Arm C, a total of 9 subjects is planned to be enrolled to evaluate at least two dose levels of CyPep-1 based on results of Phase I.

For Arm D, a total of approximately 30 subjects are planned to be enrolled.

OBJECTIVES:

The primary objectives are:

- To evaluate the safety and tolerability of IT administration of CyPep-1, as monotherapy and in combination with pembrolizumab.
- To identify the recommended phase II dose (RP2D) of CyPep-1, as monotherapy and in combination with pembrolizumab.

The secondary objectives are:

- To assess the preliminary anti-tumor efficacy of CyPep-1, as monotherapy and in combination with pembrolizumab.
- To characterize the pharmacokinetics (PK) of CyPep-1.

The additional exploratory objectives are:

- To assess the preliminary anti-tumor efficacy of CyPep-1, as monotherapy and in combination with pembrolizumab, in injected lesions and non-injected lesions, separately.
- To assess survival after treatment with CyPep-1, as monotherapy and in combination with pembrolizumab.
- To assess the immune modulating properties of treatment with CyPep-1, as monotherapy and in combination with pembrolizumab.

TRIAL ENDPOINTS:

Primary endpoints:

- Type and number of adverse events (AEs) according to National Cancer Institute (NCI) – Common Terminology Criteria for Adverse Events (CTCAE) criteria v5.0, and additional safety parameters of CyPep-1 as monotherapy and in combination with pembrolizumab.
- Dose limiting toxicities (DLTs) and the maximum tolerated dose (MTD) for determination of RP2D of CyPep-1 as monotherapy and treatment-limiting toxicities (TLTs) of CyPep-1 in combination with pembrolizumab.

Secondary endpoints:

- Objective response rate (ORR), defined by complete and partial responses, according to immune Response Evaluation Criteria in Solid Tumors (iRECIST) based on investigator's assessment.
- Time to and duration of response and duration of stable disease.
- The plasma concentration time profile of CyPep-1 and, if detectable, the derived PK parameters (i.e., area under the curve [AUC], peak plasma concentration [C_{max}], time to reach C_{max} [t_{max}], systemic clearance (CL), elimination half-life ($t_{1/2}$) and volume of distribution [VD]).

Exploratory endpoints:

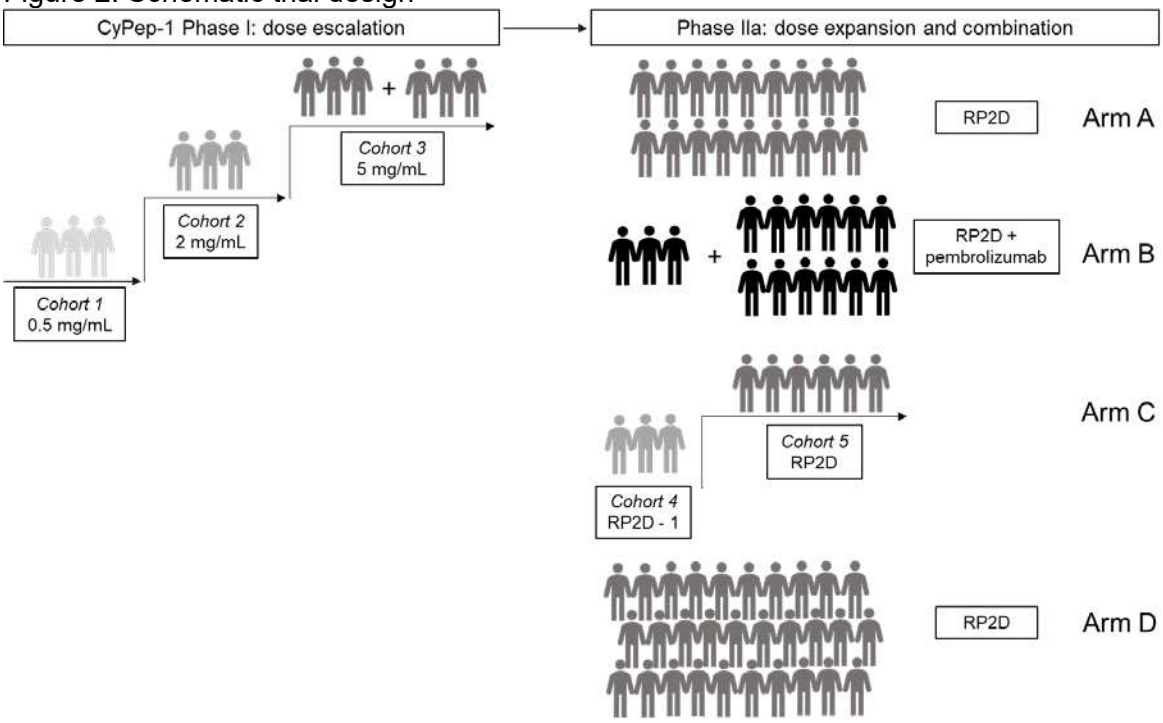
- For all Phase IIa arms: ORR in injected lesions and non-injected lesions, separately, per itRECIST* [Goldmacher 2020]).
- Progression-free survival (PFS) per iRECIST based on investigator's assessment.
- Overall survival (OS).
- The relative change in number of tumor infiltrating CD8+ T-cells in the injected and, whenever available, non-injected tumor biopsies.
- The association between the relative change in tumor infiltrating CD8+ T-cells and response rate in the injected lesions (per itRECIST) and all lesions (per iRECIST).
- The change in T-cell receptor (TCR) clonality levels in peripheral blood and (when available) biopsied lesions.
- Changes in the tumor microenvironment (injected and, whenever available, non-injected tumor biopsies) via expression of selected candidate immune markers: [redacted]
- For Arms A, B, and C: [redacted]
- Peripheral blood phenotyping of selected immune cell markers.

*itRECIST will be programatically calculated using data obtained from iRECIST assessments following the itRECIST principle summarized in Appendix F Figure 1.

OVERALL TRIAL DESIGN:

This is a combined Phase I/IIa, open-label, dose escalation followed by dose expansion trial in subjects with advanced solid cancers. The trial consists of two phases and multiple arms, as shown in the diagram below (Figure 2).

Figure 2. Schematic trial design



Phase I (dose escalation, N=12) and Phase IIa (dose expansion Arm A, N=9 up to 18; combination Arm B, N=15; dose expansion liver metastases Arm C, N=9; dose expansion melanoma Arm D, N=30).
Abbreviations: RP2D: recommended phase 2 dose.

Phase I – dose escalation:
In this phase of the trial, safety and tolerability will be documented and the MTD/RP2D will be determined. Cohorts of 3 subjects will receive IT injections with CyPep-1. The DLT observation period for each dose level will be 6 weeks (5 weeks of trial treatment and 1 week of safety follow-up).

Screening will occur during the 4 weeks prior to start of CyPep-1 treatment.
In three dose cohorts of 3 subjects each, subjects will receive CyPep-1 IT at different concentrations (dose escalation) and volumes (depending on tumor size), i.e., 0.5 mg/mL, 2 mg/mL, or 5 mg/mL, respectively. These dose concentrations have been selected based on previous pre-clinical experience with CyPep-1.

Each subject will receive IT injection(s) with CyPep-1 on Day 1 of Weeks 1, 3, and 5, respectively. After each CyPep-1 administration, subjects will be required to stay at the clinic for at least 4 hours for safety and, for those in which PK will be assessed, also for PK monitoring. The data from the additional three subjects in cohort 3 will be used for further confirmation of RP2D. The following CyPep-1 administrations are planned as continuous Q2W administrations.

For each dose cohort, at least 24 hours must elapse between each subject to start treatment with CyPep-1. No intra-subject dose escalation is allowed.

The decisions on dose escalation and MTD will be taken by the Dose Escalation Committee (DEC) after reviewing safety data (including DLTs) from all subjects who have entered the

previous dose cohort and have completed the DLT observation period. The DEC is comprised of all the investigators or designees, as well as the medical monitor and representatives of the sponsor.

In Phase I, subject replacement for subjects who drop out for any reason, except DLTs, will only occur before the DLT observation period is completed and is allowed if a subject does not receive all three CyPep-1 administrations, unless due to CyPep-1-related toxicity. No subject replacement will occur for subjects who withdraw later.

After completion of Phase I, all results will be evaluated by the DEC. This will include safety data and if available, other supportive clinical data (i.e., efficacy, pharmacokinetics, tumor biopsy analyses) from all subjects included in Phase I of the trial. The DEC will confirm the MTD or RP2D (in case the MTD is not reached).

All additional arms can start once Phase I data has been evaluated by the DEC and an RP2D is determined for CyPep-1.
Phase I has been completed in August 2021 and the RP2D of CyPep-1 was determined at 5 mg/mL.

Phase IIa – dose expansion (Arm A):
In this phase, safety and tolerability will be further evaluated in an expanded cohort of 9 subjects at the RP2D of CyPep-1, determined in Phase I. Screening- and treatment-schedules will be the same as in Phase I, except for the 24-h observation period before start of treatment of the next subject, which is not applicable.

At the RP2D, in case of a responder per iRECIST or of 2 subjects with stable disease per iRECIST, the number of subjects will be expanded to a total of 18 (to evaluate overall data from a total of 24 subjects treated at RP2D for CyPep-1 monotherapy taking the 6 subjects from cohort 3 into account).

Replacement of subjects in the Phase IIa expansion monotherapy arms is not planned.

Phase IIa – combination arm (Arm B):
The safety and tolerability of CyPep-1 in combination with pembrolizumab will be evaluated in a cohort of 15 subjects in total, using a staggered approach. Initially, 3 subjects will receive CyPep-1 at RP2D in combination with pembrolizumab Q6W and each subject will be observed for 24 h before the next subject can be administered with CyPep-1. After the first 3 subjects completed the TLT observation period of 6 weeks with no TLTs observed and after reviewing the safety data (including TLTs) by the DEC, the inclusion of all remaining subjects at RP2D of CyPep-1 in combination with pembrolizumab can be initiated. In the absence of any TLTs in the initial 3 subjects, the 24-h observation period before start of treatment of the next subject is no longer applicable. Should any TLTs be observed in the first 3 subjects at the RP2D of CyPep-1 in combination with pembrolizumab, an additional 3 subjects in this dose group will be included. If ≤1 out of 6 subjects have a TLT, the remainder of the 15 subjects will be treated at the RP2D. In the unlikely case that 2 or more of the 6 subjects experience any TLTs at RP2D of CyPep-1, a recommendation to dose de-escalate CyPep-1 and to what dose or to continue the combination part of the trial will be made by the DEC. The same screening schedule is planned and CyPep-1 will be administered according to the same treatment schedule as in Phase I.

For arm B, replacement of the first three subjects is allowed if a subject drops out for any reason, and does not receive all three CyPep-1 injections during the TLT observation period, unless due to CyPep-1- and/or pembrolizumab-related toxicity. No subject replacement will occur for subjects who discontinue later.

Phase IIa – dose expansion liver metastases (Arm C):
The safety and tolerability of at least two dose levels of CyPep-1, the RP2D and the dose immediately below that, are planned to be evaluated when CyPep-1 is administered intratumorally using ultrasound guidance to one metastatic lesion in the liver. The two dose levels are planned to be investigated adhering to the "3+3" design and will follow the procedures as described for the dose escalation (Phase I) (Section 3.1). The first three subjects will receive CyPep-1 at the lower dose level and each subject will be observed for 24 h before CyPep-1 administration to a next subject (cohort 4). After the third subject at that dose finished the DLT observation period of 6 weeks (5 weeks CyPep-1 treatment plus 1 week safety follow-up), the DEC will recommend on the start of the cohort to be administered at RP2D based on the review of the safety data (cohort 5). After the DLT observation period for the first three subjects at RP2D for CyPep-1 and the review of all safety data, the DEC will recommend on the inclusion of all remaining subjects. In the absence of any DLTs in the first 3 subjects, the 24-h observation period before start treatment of the next subject is not applicable. Should a DLT be observed in the first three subjects at the RP2D of CyPep-1, an additional three subjects in this dose group will be included. In case that two or more subjects experience DLTs at RP2D of CyPep-1, a decision to dose de-escalate and to what dose and how to continue Arm C will be made by the DEC.

For Arm C, replacement for subjects who drop out for any reason, except DLTs, before the DLT observation period is completed is allowed when a subject did not receive all three CyPep-1 administrations. No subject replacement will occur for subjects who discontinue later.

Phase IIa – dose expansion melanoma (Arm D):
The safety and tolerability of CyPep-1 at RP2D will be further evaluated with focus on assessing efficacy signals of CyPep-1 monotherapy in up to 30 subjects with melanoma. Although safety information will be collected, there will not be a formal DLT observation period.

As part of the continuous safety monitoring of the trial, reports with cumulative safety data of Arm D subjects will be shared with all investigators, competent authorities, and ethical committees of countries where the trial is conducted at the following time points:

- After the first three subjects completed three weeks of treatment with CyPep-1.
- After the first three, six, and 12 subjects completed six weeks of treatment with CyPep-1.

In the occurrence of AEs fulfilling DLT criteria or other safety alerts, the DEC will convene to review the safety data and provide recommendations on the continuation of dosing.

Replacement of subjects who dropped out before V8 (at week 8) due to any reasons other than CyPep-1 toxicity is allowed. No subject replacement will occur for subjects who withdraw later than V8.

For both phases and all trial arms, subjects will stay in the trial until end of trial or until confirmed disease progression, unacceptable toxicity, death or discontinuation for any other reason.

DOSE ESCALATION AND SCHEDULE:

Dose escalation (Phase I of the trial) will be conducted to determine the MTD or RP2D using a standard "3+3" design, with at least three subjects treated at each dose-level. Arm C in the dose expansion (phase IIa of the trial) will follow a similar dose escalation for the two dose levels planned (RP2D and the dose immediately below). Dose escalation will proceed according to the following rules:

Number of subjects with DLT*	Escalation decision rule
0 out of 3 subjects	Enter 3 subjects at the next planned dose level.
1 out of 3 subjects	Enter 3 additional subjects at this dose level: • If 0 of these additional subjects experience a DLT, proceed to the next dose level. • If 1 or more of the additional 3 subjects experience a DLT, then dose escalation to higher dose levels is stopped. Three additional subjects may be entered at the second highest dose if only 3 subjects have been treated at that dose level. Alternatively, a new dose level may be introduced between the second highest dose and the MTD (3 additional subjects).
≤ 1 out of 6 subjects at the highest dose level below the MTD	This dose level is declared the MTD or RP2D.

*Assessed as related to the trial drug (CyPep-1) at any given dose level.

Scheduling of treatment of CyPep-1 and pembrolizumab (Arm B) will be as follows: CyPep-1 will be administered every 2 weeks (Q2W) and pembrolizumab will be administered following a Q6W schedule.

For Arm B, on the visits that CyPep-1 and pembrolizumab are administered on the same day, CyPep-1 is planned to be administered 30 to 60 min after pembrolizumab has been administered. In case of pembrolizumab-related toxicity, treatment with CyPep-1 will be delayed and planned after a review by the investigator and medical monitor. If CyPep-1 treatment is delayed > 7 days, that dose will be omitted. If the delay of > 7 days is during the TLT observation period, the subject will be replaced.

For Phase I, Phase IIa Arms A and C, CyPep-1 administration is planned as Q2W injections. If CyPep-1 treatment is delayed > 7 days, that dose will be omitted. If CyPep-1 treatment is delayed for > 6 weeks during the DLT period (first 3 injections), the subject should be discontinued from the trial.

For Arm D, CyPep-1 administration is planned as QW injections until the second iRECIST/itRECIST assessment at week 16, followed by a Q2W dosing scheme. In case CyPep-1 treatment is delayed for > 3 weeks (first 3 injections), the investigator and the Medical Monitor must assess on a case-by-case basis the decision whether the subject should be discontinued from the trial. Treatment delay due to TEAEs will be considered for treatment discontinuation.

Radiological assessment to determine occurrence of (un)confirmed PD will be performed Q8W from the start of treatment for all trial phases and arms. Treatment will continue until confirmed PD, unacceptable toxicity, death or consent withdrawal, loss to follow up, or trial end, whichever occurs first.

DOSE LIMITING TOXICITY:

A DLT is defined by the occurrence of any of the following toxicities according to CTCAE v5.0 during treatment Cycle 1 and that are considered by the investigator to be possibly, probably, or definitely related to CyPep-1:

1. Any Grade ≥ 3 AEs of any etiology, except:
   - Nausea, vomiting, or diarrhea will be considered a DLT only if it persists at Grade ≥ 3 for > 3 days despite adequate supportive care measures. At the investigator's

discretion, subjects who experience nausea, vomiting, or diarrhea after receiving CyPep-1 may receive antiemetic or anti-diarrheal medication prior to subsequent doses of CyPep-1.

- Isolated laboratory abnormalities Grade ≥ 3 (not present at baseline) that resolve to Grade ≤ 1 in ≤ 7 days without clinical sequelae or need for therapeutic intervention.
- Fatigue Grade ≥ 3 for ≤ 7 days.
- For Phase I, Arms A and C: Alanine amino transferase (ALAT) or aspartate amino transferase (ASAT) toxicities:
    - ALAT or ASAT > 5 x upper limit of normal (ULN) (> 7.5 x ULN for subjects with liver metastases), unless greater than 14 days.
    - ALAT or ASAT > 5 x ULN (> 7.5 x ULN for subjects with liver metastases) accompanied by an elevation in total bilirubin of > 2.5 x ULN (not explained by obstruction), regardless of duration, will be considered a DLT.

2. Any other toxicity occurring at any time during the trial that in the view of the participating investigators and the medical monitor represents a clinically significant hazard to the subject.

DEFINITION OF TREATMENT LIMITING TOXICITY (CyPep-1 plus pembrolizumab combination)

All TLTs will be graded using NCI CTCAE v5.0 based on the investigator assessment. The TLT window of observation will be during Cycle 1.

The occurrence of any of the following toxicities during Cycle 1 will be considered a TLT, if assessed by the investigator to be possibly, probably, or definitely related to trial treatment administration.

1. Grade 4 nonhematologic toxicity (not laboratory).
2. Grade 4 hematologic toxicity lasting ≥7 days, except thrombocytopenia:
    - Grade 4 thrombocytopenia of any duration
    - Grade 3 thrombocytopenia associated with clinically significant bleeding
3. Any nonhematologic AE ≥Grade 3 in severity should be considered a TLT, with the following exceptions: Grade 3 fatigue lasting ≤ 3 days; Grade 3 diarrhea, nausea, or vomiting without use of anti-emetics or anti-diarrheal per standard of care; Grade 3 rash without use of corticosteroids or anti-inflammatory agents per standard of care.
4. Any Grade 3 or Grade 4 non-hematologic laboratory value if:
    - Clinically significant medical intervention is required to treat the participant or
    - The abnormality leads to hospitalization, or
    - The abnormality persists for >1 week.
    - The abnormality results in a Drug-induced Liver Injury (DILI)
    - Exceptions: Clinically nonsignificant, treatable, or reversible laboratory abnormalities including liver function tests, uric acid, etc.
5. Febrile neutropenia Grade 3 or Grade 4:
    - Grade 3 is defined as ANC <1000/mm^3 with a single temperature of >38.3 degrees C (101 degrees F) or a sustained temperature of ≥38 degrees C (100.4 degrees F) for more than 1 hour
    - Grade 4 is defined as ANC <1000/mm^3 with a single temperature of >38.3 degrees C (101 degrees F) or a sustained temperature of ≥38 degrees C (100.4 degrees F) for more than 1 hour, with life-threatening consequences and urgent intervention indicated.
6. Prolonged delay (>2 weeks) in initiating Cycle 2 due to treatment-related toxicity.
7. Any treatment-related toxicity that causes the participant to discontinue treatment during Cycle 1.

8. Missing >25% of CyPep-1 doses as a result of drug-related AE(s) during the first cycle.
9. Grade 5 toxicity.

DURATION OF SUBJECT PARTICIPATION:

Each subject will undergo up to 4 weeks of screening. For Phase I, Phase IIa Arms A, B and C subjects will undergo a DLT/TLT observation period of 6 weeks (5 weeks of CyPep-1 ± pembrolizumab treatment plus 1 week observation). In absence of DLT/TLTs, treatment with CyPep-1 continues until confirmed PD, unacceptable toxicity, death or consent withdrawal, loss to follow up, or trial end, whichever occurs first.For the combination arm (Arm B), Q6W administration of pembrolizumab is allowed up to 24 months (18 cycles). For Arm D, without a DLT period, each subject will undergo QW CyPep-1 treatment until the second iRECIST/itRECIST assessment at week 16, followed by a Q2W dosing scheme. Hereafter, there will be efficacy (PFS) follow-up every 8 weeks, or until confirmed PD, unacceptable toxicity, death, withdrawal of consent, loss to follow-up, or trial end, whichever occurs first.

- Start of inclusion: Q1 2020
- Planned last subject last trial visit: last subject's last follow-up visit

The end of treatment is defined as the last subject's end-of-treatment visit. The end of trial is defined as the last subject's last visit. For the purposes of data summarization, data analyses will be performed after the last enrolled subject has completed 3 months of trial participation. Subjects may still be in the trial at the time of data summarization, as all subjects may continue to participate until confirmed disease progression, unacceptable toxicity, or discontinuation for any other reason.

INCLUSION AND EXCLUSION CRITERIA:

Inclusion criteria:

For Phase I and Phase IIa Arms A and C:

1. Histologically or cytologically confirmed locally advanced (unresectable) or metastatic tumors (solid tumor or lymphoma) with an accessible tumor lesion for intratumoral injection of CyPep-1 malignancy (including lymphomas) that is either:
   a. Refractory to standard-of-care treatment
   b. Have a disease for which there is no standard therapy considered appropriate. Metastatic deposits (including cutaneous/subcutaneous lesions and metastatic deposits in lymph nodes) of tumors for which IT injections may be performed are eligible. Pure cutaneous infiltrations (e.g., breast cancer cutaneous carcinomatosis) are ineligible.
2. 1 to 3 non-ulcerated transcutaneously accessible lesion(s) for injection and measurable as defined by iRECIST. All other tumor lesion(s) may be selected for efficacy follow-up but will not be subjected to treatment with CyPep-1.
3. Presence of tumor lesion(s) (that have not been previously irradiated) suitable for biopsy at screening and at Week 6.

For Arm C:

4. Confirmation of the presence of at least 1 liver metastasis by imaging.
5. Subjects must have measurable disease which is equal to one or more metastatic liver lesions that can be accurately and serially measured that are greater than 1 cm dimension and for which the longest diameter is greater or equal to 1 cm as measured by CT (computed tomography)scan or magnetic resonance imaging (MRI). The metastatic liver lesion must not be in an area that received prior localized therapies.

6. Metastatic liver lesion for injection with >50% radiological visible necrosis must be avoided and the lesion must be located where any tumor swelling will not lead to gall bladder tract obstruction or lead to bleeding risk.

For Arm D:

7. Histologically or cytologically confirmed diagnosis of advanced (unresectable Stage III) or metastatic (Stage IV: M1a and/or M1b) melanoma considered incurable by Standard of Care. For metastatic melanoma, only cutaneous, subcutaneous, lymph node, or lung metastases are allowed.
8. Previously exposed to ICI(s) and be categorized following the SITC Immunotherapy Resistance Taskforce (Kluger 2020) meeting one of the following:
    a. Have primary resistance: PD-(L)-1 inhibitor exposure ≥6 weeks and have the best response as one of the following:
        i. PD,
        ii. SD for <6 months.
    b. Have secondary resistance: PD-(L)-1 inhibitor exposure ≥6 weeks and best response CR, PR, or SD >6 months.
    c. Have adjuvant therapy resistance: recurrence subcategorized into primary resistance/early relapse occurred <12 weeks after the last dose, and late relapse occurred ≥12 weeks after the last dose. If BRAF mutated, patients must have progressed to treatment with BRAF inhibitors.
    d. Have neoadjuvant therapy resistance including subjects with or without major pathologic response and subsequent PD that fulfills criteria for primary or secondary resistance
    e. Discontinued from ICI(s) therapy due to immune-related adverse events grade 3 or 4 other than endocrine insufficiencies treatable with hormonal replacement therapy, and meet one of the following:
        i. Remain on SD at discontinuation of PD-(L)1 inhibitor in combination with ipilimumab or show regrowth after <12 weeks of the last dose
        ii. Have not achieved a CR with single-agent PD-(L)1 inhibitor or combination of PD-(L)1 with LAG-3 inhibitor
9. At least 1 non-ulcerated lesion, not exceeding 5 cm in (the longest) diameter, for intratumoral injection(s) and measurable as defined by iRECIST.
10. Resolution of toxicity due to prior therapy returned to baseline or < Grade 2, except for alopecia or other irreversible immune-mediated AEs, as defined by CTCAE v5.0. and SITC ICI-related AEs (Brahmer et al, 2021).
11. Prior treatment(s) delivered by IT injection to the to-be injected lesion(s), including investigational agents, is allowed.

For Phase I and Phase IIa Arms A, C, and D in addition:

12. Age ≥ 18 years.
13. Estimated life expectancy of at least 3 months.
14. Eastern Cooperative Oncology Group (ECOG) Performance Status of 0 or 1 (Appendix B).
15. Resolution of toxicity due to prior therapy to Grade < 2 (except for alopecia and transaminases in case of liver metastases) as defined by CTCAE v5.0 (Appendix C).
16. Ability to give written informed consent and to comply with the protocol.
17. All subjects of childbearing potential (defined as < 2 years after last menstruation or not surgically sterile) must have a negative highly sensitive pregnancy test at screening (urine/serum) and agree to use highly effective method for contraception according to the EU Clinical Trial Facilitation Group guidance from time of signing the informed consent form (ICF) until at least 120 days after the last administration of

CyPep-1. The partners of subjects with childbearing potential must also apply contraceptive methods and are recommended not to donate sperm.

18. A male participant must agree to use contraception and refrain from sperm donation during the treatment period and for at least 120 days after the last dose of trial medication.
19. Adequate bone marrow, liver, and renal function:
    a. Platelet count ≥ 100 x 10^9/L
    b. Hemoglobin ≥ 6.0 mmol/L or 9.67 g/dL
    c. Absolute Neutrophil Count (ANC) ≥ 1.5 x 10^9/L
    d. Total bilirubin ≤ 1.5 x ULN, except for subjects with familial bilirubinemia (Gilbert's disease)
    e. Serum ASAT and ALAT ≤ 2.5 x ULN (≤ 5 x ULN in case of liver metastases)
    f. Creatinine clearance ≥ 30 mL/min (Glomerular Filtration Rate [GFR] to be calculated by CKD-EPI formula).

For Phase IIa Arm B:
Participants are eligible to be included in Arm B of the trial only if all of the following criteria apply:

Type of Participant and Disease Characteristics

20. The participant provides written informed consent for the trial.
21. Be ≥ 18 years of age on day of signing informed consent.
22. Participant with histologically or cytologically confirmed diagnosis of advanced (unresectable Stage III) or metastatic (Stage IV) solid tumor malignancy (including lymphomas) that is refractory to standard-of-care treatment or for which there is no standard therapy considered appropriate. Metastatic deposits (including cutaneous/subcutaneous lesions and metastatic deposits in lymph nodes) of tumors for which IT injections may be performed are eligible. Pure cutaneous infiltrations (e.g., breast cancer cutaneous carcinomatosis) are ineligible.
23. Subjects must have progressed on treatment with an anti-PD1/L1 monoclonal antibody (mAb) administered either as monotherapy, or in combination with other checkpoint inhibitors or other therapies. Treatment progression is defined by meeting all of the following criteria:
    a. Has received at least 2 doses of an approved anti-PD-1/L1 mAb.
    b. Has demonstrated clinical or radiological disease progression (PD) after PD-1/L1
24. A male participant must agree to use contraception and refrain from sperm donation during the treatment period and for at least 120 days after the last dose of trial medication.
25. A female participant is eligible to participate if she is not pregnant, not breastfeeding, and at least one of the following conditions applies:
    a. Not a woman of childbearing potential (WOCBP)
    b. A WOCBP (defined as < 2 years after last menstruation or not surgically sterile) must have a negative highly sensitive pregnancy test at screening (urine/serum) and must follow contraceptive guidance (highly effective method for contraception according to the EU Clinical Trial Facilitation Group guidance) from time of signing the ICF until at least 120 days after the last administration of trial medication. The partners of subjects with childbearing potential must also apply contraceptive methods and are recommended not to donate sperm.

26. 1 to 3 non-ulcerated transcutaneously accessible lesion(s) for injection and measurable as defined by iRECIST. All other tumor lesion(s) may be selected for efficacy follow-up but will not be subjected to treatment with CyPep-1.
27. Presence of tumor lesion(s) (that have not been previously irradiated) suitable for biopsy at screening and at Week 6.
28. Have an ECOG performance status of 0 to 1 (Appendix B).
29. Have adequate organ function as defined in the following table. Specimens must be collected within 10 days (or less) prior to the start of trial treatment.

Adequate Organ Function Laboratory Values:

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1500/µL
Platelets	≥100 000/µL
Hemoglobin	≥9.0 g/dL or ≥5.6 mmol/L[1]
Renal	
Creatinine OR Measured or calculated[2] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 × ULN OR ≥30 mL/min for participant with creatinine levels >1.5 × institutional ULN
Hepatic	
Total bilirubin	≤1.5 × ULN OR direct bilirubin ≤ ULN for participants with total bilirubin levels >1.5 × ULN
AST (SGOT) and ALT (SGPT)	≤2.5 × ULN (≤5 × ULN for participants with liver metastases)

ALT (SGPT)=alanine aminotransferase (serum glutamic pyruvic transaminase); AST (SGOT) =aspartate aminotransferase (serum glutamic oxaloacetic transaminase); GFR=glomerular filtration rate; ULN=upper limit of normal.

[1] Criteria must be met without packed red blood cell (pRBC) transfusion within the prior 2 weeks. Participants can be on stable dose of erythropoietin (≥ approximately 3 months).

[2] Creatinine clearance (CrCl) should be calculated per institutional standard.

Note: This table includes eligibility-defining laboratory value requirements for treatment; laboratory value requirements should be adapted according to local regulations and guidelines for the administration of specific chemotherapies.

30. Only for subjects with lymphoma: have measurable disease defined as at least one lesion that can be accurately measured in at least two dimensions with spiral CT scan. Minimum measurement must be > 15 mm in the longest diameter by > 10 mm in the short axis.
31. Estimated life expectancy of at least 3 months.
32. HIV infected participants must be on anti-retroviral therapy (ART) and have a well-controlled HIV infection/disease defined as:

    a. Participants on ART must have a CD4+ T-cell count ≥ 350 cells/mm^3 at time of screening.
    b. Participants on ART must have achieved and maintained virologic suppression defined as confirmed HIV RNA level below 50 copies/mL or the lower limit of qualification (below the limit of detection) using the locally available assay at the time of screening and for at least 12 weeks prior to screening.

c. Participants on ART must have been on a stable regimen, without changes in drugs or dose modification, for at least 4 weeks prior to study entry (Day 1).

Exclusion criteria:

For Phase I and Phase IIa Arms A, C, and D: subjects who meet ANY of the following criteria at screening will be excluded from trial entry:

1. There is no limit to the number of prior treatment regimens, but prior treatment(s) should not include compounds delivered by IT injection to the to-be injected lesion(s), including investigational agents. Subjects with prior IT therapies are allowed in Arm D.
2. Participation in another clinical trial within 4 weeks prior to first dose of CyPep-1.
3. Anti-cancer therapy within 4 weeks prior to the first dose of CyPep-1 (within 2 weeks for palliative radiotherapy, within 1 week for endocrine therapy).
4. Major surgical procedure within 14 days prior to the first dose of CyPep-1.
5. Live vaccine within 30 days prior to first dose of CyPep-1.
6. Expected to require any other form of systemic or localized antineoplastic therapy while in this trial. Localized palliative radiotherapy for pain relief is allowed on tumor lesions that are not selected for evaluation of treatment response.
7. Clinical evidence of an active second malignancy that is progressing or requires active treatment, except for curatively treated early stage (carcinoma *in situ* or stage 1) carcinomas or non-melanoma skin cancer.
8. Active autoimmune disease requiring immunosuppressive therapy.
9. Any condition requiring continuous systemic treatment with either corticosteroids (> 10 mg daily prednisone equivalents) or other immunosuppressive agents within 2 weeks prior to first dose of CyPep-1. Inhaled, intranasal or topical (only on areas outside the injected lesion[s]) and physiological replacement doses of up to 10 mg daily prednisone equivalent are permitted in the absence of active auto-immune disease.
10. Abnormal or clinically significant coagulation parameters:
    a. Prothrombin Time – International Normalized Ratio (PT-INR) ≥ 1.5 ULN
    b. Activated Partial Thromboplastin Time (APTT) ≥ 1.5 ULN

    Subjects being treated with anticoagulants are excluded if the coagulation parameters are outside the therapeutic intervals as described in the SmPC for the administered treatment.
11. Subjects on anticoagulants with temporarily stop and start, supported by low molecular weight heparin (or other anticoagulation therapy at the discretion of the investigator and/or per local standard of care) during treatment period.
12. Known hypersensitivity to any component of CyPep-1.
13. Prior allogeneic tissue/solid organ transplant, stem cell or bone marrow transplant.
14. Known active human immunodeficiency virus (HIV). Subject is eligible when normal levels of CD4 are present.
15. Central nervous system (CNS) metastasis that is symptomatic or progressing or that requires current therapy (e.g., evidence of new or enlarging CNS metastasis, carcinomatous meningitis or new neurological symptoms attributable to CNS metastasis).
16. QTcF > 480 ms, history of long or short QT syndrome, Brugada syndrome, or known history of QTc prolongation, or Torsade de Pointes.
17. Women who are pregnant or breastfeeding.

18. Any serious and/or unstable pre-existing medical, psychiatric or other condition which in the investigator's opinion could interfere with subject safety, obtaining written informed consent, or compliance with the trial protocol.
19. Has an active acute or chronic infection requiring systemic therapy at the time of CyPep-1 injection. Note: Subjects treated for mild/moderate infection with oral antibiotics only may be included based on consultation with the study medical monitor and the sponsor.

Additional exclusion criteria for Phase IIa Arm C:

20. Subject is a candidate for hepatic surgery or local regional therapy of liver metastases with curative intent.
21. More than one third of the liver is estimated to be involved with metastases.
22. There is invasion by cancer into the main blood vessels such as the portal vein, hepatic vein or the vena cava.
23. Subject is currently receiving or has received liver metastatic-directed therapy (eg: radiation, ablation, embolization) less than 4 weeks prior to enrolment or hepatic surgery.

Exclusion criteria specific for Phase IIa Arm B:

Participants are excluded from the trial if ANY of the following criteria apply at screening:

Pregnancy Exclusion

24. A WOCBP who has a positive urine pregnancy test (within 72 hours) prior to trial treatment. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

Prior/Concomitant Therapy

25. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti PD-L2 agent or with an agent directed to another stimulatory or co-inhibitory T-cell receptor (e.g., CTLA-4, OX 40, CD137), and was discontinued from that treatment due to a Grade 3 or higher irAE.
26. Has received prior systemic anti-cancer therapy including investigational agents within 4 weeks (within 1 week for endocrine therapy) prior to first dose of CyPep-1.
    Note: Participants must have recovered from all AEs due to previous therapies to ≤Grade 1 or baseline as defined by CTCAE v5.0 (Appendix C). Participants with ≤Grade 2 neuropathy may be eligible. Participants with endocrine-related AEs Grade ≤2 requiring treatment or hormone replacement may be eligible.
    Note: If the participant had major surgery, this should not have been within 14 days prior to the first dose of CyPep-1 and the participant must have recovered adequately from the procedure and/or any complications from the surgery prior to starting study intervention.
27. Has received prior (palliative) radiotherapy within 2 weeks of start of trial treatment. Participants must have recovered from all radiation-related toxicities, not require corticosteroids, and not have had radiation pneumonitis. A 1-week washout is permitted for palliative radiation (≤2 weeks of radiotherapy) to non-CNS disease.
28. Has received a live or live-attenuated vaccine within 30 days prior to the first dose of CyPep-1. Note: Administration of killed vaccines are allowed.
29. Has received prior compounds delivered by IT injection to the to-be injected lesion(s), including investigational agents.
30. Expected to require any other form of systemic or localized antineoplastic therapy while in this trial. Localized palliative radiotherapy for pain relief is allowed on tumor lesions that are not selected for evaluation of treatment response.
31. Ongoing pembrolizumab-related toxicity event(s) as per TLT definition.

Prior/Concurrent Clinical Trial Experience

32. Is currently participating in or has participated in a trial of an investigational agent or has used an investigational device within 4 weeks prior to the first dose of trial treatment.
    Note: Participants who have entered the follow-up phase of an investigational trial may participate as long as it has been 4 weeks after the last dose of the previous investigational agent.
33. Has had an allogeneic tissue/solid organ transplant.

Diagnostic assessments

34. Has a diagnosis of immunodeficiency or is receiving chronic systemic steroid therapy (in dosing exceeding 10 mg daily of prednisone equivalent) or any other form of immunosuppressive therapy within 7 days prior the first dose of CyPep-1.
35. Has a known additional malignancy that is progressing or has required active treatment within the past 3 years, except for curatively treated early stage (carcinoma *in situ* or stage 1) carcinomas or non-melanoma skin cancer.
    Note: Participants with basal cell carcinoma of the skin, squamous cell carcinoma of the skin or carcinoma in situ (e.g., breast carcinoma, cervical cancer in situ) that have undergone potentially curative therapy are not excluded.
36. Has known active CNS metastases and/or carcinomatous meningitis. Participants with previously treated brain metastases may participate provided they are radiologically stable, i.e., without evidence of progression for at least 4 weeks by repeat imaging (note that the repeat imaging should be performed during study screening), clinically stable and without requirement of steroid treatment for at least 14 days prior to first dose of study treatment.
37. Has severe hypersensitivity (≥Grade 3) to pembrolizumab and/or any of its excipients or to another mAb, as well as any known hypersensitivity to any component of CyPep-1.
38. Has an active autoimmune disease that has required systemic treatment in past 2 years (i.e., with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency) is not considered a form of systemic treatment and is allowed.
39. Has a history of (non-infectious) pneumonitis / interstitial lung disease that required steroids or has current pneumonitis / interstitial lung disease.
40. Has an active infection requiring systemic therapy.
41. HIV-infected participants with a history of Kaposi sarcoma and/or Multicentric Castleman Disease.
42. Has a known history of Hepatitis B (defined as HBsAg reactive) or known active Hepatitis C virus (defined as HCV RNA [qualitative] is detected) infection.
    Note: No testing for Hepatitis B and Hepatitis C is required unless mandated by a local health authority.
43. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the participant's participation for the full duration of the trial, or is not in the best interest of the participant to participate, in the opinion of the treating investigator.
44. Has a known psychiatric or substance abuse disorder that would interfere with the participant’s ability to cooperate with the requirements of the study.
45. Has received radiation therapy to the lung that is >30 Gy within 6 months of the first dose of trial treatment (applicable only for subjects with non-small cell lung cancer [NSCLC], mesothelioma and small cell lung cancer [SCLC]).

TREATMENT REGIMENS:

CyPep-1 Treatment Regimen and Dose Levels:

For Phase I and Phase IIa, Arms A, B and C, CyPep-1 will be administered intratumorally Q2W, on Day 1, 15, and 29 of each cycle. In the absence of DLT/TLTs during Cycle 1, treatment continues with Q2W IT injections of CyPep-1. For Arm D, CyPep-1 will be administered intratumorally QW until the second iRECIST/itRECIST assessment at week 16, followed by a Q2W dosing scheme. For all phases/arms, treatment will continue until confirmed PD, unacceptable toxicity, death, or consent withdrawal, loss to follow up, or trial end, whichever occurs first (for discontinuation refer to Section 4.3.1).

Administration will be through a needle inserted trans-dermally. The needle should be redirected along multiple tracks to ensure even dispersion of CyPep-1 throughout the lesion. For Arm C, CyPep-1 will be administered via intralesional injection with ultrasound guidance into one metastatic liver lesion. A detailed guidance for intratumoral administration of CyPep-1 is presented in the Study Procedural Manual.

The CyPep-1 concentration for each dose cohort during dose escalation (Phase I) is as follows:

Dose cohort	**CyPep-1 concentration**
1	0.5 mg/mL
2	2 mg/mL
3	5 mg/mL

The total amount of CyPep-1 injected will depend on the tumor volume(s). Effort should be made to administer the maximum planned injection volume of CyPep-1 as planned per lesion size.

CyPep-1 Injection Volume:

The volume delivered to each lesion selected for injection will be determined based on the longest diameter measured by the most accurate measurement technique available for each lesion (e.g. caliper, CT, or MRI) at screening and every 8 weeks during the iRECIST/itRECIST assessments. A chosen technique used at screening will be consistently used throughout the study. Lesion sizes need to be checked before each injection by either caliper (mandatory for Arm D and to-be-injected cutaneous/subcutaneous lesions for Phase IIa Arms A and B) or other imaging modalities used to guide CyPep-1 injections, i.e. ultrasound, CT, or MRI. For Arm C, the lesion size measurement will be performed by the same imaging modality used to guide CyPep-1 injections. To ensure consistency of the measurements, the sites need to strive for having the same operator to perform all measurement for a chosen measuring technique. If there are major changes in the lesion size, the injection volume may be adjusted based on the ultrasound measurements per investigator's opinion and consultation with the Medical Monitor is possible.

For both phases of the trial, the cumulative maximal injected volume will be 4 mL per treatment day, corresponding to a cumulative maximal dose of 20 mg of CyPep-1 (depending on the dose concentration). Effort should be made to administer the maximum planned injection volume of CyPep-1 as planned per lesion size (see tables below).

For Phase I and Phase IIa Arms A and B, the to-be-injected volume may be divided for injection over 1-3 tumor lesions (e.g., one large lesion or 2-3 relatively small lesions). In case more than 3 lesions are good candidates for injection, the to be injected lesions selection can be discussed with the sponsor and the medical monitor.

For Arm C, CyPep-1 will be administered with ultrasound guidance into one metastatic liver lesion. The same single metastatic lesion is to be injected with CyPep-1 based on lesion size.

For Arms A and B, for a lesion > 7 cm, the same lesion should be considered to be injected weekly with up to 4 mL of CyPep-1 for the first 6 weeks (on Day 1, 8, 15, 22, 29 and 36), independent of the potential change in lesion size, followed by Q2W 4 mL administrations of CyPep-1.

For Arm D, the to-be-injected volume for injection is preferably divided over as many lesions as possible. The longest diameter of the lesions for injection is ideally smaller than 2 cm and should not exceed 5 cm.

For subjects in Arms A, B and C, the injection volume will be:

Measured Lesion Diameter (cm)	**Injected volume (mL)**
≤ 1	1
> 1 and ≤ 2	2
> 2	4

For subjects in Arm D, the injection volume will be:

Measured Lesion Diameter (cm)	**Injected volume (mL)**
≤ 0.79	0.1
0.80 to 0.99	0.2
1.00 to 1.24	0.5
1.25 to 1.50	1.5
1.51 to 2.49	3.0
≥ 2.50	4.0

For all subjects, if a lesion disappears at any time, the investigator may choose another lesion for subsequent injections. Previously non-injected lesions may also be injected if necessary (e.g. safety reasons), per investigator's discretion.

Sequence of CyPep-1 Administration for Phase IIa Arm D only:

Lesion Map
A lesion map that will guide the preferred sequence of injection of the identified Lesion Sets must be created prior to the first CyPep-1 injection (detailed in Study Procedural Manual). A lesion map will contain the following information:
For superficial lesions:

- Digital color photography of the lesions must be taken and the mark-ups of individual lesions (or lesion groups) with lesion ID are overlayed on top of the digital photography.
- Silhouette of body parts must be used to present the relative location of the lesions.
- All digital color photography containing a ruler held next to the longest diameter of the lesion to indicate the size of the lesion, and the lesions marked-up with lesion IDs, together with the silhouette of body parts indicating the location of the lesions, need to be submitted to eCRF with inspection readiness for the reported responses.
- In the case of bilateral lesions, the laterality of the lesions must be documented on the lesion map.

For deep lesions:

- CT or MRI scan will be used for iRECIST/itRECIST assessment at baseline, and the location of lesions provided by the CT/MRI scan will be used.
- In the case of bilateral lesions, the laterality of the lesions must be documented on the lesion map.

Once the lesion map is created, Lesion Sets for sequential CyPep-1 injections will be planned and documented in the source. The size of the injected lesions and the lesion map should be updated when assessments of local measurements of tumor lesions, iRECIST and itRECIST assessments are performed during the treatment period.

Lesion Sets
A Lesion Set will be composed of multiple lesions, and will be administered with CyPep-1 during the same treatment visit for three consecutive doses. Unless there are no other lesion sets to be injected, each Lesion Set will not receive more than three consecutive doses of CyPep-1. In the case of bilateral lesions, both of the bilateral lesions should be included in the same Lesion Set if feasible considering the maximum injected volume allowed per treatment visit (4 mL).

The sequence of CyPep-1 Administration
After three doses of CyPep-1, the next lesion set will be administered with CyPep-1. The ideal next to-be-injected Lesion Set should be located as far as possible from the last injected Lesion Set on the lesion map to activate different draining lymph nodes and thus optimize a systematic immune response against the tumor. Once all injectable lesions have been injected and the subject remains on treatment, a new lesion map that permits reprioritizing of the to-be-injected Lesion Sets will be created, taking into account size changes of individual lesions, e.g., an individual lesion enlarged or regressed after initial 3 doses of CyPep-1 injections.

Pembrolizumab Treatment Regimen for Phase IIa Arm B only:

The dose of pembrolizumab in combination with CyPep-1 in the combination arm will be 400 mg Q6W. Dosing of CyPep-1 will be aligned such that subjects will receive both pembrolizumab and the first dose of CyPep-1 on the same day (C1V1). After C1V1, pembrolizumab dosing will continue following a Q6W dosing scheme until 18 cycles, confirmed PD, unacceptable toxicity or consent withdrawal.

DOSE-MODIFICATION CRITERIA:

CyPep-1: No major toxicity is expected. There may be minor local toxicity or tumor ulceration due to the treatment procedure, which can be treated according to Standard of Care.

In case of toxicity, the administration of CyPep-1 may be delayed at the discretion of the investigator and is to be discussed with the medical monitor. Neither a dose reduction, nor intra-subject dose escalation of CyPep-1 is allowed.

Dose modification of pembrolizumab in Arm B will be according to the provided dose modification and toxicity management guidelines (refer to Section 5.3.6).

SAFETY ASSESSMENTS:

Safety will be assessed by means of physical examinations, body weight, vital signs, ECOG performance status, laboratory evaluations (hematology, biochemistry, coagulation, urinalysis), electrocardiograms (ECG), and recording of concurrent ilness/therapy and adverse events (as defined by CTCAE v5.0). All assessments will be performed as indicated in the Schedule of Assessments (SoA, Table 1). Additional assessments may be performed as clinically indicated.

EFFICACY ASSESSMENTS:

Clinical efficacy of CyPep-1 monotherapy (Phase I dose escalation and Phase IIa Arms A, C, and D) and in combination with pembrolizumab (Phase IIa Arm B) will be measured by radiologic assessments (i.e., caliper, CT, MRI) every 8 weeks, starting from C1V1 for all phases and arms as baseline. The imaging modality must be the same for all efficacy measurements.

Radiologic assessments will be evaluated based on the investigator's assessments at the clinical sites according to the consensus guideline iRECIST (Appendix D, based on modified

RECIST*)* and response (iCPD, iUPD, iSD, iPR, iCR) will be defined taking into account the total disease burden (as required per iRECIST using modified RECIST).

ORR in injected and non-injected lesions per itRECIST will be estimated by statistical programming using data collected from iRECIST assessments.

The following tumor lesion(s) will be followed-up for efficacy:

- One or more injected lesion(s) that are treated with CyPep-1 injection.
- All other non-injected lesion(s) that are not treated with CyPep-1 injection.
- For Phase I Cohort 3 and all Phase IIa arms, in addition to iRECIST, (exploratory) ORR in injected and non-injected lesions will be assessed per itRECIST (Appendix F). For Phase I Cohort 3, Phase IIa Arms A, B, and C, data will be extracted retrospectively from eCRF.

Upon start of CyPep-1 treatment, subjects will be followed for PFS (every 8 weeks) and OS. PFS will be radiologically assessed at the trial sites until disease relapse or progression (based on all lesions), death due to any cause, withdrawal of consent, loss to follow-up, or until the end of the trial, whichever occurs first. A phone call for survival status (OS) will be done every 3 months after confirmation of PD and until the end of the trial.

Confirmation of clinical progressive disease:

In order to account for the possibly delayed onset of CyPep-1 action and for subjects who are of acceptable clinical performance status (as deemed by the investigator), if PD is recorded and provided that the tumor enlargement is smaller than 50% compared to the previous measurement, the subject will remain in the trial and will be reassessed radiologically after 4 weeks. All lesion(s) are to be evaluated for clinical progression:

- If the lesion(s) continue(s) to enlarge, the original PD will be considered as the “first documented PD” and there will be an End of Treatment (EoT) visit.
- If lesion(s) remain(s) unchanged or decrease(s) in size, assessments should continue as per protocol (i.e., the next radiological assessment will be performed after an 8-week interval). In this case, the first measured PD will be disregarded.

In case of unconfirmed PD after the first (or subsequent) 3 Q2W injections of CyPep-1, the subject should continue Q2W administrations (start of another cycle of CyPep-1) until confirmed PD. If PD is confirmed 4 weeks later, then the subject will be taken off-trial and move to the EoT visit.

PHARMACOKINETIC ASSESSMENTS:

During the trial treatment period, the plasma concentration time profile of CyPep-1 will be evaluated and, if detectable plasma drug levels are identified, the derived PK parameters will be assessed. These include area under the curve (AUC), peak plasma concentration (C_{max}), time to reach C_{max} (t_{max}), systemic clearance (CL), elimination half-life ($t_{1/2}$) and volume of distribution (VD). PK assessment is planned for the first 9 subjects of Phase I, all subjects of Phase IIa Arm C, and the first 12 subjects of Phase IIa Arm D.

EXPLORATORY ASSESSMENTS:

A tumor biopsy of the injected lesion(s) and, if applicable, of additionally selected lesion(s) that are not to be injected, will be obtained at screening and at Week 6 (one week after 3rd CyPep-1 injection). The screening tumor biopsy is preferably as fresh as possible, but may have been obtained within 90 days of first administration of CyPep-1.

At least one biopsy of the injected tumor lesion(s) should be available at screening and at Week 6 (one week after 3rd CyPep-1 injection from the start of treatment). If the subject is willing to provide additional tumor material for exploratory analysis, also a biopsy of non-injected tumor lesion(s) will be taken.

After receiving additional cycle(s) of treatment with Q2W injections of CyPep-1, a biopsy of any new injected lesion(s) can be taken (not mandatory) before the first treatment and after 6 weeks.

The following will be assessed in the tumor biopsies:

- Number and relative change of tumor infiltrating CD8+ T-cells.
- Expression of immune markers: [redacted]

The following will be assessed in peripheral blood (timepoints defined in SoA):

- [redacted]
- Peripheral blood phenotyping of selected immune cell markers.

Additional analyses for immune markers may be performed.

STATISTICAL METHODS:

A statistical analysis plan (SAP) will provide details of the methods of analysis to address all trial objectives.

Phase IIa Arm A: At RP2D in case a responder per iRECIST is observed or 2 subjects with stable disease (SD) per iRECIST are observed in the first 12 subjects, the number of subjects will be expanded to a total of 24 (CyPep-1 monotherapy). Response evaluation will be based on the sum of diameter of all target lesions. ORR is defined as best response (CR/PR) or SD as >16 weeks from onset of SD. The first 12 subjects are the number of subjects at RP2D taking the subjects from the dose escalation part into account.

Formal assessment of response will be based on the first 12 evaluable subjects as follows: in case of no responders in 12 subjects, the likelihood of a 20% ORR is 6.9%; in case of one responder in 12 subjects, the likelihood of a 20% ORR is 26.5%. Assuming stable disease to occur in 30% of subjects, in case no subject has stable disease in the first 12 evaluated subjects, the likelihood is 1.4%; in case one subject has stable disease in the first 12 evaluated subjects, this becomes 8.5%, while with two subjects having stable disease, this becomes 25.2%. Therefore, in case of either one of 12 subjects having an objective response or two of 12 subjects having stable disease, the number of subjects in Arm A may be increased to a total of 24 subjects (meaning 18 subjects in Arm A; based on the total number of subjects at RP2D during dose escalation plus the number of subjects at RP2D in Arm A). In case, these outcomes are not observed, Arm A will enrol the planned 9 subjects.

The sample size for Phase IIa Arm D is based on a desired precision by which preliminary clinical efficacy (ORR for injected lesions) may be evaluated. Using 90% 1-sided Clopper-Pearson exact confidence intervals, lower bounds identified in the table below would apply.

90% 1-sided Confidence Intervals for the Number of Responders in N=30 Subjects

Number or Responders	Observed ORR	One-sided 90% Lower Bound
1	3.3%	0.4%

2	6.7%	1.8%
3	10.0%	3.7%
4	13.3%	5.9%
5	16.7%	8.3%
6	20.0%	10.9%
7	23.3%	13.5%
8	26.7%	16.2%
9	30.0%	19.0%

For example, the lower bound for an observed ORR of 13.3% when there are 4 responders would be higher than 5% (i.e. 5.9%); the lower bound if 6 responders are observed would be 10.9%, which is greater than 10%, allowing for a sufficient sample to review preliminary effectiveness of CyPep-1 monotherapy for Arm D subjects.

Safety parameters:

All subjects receiving at least one IT injection with CyPep-1 or one pembrolizumab infusion will be evaluable for safety.

AE incidence rates will be described by the frequency of AEs, categorized by preferred term and system organ class according to CTCAE v5.0. AEs will be summarized by severity and relationship to CyPep-1. The incidence of AEs and DLTs/TLTs will be evaluated for each dose level, and for all subjects combined.

Vital signs and body weight, 12-lead ECG parameters, laboratory data (hematology, biochemistry, coagulation, urinalysis) will be summarized using descriptive statistics. Shift tables showing changes from baseline will be generated where appropriate.

Efficacy parameters:

Efficacy will be determined from the number of subjects with complete response, partial response, stable disease or progressive disease, according to iRECIST based on the investigator's assessments. PFS (percentage of subjects alive and progression-free after trial treatment start) will also be determined. These clinical response data will be summarized.

ORR (proportion of subjects with a best overall response of PR or, CR) will be evaluated based on iRECIST.

- For Phase I Cohort 3 and all Phase IIa arms, ORR (proportion of subjects with a best overall response of PR or, CR) will be evaluated by two additional approaches based on itRECIST:Injected response: considering only the targeted-injected lesions.
- Non-injected response: considering only the targeted-non-injected lesions.

Pharmacokinetic parameters:

Subjects who have received CyPep-1 and have provided one evaluable pre-dose and at least one post-dose PK blood sample will be evaluable for PK.

Standard methodology will be used to estimate PK parameters. Concentration-time profiles will be derived for subjects with pre- and post-dose samples.

Immunological parameters:

The relative changes in CD8+ T-cell infiltration (CD8+ expression) in the biopsied injected, and whenever available, non-injected lesion(s) between screening and Week 6 will be assessed.

The association between the relative changes in CD8+ T-cell infiltration and response rate in the injected lesions (per itRECIST) and all lesions (per iRECIST) will be assessed.

The change in T-cell receptor (TCR) clonality levels will be assessed in the available peripheral blood samples. To assess the hypothesis that CyPep-1 increases the TCR clonality systematically, which suggests that T-cells are targeting the tumor, the clonality diversity metric will be computed for all subjects providing peripheral blood samples at screening and/or Week 6 of every CyPep-1 treatment cycle. Based on preliminary on file data of TCR clonality analysis in peripheral blood dated Nov 2022, the dataset collected is sufficient for the intended analysis and further sampling will provide no additional insights. Uncertainty about the representativeness of data generated with blood samples regarding TCR changes in the tumor microenvironment has lead Cytovation to reconsider this scientific relevance of this assay. Therefore, Cytovation decided to stop collecting blood samples for TCR clonality analysis for all subjects. TCR sequencing might be performed on the available remaining tumor biopsy material from the analyses of changes in the tumor microenvironment. Overlaps of T cell clones in respect to expansion and breadth of T cell clonality before and after intratumoral injection with CyPep-1, as monotherapy and in combination with pembrolizumab (central analysis) will be analyzed.

The change in tumor microenvironment is examined via the expression of selected candidate immune markers by immunohistochemistry. For each marker, a difference in expression will be explored.

Changes in cytokine levels and in frequency of various immune cell types in peripheral blood (immunophenotyping) will be explored.

SCHEDULE OF ASSESSMENTS

The schedule of all assessments performed within the context of this clinical trial is provided in Tables 1a to 1d.

Table 1. Schedule of Assessments

Table 1a. Schedule of Assessments Phase I Dose Escalation and Phase IIa Arms A and C for Cycle 1 (CyPep-1 Monotherapy)

	Screening[1]	DLT observation period				Continued Treatment	EoT[2]	FU[3]	PFS-FU[4]	OS-FU[5]
Week		**1**	**3**	**5**	**6**		**-**	**-**	**-**	**-**
Day	**-28 to 1**	**D1**	**D15**	**D29**	**D36**		**-**	**-**	**-**	**Every 3 months**
Visit no.	**Screening**	**C1V1**	**C1V2**	**C1V3**	**C1V4**		**-**	**-**	**-**	**-**
Visit window	**NA**	**-**	**± 3 d**	**± 3 d**	**± 3 d**		**± 3 d**	**+ 1 wk**	**± 1 wk**	**± 1 wk**
Informed consent	X					See Table 1b Subsequent cycles[6]				
Demography	X									
Medical and surgical history[7]	X									
Eligibility	X	X								
Tumor biopsy[8]	X				X[28]					
Performance (ECOG) status[9]	X	X	X	X	X		X	X	X	
Height[9]	X									
Weight[9]	X	X	X	X	X		X	X		
Physical examination[9]	X	X	X	X	X		X	X	X	
Vital signs[10]	X	PRE- & 15m, 30m, 1h, 2h, 4h POST-dose	PRE- & 15m, 30m, 1h, 2h, 4h POST-dose	PRE- & 15m, 30m, 1h, 2h, 4h POST-dose	X		X	X		
Electrocardiogram[11]	X	X	X	X						
Hematology and biochemistry[12]	X	X	X	X	X		X			

	Screening[1]	DLT observation period				Continued Treatment	EoT[2]	FU[3]	PFS-FU[4]	OS-FU[5]
Week		**1**	**3**	**5**	**6**		**-**	**-**	**-**	**-**
Day	**-28 to 1**	**D1**	**D15**	**D29**	**D36**		**-**	**-**	**-**	**Every 3 months**
Visit no.	**Screening**	**C1V1**	**C1V2**	**C1V3**	**C1V4**		**-**	**-**	**-**	**-**
Visit window	**NA**	**-**	**± 3 d**	**± 3 d**	**± 3 d**		**± 3 d**	**+ 1 wk**	**± 1 wk**	**± 1 wk**
Creatinine clearance[13]	X									
Coagulation[12]	X	X	X	X						
Urinalysis[12]	X	X	X	X	X		X			
Pregnancy test[14]	X	X	X	X			X			
PK (first 9 subjects Phase I + all subjects Arm C)[15]		PRE- & 15m, 30m, 1h, 2h, 4h POST-dose								
Cytokines[16]	X		X		X		X			
Blood sampling for immune cell phenotyping[17]	X		X		X					
Local measurement of tumor lesion(s)[18]		X	X	X						
Administration of CyPep-1[19]		X	X	X						
Concomitant medication[20]	X	X	X	X	X		X	X		
AEs[21]	X	X	X	X	X		X	X		
iRECIST assessment[22]	At 8-week (±3 days) intervals while on trial treatment[23]								X	
Evaluation of survival and subsequent anti-cancer therapies										X

Table 1b. Schedule of Assessments Phase I Dose Escalation and Phase IIa Arms A and C (Starting Cycle 2 and all subsequent cycles monotherapy)[24]

Week	**1**	**3**	**5**	**6**
Day	**D1**	**D15**	**D29**	**D36**
Visit no.	**Cycle X Visit 1[25] (CXV1)**	**CXV2**	**CXV3**	**CXV4**
Visit window	**± 3 d**	**± 3 d**	**± 3 d**	**± 3 d**
Weight	X			
Physical examination	X	X	X	
Vital signs[10]	PRE- & 15m, 30m, 1h, 2h, 4h POST-dose	X	X	
Tumor biopsy	X[26]			X[26]
Performance (ECOG) status[9]	X			
Hematology and biochemistry[12]	X			
Urinalysis[12]	X			
Pregnancy test[14]	X	X	X	
Cytokines[16]				X[27]
Blood sampling for immune cell phenotyping[17]				X
Local measurement of tumor lesion(s)[18]	X	X	X	
Administration of CyPep-1[19]	X	X	X	
Concomitant medication[20]	X	X	X	X
AEs[21]	X	X	X	X
iRECIST assessment[22]	At 8-week (±3 days) intervals while on trial treatment[23]			

Table 1c. Schedule of Assessments Phase IIa Arm B (Cycle 1 CyPep-1 plus pembrolizumab combination)

	Screening[1]	TLT observation period				Continued Treatment	EoT[2]	FU[3]	PFS-FU[4]	OS-FU[5]
Week		**1**	**3**	**5**	**6**		**-**	**-**	**-**	**-**
Day	**-28 to 1**	**D1**	**D15**	**D29**	**D36**		**-**	**-**	**-**	**Every 3 months**
Visit no.	**Screening**	**C1V1**	**C1V2**	**C1V3**	**C1V4**		**-**	**-**	**-**	**-**
Visit window	**NA**	**-**	**± 3 d**	**± 3 d**	**± 3 d**		**± 3 d**	**± 1 wk**	**± 1 wk**	**± 1 wk**
Informed consent	X					Continued Treatment see Table 1d Subsequent cycles[6]				
Demography	X									
Medical and surgical history[7]	X									
Eligibility	X	X								
Tumor biopsy[8]	X				X[28]					
Performance (ECOG) status[9]	X	X	X	X	X		X	X	X	
Height[9]	X									
Weight[9]	X	X	X	X	X		X	X		
Physical examination[9]	X	X	X	X	X		X	X	X	
Vital signs[10]	X	PRE- & 15m, 30m, 1h, 2h, 4h POST-dose	PRE- & 15m, 30m, 1h, 2h, 4h POST-dose	PRE- & 15m, 30m, 1h, 2h, 4h POST-dose	X		X	X		
Electrocardiogram[11]	X	X	X	X						
Hematology and biochemistry[12]	X	X	X	X	X		X			
Creatinine clearance[13]	X									
Coagulation[12]	X	X	X	X						
Urinalysis[12]	X	X	X	X	X		X			
Pregnancy test[14]	X	X	X	X			X			
Cytokines[16]	X		X		X		X			

	Screening[1]	TLT observation period				Continued Treatment	EoT[2]	FU[3]	PFS-FU[4]	OS-FU[5]
Week		**1**	**3**	**5**	**6**		**-**	**-**	**-**	**-**
Day	**-28 to 1**	**D1**	**D15**	**D29**	**D36**		**-**	**-**	**-**	**Every 3 months**
Visit no.	**Screening**	**C1V1**	**C1V2**	**C1V3**	**C1V4**		**-**	**-**	**-**	**-**
Visit window	**NA**	**-**	**± 3 d**	**± 3 d**	**± 3 d**		**± 3 d**	**± 1 wk**	**± 1 wk**	**± 1 wk**
Blood sampling for immune cell phenotyping[17]	X		X		X					
Local measurement of tumor lesion(s)[18]		X	X	X						
Administration of CyPep-1[19]		X	X	X						
Administration of pembrolizumab[29]		X								
Thyroid function testing[30]	Every 6 weeks while on trial treatment									
Concomitant medication[20]	X	X	X	X	X		X	X		
AEs[21]	X	X	X	X	X		X	X		
iRECIST assessment[22]		At 8-week (±3 days) intervals while on trial treatment[23]							X	
Evaluation of survival and subsequent anti-cancer therapies										X

Table 1d. Schedule of Assessments Phase IIa Arm B (Starting Cycle 2 and all subsequent cycles of CyPep-1 plus pembrolizumab combination)[23]

Week	**1**	**3**	**5**	**6**
Day	**D1**	**D15**	**D29**	**D36**
Visit no.	**Cycle X Visit X[25] (CXV1)**	**CXV2**	**CXV3**	**CXV4**
Visit window	**± 3 d**	**± 3 d**	**± 3 d**	**± 3 d**
Weight	X			
Physical examination	X	X	X	
Vital signs	PRE- & 15m, 30m, 1h, 2h, 4h POST-dose	X	X	
Electrocardiogram[11]	X			
Tumor biopsy	X[26]			X[26]
Performance (ECOG) status[9]	X			
Hematology and biochemistry[12]	X			
Urinalysis[12]	X			
Pregnancy test[14]	X	X	X	
Cytokines[16]				X[27]
Blood sampling for immune cell phenotyping[17]				X
Local measurement of tumor lesion(s)[18]	X	X	X	
Administration of CyPep-1[19]	X	X	X	
Administration of pembrolizumab[29]	Every 6 weeks starting C1V1			
Thyroid function testing[30]	Every 6 weeks while on trial treatment			
Concomitant medication[20]	X	X	X	X
AEs[21]	X	X	X	X
iRECIST assessment[22]	At 8-week (±3 days) intervals while on trial treatment[23]			

Table 1e. Schedule of Assessments Phase IIa Arm D (CyPep-1 Monotherapy)

	Screening[1]	Treatment			Continued Weekly Treatment until Week 16	Bi-weekly Treatment	EoT[2]	FU[3]	PFS-FU[4]	OS-FU[5]
Week		1	2	3	4 to 16	17 onwards	-	-	-	-
Day	-28 to 1	D1	D8	D15	D22 to D106	D113 onwards	-	-	-	Every 3 months
Visit no.	Screening	V1	V2	V3	VN	VN'	-	-	-	-
Visit window	NA	-	± 3 d	± 3 d	± 3 d	± 3 d	± 3 d	+ 1 wk	± 1 wk	± 1 wk
Informed consent	X									
Demography	X									
Medical and surgical history[7]	X									
Eligibility	X	X								
Tumor biopsy[8]	X	X[31]								
Performance (ECOG) status[9]	X	X	X	X	X[32]		X	X	X	
Height[9]	X									
Weight[9]	X	X	X	X	X[32]		X	X		
Physical examination[9]	X	X	X	X	X[32]		X	X	X	
Vital signs[10]	X	PRE- & 15m, 30m, 1h, 2h, 4h POST-dose					X	X		
Electrocardiogram[11]	X	PRE- & 30 min POST-dose	PRE- & 30 min POST-dose	PRE- & 30 min POST-dose	X[32]					
Hematology and biochemistry[12]	X	X	X	X	X[32]		X			
Creatinine clearance[13]	X									
Coagulation[12]	X	X	X	X	X[32]					

	Screening[1]	Treatment			Continued Weekly Treatment until Week 16	Bi-weekly Treatment	EoT[2]	FU[3]	PFS-FU[4]	OS-FU[5]
Week		**1**	**2**	**3**	**4 to 16**	**17 onwards**	**-**	**-**	**-**	**-**
Day	**-28 to 1**	**D1**	**D8**	**D15**	**D22 to D106**	**D113 onwards**	**-**	**-**	**-**	**Every 3 months**
Visit no.	**Screening**	**V1**	**V2**	**V3**	**VN**	**VN'**	**-**	**-**	**-**	**-**
Visit window	**NA**	**-**	**± 3 d**	**± 3 d**	**± 3 d**	**± 3 d**	**± 3 d**	**+ 1 wk**	**± 1 wk**	**± 1 wk**
Urinalysis[12]	X	X	X	X	X[32]		X			
Pregnancy test[14]	X	X	X	X	X[33]		X			
PK (first 12 subjects only)[15]		PRE- & 15m, 30m, 1h POST-dose								
Blood sampling for immune cell phenotyping[17]	X	At 6-week (±3 days) intervals while on trial treatment								
Local measurement of tumor lesion(s)[18]		X	X	X	X	X				
Lesion mapping[34]	X	X	X	X	X	X				
Administration of CyPep-1[19]		X	X	X	X	X				
Concomitant medication[20]	X	X	X	X	X	X	X	X		
AEs[21]	X	X	X	X	X	X	X	X		
iRECIST and itRECIST assessment[22]	At 8-week (±3 days) intervals while on trial treatment[23]								X	
Evaluation of survival and subsequent anti-cancer therapies										X

List of footnotes

1. Re-screening may be allowed on a case-by-case basis. In the case of out-of-range laboratory results during the screening period, a re-test may be allowed within the screening period. The investigator must contact the medical monitor to discuss each re-screening case.
2. The EoT visit will be done within 7 days after the subject is taken off CyPep-1.

3. The FU visit will occur at least 30 days after the last CyPep-1 or pembrolizumab (combination arm) administration (if the EoT visit occurs more than 30 days after the last treatment administration, the FU visit will be skipped).
4. For subjects who leave the trial early and do not receive any other anti-cancer therapy or any other investigational therapy, PFS visits should continue to be performed every 8 weeks (from D1) until occurrence of confirmed progressive disease (PD) or until start of any further line of anticancer treatment
5. Every 3 months after confirmed PD and until the end of the trial, by phone call. In case of unconfirmed PD (iUPD) by iRECIST and subject has started further line of anti-cancer treatment, the first OS-FU visit will be done 90 days after the iUPD event occurred. Besides overall survival status, any next line of anti-cancer therapies, ORR and duration of response to next line anti-cancer therapies, and additional relevant information will be recorded. If necessary, patients may be contacted occasionally outside of this FU window, and data regarding next line anti-cancer therapies can be collected, retrospectively.
6. Additional cycles of CyPep-1 are planned with continued Q2W administration, please see Table 1 b/e for schedule of assessments (in this case, end of treatment [EoT], FU, PFS-FU, and OS-FU are relative to each cycle).
7. Medical history should include details of cancer onset, prior surgery and past cancer treatments.
8. **For Arms A, B and C**: Arrangements to be made for any tumor material to be sent for analysis are described in the laboratory manual. Tumor biopsies (should be available from at least one injected lesion at screening and at Week 6 of Cycle 1) will be taken from injected and, whenever available, non-injected lesions at screening. At Week 6 (±1 day), the same lesion(s) has to be used for the biopsy sampling. No biopsy is to be taken if tumor is inaccessible, biopsy not in subject's best interest or thought to be dangerous or if subject declines to consent to biopsy. **For Arm D**: Tumor biopsy from at least one injected lesion at screening and at Week 6 is highly desired but not mandatory.
9. ECOG performance status assessment, height, weight, and physical examination may be done the day before CyPep-1 administration or pembrolizumab administration (if pembrolizumab administration day is different from CyPep-1 scheduled day) before dosing for logistical reasons.
10. For vital signs (blood pressure, pulse and temperature), a time window of ± 5 min is allowed. **For Arms A, B and C:** Vital signs before and 15 min, 30 min, 1 h, 2 h and 4 h after treatment will be recorded for all subjects during Cycle 1 on Days 1, 15, 29 and 36 (DLT/TLT period). For all subsequent cycles, vital signs will be recorded on Day 1 before and 15 min, 30 min, 1 h, 2 h and 4 h after treatment. For all other Days of each cycle, only before treatment and if abnormal to be followed up at the investigator's discretion. **For Arm D:** Vital signs before and 15 min, 30 min, 1 h, 2 h and 4 h after treatment will be recorded for all subjects on each dosing day. For subjects who have no clinically significant abnormality with vital signs reported during the first four doses of CyPep-1 (on V1 (D1), V2 (D8), V3 (D15) and V4 (D22), vital signs will be recorded before and 15 min, 30 min, and 1 h after treatment on all the subsequent dosing days.
11. Single 12-lead ECG recordings will be taken pre-dose of CyPep-1 or pembrolizumab administration (if pembrolizumab administration day is different from CyPep-1 scheduled day). Subjects must be supine for approximately 10 minutes before ECG collection and remain supine but awake during ECG collection. Blood sampling can be performed before ECG procedure. **For Arm D:** ECG recordings will be performed both pre-dose and 30 minutes after CyPep-1 administration at screening, V1 (D1), V2 (D8), V3 (D15), and every 6 weeks from V4 (D22).
12. The safety laboratory includes hematology, biochemistry, coagulation, and urinalysis (performed by dipstick) to be performed pre-dose of CyPep-1 or pembrolizumab administration (if pembrolizumab administration day is different from CyPep-1 scheduled day). Laboratory values assessed during screening less than 72 hours before CyPep-1 administration do not need to be repeated at Visit 1. Further assessments may be performed up to 1 day prior to the visit in order to have the results (of at least Hb, WBC, platelets, PT-INR, and APTT) available on the visit day prior to CyPep-1 and/or pembrolizumab administration.
13. Creatinine clearance will be measured using CKD-EPI formula pre-dose of CyPep-1 administration or pembrolizumab administration. Assessment less than 72 hours before CyPep-1 administration do not need to be repeated at Visit 1.
14. Women of childbearing potential must have a highly sensitive negative pregnancy test at screening (serum). Pregnancy testing (highly sensitive urine/serum) should be performed before each administration of trial treatment for the duration of the trial and at EoT, with an exception for Arm D, where the frequency of pregnancy testing should be performed before each administration during the first three visits followed by every 4 weeks counting from V4 ([D22], ±3 days) (see footnote 33).
15. PK assessment will be done at the following time-points: **For Phase I, and Arm C:** (up to 1 hour) pre-dose, and 15 min, 30 min, 1 hour, 2 hours, and 4 hours post-dose. **For Arm D**: (up to 1 hour) pre-dose, and 15 min, 30 min, and 1 hour post-dose. A time window of ± 5 min is allowed for the PK blood samples.
16. [redacted]
17. Peripheral blood phenotyping of selected immune cell markers will be performed by a central laboratory.
18. **For Phase I, and Phase IIa Arms A, B, and D**: Local measurements of accessible (to-be) injected cutaneous/subcutaneous tumor lesion(s) must be done within one day (<24h) prior to each CyPep-1 injection by caliper and color digital photography, using a ruler held flush to the skin next to the longest diameter of the lesion to indicate the size of the lesion. If needed, guidance with ultrasound is permitted. The (to-be) injected lesions under ultrasound control must be measured with ultrasound guidance before each CyPep-1 injection and only on dates of CyPep-1 injection. All ultrasound and color digital photography image files should be stored and available in the patient file). Lesion(s) that disappeared will be checked on the days of CyPep-1 injections. **For Arm C:** If lesion injections are performed under CT-guidance, the volume measurement will be performed by CT **For Arm D:** Lesions identified as non-injected lesions will be measured following the procedures as the (to-be) injected lesions above in a 4-week interval starting at D1. Details on local measurement to determine the injection volume see Section 5.3.1.3.
19. Premedication is mandatory prior to the first IT dose of CyPep-1. Premedication is started 30-60 minutes before the IT injection, and continues for up to 24-48 hours after the procedure was completed, if necessary. An antihistamine (e.g., 25 to 50 mg clemastine) is mandatory with optional anxiolytics if deemed appropriate by the investigator. Only for subjects experience pain

during or after IT administration, it is recommended to provide celecoxib 400 mg as premedication every 6 hours for 24-48 hours.Premedication should be administered for subsequent CyPep-1 doses based upon clinical judgment and presence/severity of prior reactions. This regimen may be modified based on local treatment standards and guidelines as appropriate. To ensure prophylactic hydration, subjects should consume ≥2 L of fluid intake per day, starting ≥48 hours prior to first dose/each dose escalation and for ≥24 hours thereafter. For subjects who have difficulty to maintain adequate oral hydration, intravenous hydration is recommended, at an infusion rate of 150-200 mL/hr (i.e., 2-3 L per day) for initial dosing; for following dose escalations, an intravenous hydration with 1.5-2 L per day is advisable. For lesions > 7 cm, weekly CyPep-1 administration should be considered by the Investigator for the first 6 weeks, on Days 1, 8, 15, 22, 29 and 36 of cycle 1. The assessments are as per scheduled visit for Day 1, 15 and 29. Visits on Day 8 and 22 will follow the assessments as per D15 (C1V2) with the exclusion of central lab sampling for cytokine analysis and blood sampling for immune cell phenotyping. For Day 36 assessments as specified in the schedule need to be performed, with additional assessments: Pre-dose ECG, Vital Signs PRE- & 15m, 30m, 1h, 2h, 4h POST-dose, Coagulation and Pregnancy test (if applicable).

20. All concomitant medication/procedures should be reported beginning upon ICF signature until FU (or EoT, if it occurs more than 30 days after the last CyPep-1 or pembrolizumab administration).
21. Reporting period is from ICF signature until FU (or EoT, if it occurs more than 30 days after the last CyPep-1 or pembrolizumab administration). After the FU visit (or EoT, if it occurs more than 30 days after the last CyPep-1 or pembrolizumab administration), only ongoing (S)AEs related to CyPep-1 or pembrolizumab administration will be collected. Adverse events will be assessed using CTCAE v5.0, including start and stop dates, severity, relationship to CyPep-1 or pembrolizumab, outcome and action taken.
22. Radiological assessment should include chest, abdomen, pelvis and all other known sites of disease. The baseline scan for radiological assessment is to be done within 4 weeks prior to C1V1 (V1 for Arm D). If radiological assessments has been done in clinical routine within 4 week before Visit 1 and scan fulfills requirements for iRECIST (and additionally itRECIST for Arm D) evaluation, this scan can be used for baseline evaluation (not need to be repeated during screening). Follow-up scans are to be performed every 8 weeks starting C1V1 (V1 for Arm D). The window for follow up radiological assessments during the trial is ± 3 days. Once a tumor lesion(s) is documented, the target area should be recorded at every subsequent time-point for the duration of the trial. Efficacy assessment will be based on iRECIST (and additionally itRECIST for Arm D) for up to 10 target lesions. All skin lesions must be assessed by caliper measurement during clinical exam on the dates corresponding to iRECIST/itRECIST assessment. Documentation by color photography including a ruler to estimate the size of the lesion should be stored in the patient file.
23. A confirmatory radiological assessment only has to be done if PD was observed with the previous radiological assessment. If the lesion(s) continue(s) to enlarge, the original PD will be considered as the “first documented PD”. If lesion(s) remain(s) unchanged or decrease(s) in size, assessments should continue as per protocol (i.e., the next radiological assessment will be performed after an 8-week interval). In this case, the first measured PD will be disregarded. In case of unconfirmed PD after the first (or subsequent) cycle, the subject may continue Q2W administrations of CyPep-1 and Q6W administrations of pembrolizumab in the combination arm. If PD is confirmed 4 weeks later, then the subject will be taken off-trial and move to the EoT visit.
24. Continuous Q2W CyPep-1 treatment is planned and until confirmed PD, unacceptable toxicity or consent withdrawal, loss to follow up, or trial end, whichever occurs first. Lesions not originally planned to be injected with CyPep-1 at screening may also be injected per investigator’s discretion, if necessary and after consulting with medical monitor. Follow-up visits will follow the interval as defined in Table 1a/c. For subjects in the combination arm treatment with pembrolizumab will continue as per SoC following Q6W dosing until PD, unacceptable toxicity or consent withdrawal.
25. Per additional cycles: C2, C3, …Cn. CXV1 is scheduled 2 weeks after the last CyPep-1 administration (to follow a Q2W administration schedule).
26. If a subject is receiving additional Q2W treatment with CyPep-1, a biopsy of any new injected lesion(s) can be taken (not mandatory) after 6 weeks for each subsequent cycle. A biopsy at CxD1 (pre-dose) is only applicable if any new lesion(s) are selected for CyPep-1 injection (not mandatory). Arrangements to be made for any tumor material to be sent for analysis are described in the laboratory manual. The visit window for biopsies on CxV4 is ± 3 days.
27. Cytokine levels should only be assessed in the first and second cycles of CyPep-1 administration.
28. The visit window for biopsies on C1V4 is ± 3 days.
29. Pembrolizumab administration only in the combination arm (Arm B). Pembrolizumab should be administered as per SOC Q6W independent of other administrations or assessments (see Section 5.3.2 for details regarding pembrolizumab administration). CyPep-1 should be adminsitered 30 to 60 min after prembromlizumab if on the same day. All safety assessments and safety laboratory testing should be performed on all pembrolizumab administration days as specified in Table 1c. Pembrolizumab will be administered using IV infusion after all procedures and assessments have been completed. See Section 5.3.6.2 for dose modifications and toxocity management. Pembrolizumab will be administered to a maximum of 18 cycles and then stopped.
30. Thyroid panel should include: triiodothyronine (T3) or free triiodothyronine (FT3); tree thyroxine (FT4); thyroid stimulating hormone (TSH).
31. The frequency for tumor biopsy is every 6 weeks from D1 if a subject stays on treatment. The biopsy of at least one injected lesion is highly desired but not mandatory.
32. Frequency of assessments is every 6 weeks counting from V4 ([D22], ±3 days).
33. Frequency of assessments is every 4 weeks counting from V4 ([D22], ±3 days).
34. A lesion map that will guide the preferred sequence of injection of the identified Lesion Sets must be created prior to the first CyPep-1 injection (detailed in Study Procedural Manual). The lesion map should be updated at every visit during the treatment period. Once all injectable lesions have been injected and the subject remains on treatment, a new lesion map permits

reprioritizing of the to-be-injected Lesion Sets will be created, taking into account size changes of individual lesions, e.g., an individual lesion enlarged or regressed after initial 3 doses of CyPep-1 injections. For details see Section 5.3.1.3.

1. INTRODUCTION

The information given in this section is an overview of the pre-clinical and clinical development program for CyPep-1 that underpins the proposed Phase I/IIa clinical trial. Further specific and more detailed information is provided in the Investigator's Brochure (IB) for the Investigational Medicinal Product (IMP) CyPep-1 injectable solution.

Additionally, this section also contains information on the immune checkpoint inhibitor (ICI) pembrolizumab, which will be used in combination with the IMP CyPep-1 in this Phase I/II a clinical trial.

1.1 Name and Description of Investigational Medical Product and of Pembrolizumab

CyPep-1 is a synthetic linear 27-amino acid peptide with an acetylated amino group at the N-terminus and an amido group at the C-terminus. All optically active amino acid residues are in D-configuration with the exception of achiral glycine.

CyPep-1 is a white to off-white powder, formulated in a clear and colorless NaCl 0.9% aqueous solution (5 mg/mL).

Pembrolizumab is a potent humanized immunoglobulin G4 (IgG4) monoclonal antibody (mAb) with high specificity of binding to the programmed cell death 1 (PD-1) receptor, thus inhibiting its interaction with programmed cell death ligand 1 (PD-L1) and programmed cell death ligand 2 (PD-L2). Based on preclinical in vitro data, pembrolizumab has high affinity and potent receptor blocking activity for PD-1. Pembrolizumab has an acceptable preclinical safety profile and is in clinical development as an intravenous (IV) immunotherapy for advanced malignancies. Keytruda® (pembrolizumab) is indicated for the treatment of patients across a number of indications. For more details on specific indications, refer to the IB.

Refer to the IB/approved labeling for detailed background information on pembrolizumab (MK-3475).

1.2 CyPep-1 for Treatment of Advanced Solid Cancers

1.2.1 Background

Treatment with ICIs has been shown to result in long lasting anti-tumor responses in selective subjects with different tumor indications. However, only a subset of these subjects obtains durable remission. Several studies have previously indicated the presence of intratumoral CD8+ T cells to be a positive predictive factor for response to ICI therapy (Herbst et al, 2014; Kitano et al, 2018; Oliva et al, 2019; Prelaj et al, 2019). Thus, treatment strategies aimed at recruiting tumor antagonizing cellular components of the immune system hold great promise and are currently a main focus in oncology research (Zappasodi et al, 2018).

1.2.2 Clinical Rationale

Current evidence reveals an association between mutational tumor burden and tumor immunogenicity (Snyder et al, 2014; Schumacher et al, 2015; Samstein et al, 2019). In order to grow, antigenic tumors must develop mechanisms that enable them to evade immune-mediated destruction through a process termed immunoediting (Vesely et al, 2013). However, while mutational load represents a predictive factor for ICI therapy (Gibney et al, 2016; Huerter et al 2018), its predictive power is modest; rather, it seems associated with the presence of specific neoantigen-generating mutations (Snyder et al, 2014). This observation, supported by both preclinical- and clinical-evidence, suggests that a qualitative neoantigen-directed immunotherapy holds great potential. New therapeutic modalities in this category should ideally meet two critical criteria: 1) display tumor specific antigens and 2) induce an

inflamed tumor microenvironment. Furthermore, the capacity of such therapeutic modalities may result in an extended patient population of responders and potentially increase the overall efficacy of ICIs.

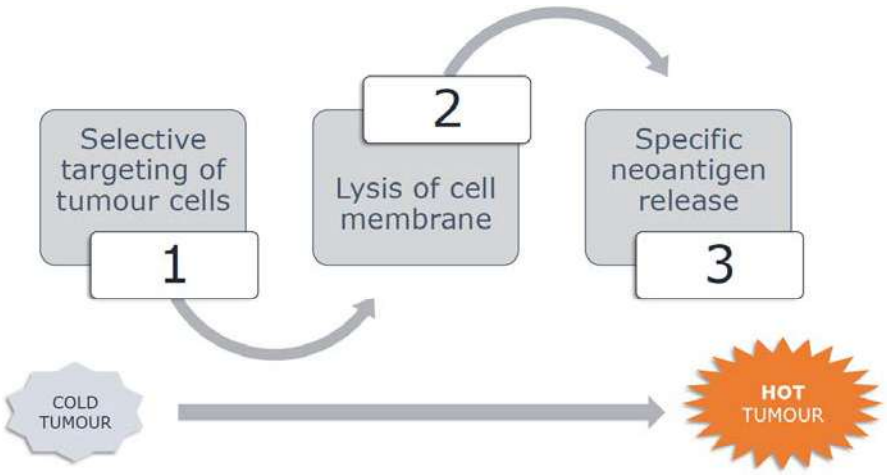
Conceptually, clinical proof-of-concept of this strategy has been established by lytic viruses, and preliminary evidence indicates that oncolytic treatment can induce systemic anti-tumoral responses (Ribas et al, 2017). Due to its unique antigen-exposing properties (please see the IB for further information), CyPep-1 could be a more potent and less toxic oncolytic therapy. In addition, CyPep-1 offers attractive pharmacological properties such as long shelf life, easy scalability, and negligible batch-to-batch variations. As such, CyPep-1 may represent a unique "tumor-agnostic" compound bolstering the effect of established immunotherapy across multiple tumor types. Lastly, preclinical studies showing that CyPep-1 can synergize with anti-PD-1 antibody treatment in terms of decreased tumor volumes and prolonged overall survival highlight the possible clinical utility of CyPep-1 in the combination setting with ICIs.

Cytovation is developing CyPep-1 for the treatment of advanced solid cancers. This Phase I/IIa trial will assess the safety, tolerability, pharmacokinetics, and pharmacodynamics of CyPep-1 when administered directly into tumor lesions, in monotherapy and in combination with the anti-PD-1 antibody pembrolizumab. Additionally, the trial will assess antitumor effects of CyPep-1 on injected lesions and distant non-injected deposits, as well as local and systemic immunological effects of CyPep-1, alone and in combination with anti-PD-1.

1.2.3 Treatment with CyPep-1

CyPep-1 is a chemically synthesized peptide with oncolytic properties, whose mode of action consists of selective targeting of tumor cell membranes based on their altered molecular composition and lysing of tumor cells by removing the surrounding cell membrane. This mode of action **1) kills cancer cells, 2) releases tumor antigens and 3) potentially induces a tumor-specific immune response by *in situ* immunization**. Preclinical toxicology studies have shown a favorable safety profile and potent anti-tumor activity of CyPep-1 in several cancer types. CyPep-1 modulates the tumor microenvironment by increasing the presence of CD8+ T cells and potentiates the effects of ICIs (please see the IB for further information). As such, intratumoral injection with CyPep-1 can transform immunological "cold" and treatment resistant tumors into "hot" and immunological active tumors that can be successfully treated with immune-modulating agents such as anti-PD-1 antibodies (Figure 1).

Figure 1. Mode of action of CyPep-1.



1.3 Pharmaceutical and Therapeutic Background of Pembrolizumab

The importance of intact immune surveillance function in controlling outgrowth of neoplastic transformations has been known for decades (Disis, 2010). Accumulating evidence shows a correlation between tumor-infiltrating lymphocytes in cancer tissue and favorable prognosis in various malignancies. In particular, the presence of CD8+ T-cells and the ratio of CD8+ effector T-cells/FoxP3+ regulatory T-cells (T-regs) correlates with improved prognosis and long-term survival in solid malignancies, such as ovarian, colorectal, and pancreatic cancer; hepatocellular carcinoma; malignant melanoma; and renal cell carcinoma. Tumor-infiltrating lymphocytes can be expanded ex vivo and reinfused, inducing durable objective tumor responses in cancers such as melanoma (Dudley et al., 2005; Hunder et al., 2008).

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene *Pdcd1*) is an immunoglobulin (Ig) superfamily member related to cluster of differentiation 28 (CD28) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) that has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2) (Greenwald et al., 2005; Okazaki et al., 2001).

The structure of murine PD-1 has been resolved (Zhang et al., 2004). PD-1 and its family members are type I transmembrane glycoproteins containing an Ig-variable–type (IgV-type) domain responsible for ligand binding and a cytoplasmic tail responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif, and an immunoreceptor tyrosine-based switch motif. Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases, SHP-1 and SHP-2, to the immunoreceptor tyrosine-based switch motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3 zeta (CD3ζ), protein kinase C-theta (PKCθ), and zeta-chain-associated protein kinase (ZAP70), which are involved in the CD3 T-cell signaling cascade (Okazaki et al., 2001; Chemnitz et al., 2004; Sheppard et al., 2004; and Riley, 2009). The mechanism by which PD-1 down-modulates T-cell responses is similar to, but distinct from, that of CTLA-4, because both molecules regulate an overlapping set of signaling proteins (Parry et al., 2005; Francisco, 2010). As a consequence, the PD-1/PD-L1 pathway is an attractive target for therapeutic intervention in subjects with locally advanced or metastatic disease.

1.4 Population to be Studied

Phase I and IIa CICILIA:

Histologically or cytologically confirmed locally advanced (unresectable) or metastatic tumors (solid tumors or lymphoma) with an accessible tumor lesion for intratumoral injection of CyPep-1 that meet one of the following criteria:

a. Relapsed following or progressed through standard therapy.

b. Have a disease for which no standard effective therapy exists.

1.5 Non-Clinical Data of CyPep-1

The nonclinical studies of CyPep-1 conducted to date, which include pharmacology, pharmacokinetics and metabolism, and toxicology studies, were designed to support the initiation of clinical trials in subjects with solid tumors (CyPep-1 solution formulation; Appendix A).

More than eleven nonclinical studies performed to date indicate the following with regard to the nonclinical profile of CyPep-1:

Pharmacology

In vitro experiments were performed with multiple cancer cell lines and normal human cells. CyPep-1 exerted a rapid cytotoxic effect on cancer cells and scanning electron microscopy demonstrated a dose dependent membranolytic effect. The compound was further tested *in vivo* in a 4T1 murine breast cancer model. A significant reduction in tumor growth was observed in the tumors of the treatment group with an average volume of 1.1 cm^3, whereas the control tumors had grown to an average size of 3.5 cm^3 ($p<0.0001$) after 24 days (Szczepanski et al, 2014). The survival analyses showed that the animals assigned to the treatment group lived significantly longer (median survival: 32 days) than those of the control group (median survival: 20 days), indicating a clear treatment effect of CyPep-1 (Szczepanski et al, 2014). Similar results were observed in the B16-F10 melanoma and CT26 colorectal adenocarcinoma mouse models, where CyPep-1 potentiated the effect of checkpoint inhibition. Treatment led to reduced tumor-volume and -weight, and increased survival. Lastly, preclinical studies in mice with B16-F10 melanoma tumors showed a reduction in both primary and contralateral tumor volumes, of which the primary tumors were injected with CyPep-1. The reduction in tumor volumes was enhanced when CyPep-1 was administered in combination with an anti-PD-1 antibody. This highlights the capacity of CyPep-1, either alone or in combination with an anti-PD-1 antibody, to delay growth of injected and non-injected tumors. No immunogenic effects have been observed with CyPep-1 in heathy animals (please see the IB for further information).

Pharmacokinetics

Radioactive labelled CyPep-1 was administered systemically to a Wistar rat model (Szczepanski et al, 2014). The basic pharmacokinetic profiles showed distribution to and accumulation in the liver, spleen, and kidneys. The half-live of CyPep-1 in plasma was 3.15 min. In a recent study (B-02852), the PK profile after a single intravenous (bolus) or subcutaneous administration of CyPep-1 to Sprague-Dawley rats was further assessed. Neither severe clinical observations, nor mortality or premature sacrifice were reported during the duration of the study. Irrespective of the type of administration, no treatment-related effects were observed up to the end of the observation period (6 hours post-dose). After a single intravenous administration at dose levels of 0.5, 1.0 and 3.0 mg/kg, the concentration of CyPep-1 in rat plasma reached the highest concentration (329.0, 724, and 3620 ng/mL, respectively) at 5 min. Thereafter, the test item concentration decreased gradually, being below the lower limit of quantification 6 hours after administration. After a single subcutaneous administration at a dose level of 3.0 mg/kg, the concentration of CyPep-1 in rat plasma reached the highest concentration (31.3 ng/mL, respectively) at 15 min after the administration. Thereafter, the test item concentration decreased gradually, being below the lower limit of quantification 2 hours after administration. The bioavailability was 4.78%.

Toxicity

In animal studies, CyPep-1 was administered IV (0.5 mg/kg to 20 mg/kg in rats and 0.1 mg/kg to 7 mg/kg in dogs; Study 507379) or topically (formulated as 1.0% cream; AB22310 and AB22311). For all Good Laboratory Practices (GLP) studies, the test products contained CyPep-1 alone or with compendial excipients that have been previously approved for use in

pharmaceutical and dermatologic products. The following conclusions were derived from the toxicity studies of IV CyPep-1 (Appendix A):

- Systemic administration of CyPep-1 was well tolerated in rats at levels of up to 10 mg/kg upon single IV bolus injection, and at levels of 3 mg/kg/day upon repeat (7-day) IV bolus administration (Study 506066)
- At a dose of 0.5 mg/kg/day, systemic administration of CyPep-1 was well tolerated for 4 weeks, with no clinical signs observed and minimal pathology findings (Study 506092).
- There were no CyPep-1-related effects on body weight, body temperature, pupil size (Study 614721), respiratory rate, tidal volume, and minute volume (Study 614716) at single IV doses of 0.5, 1, or 3 mg/kg
- CyPep-1 is not mutagenic in a standard battery of *in vitro* and *in vivo* genotoxicity tests (Studies 01027002, 01027003)
- No cardiotoxicity was observed upon CyPep-1 administration (Study 614700)
- Upon sub-cutaneous administration of 3 mg/kg in rats, CyPep-1 could not be detected in plasma after 2 hours.

Based on the non-clinical development programs completed to date, the safety profile of CyPep-1 has been established. The non-clinical program supports the use of CyPep-1 in clinical trials.

Details of the results for the pre-clinical testing of CyPep-1 can be found in the IB and in the Investigational Medicinal Product Dossier (IMPD).

1.6 Clinical Data of CyPep-1

1.6.1 Safety and Efficacy of CyPep-1

The intratumoral formulation of CyPep-1 has not yet been assessed in clinical trials. All available safety information for Phase I of this ongoing trial is summarized in the IB (please see V3.0). Overall, no DLTs or any CyPep-1-related SAE were reported.

A randomized, placebo-controlled, double-blind, single center Phase I trial to explore safety, tolerability, and pharmacodynamics of CyPep-1 as topical formulation (1.0% w/w cream) administered to subjects with cutaneous human papillomavirus (HPV) warts has been completed.

The primary objectives are to evaluate safety and tolerability of CyPep-1 when applied to healthy skin for up to one week and cutaneous warts for up to four weeks. The trial has no secondary objectives. The exploratory objectives are to evaluate the activity and explore the efficacy of CyPep-1 across wart subgroups defined by HPV-type and lesion age, after four weeks of treatment, and after six and twelve weeks of post-treatment follow-up, when applied to cutaneous common warts.

The trial has two parts: Part 1 follows a target area of 5x5 cm healthy skin to study tolerability and safety of the formulation. During this part, also a maximum of three common warts, preferably at the dorsal or palmar side of the hand / finger(s), are treated. Several assessments are performed to determine pharmacodynamics and to explore possible efficacy after a treatment period of 1 week.

Part 2 evaluates the pharmacodynamics and efficacy of CyPep-1 after a treatment period of 4 weeks and is planned to be initiated after the execution of an interim analysis (a blind data review) of Part 1.

In total, 8 subjects with at least one common wart of 3-10 mm located, preferably, at the dorsal or palmar side of the hand / finger(s) were enrolled (N=4 active, N=4 placebo). A target area of 5x5 cm of healthy skin on the upper back as well as 1-3 common warts were treated for 1 week on a daily basis with CyPep-1/placebo topical cream (0.15 mg/wart). Study medication was applied under occlusion using respectively hypoallergenic transparent film dressings for common warts (2 x 2 cm) and the upper back (6 x 7 cm). The treated areas were occluded up to 24 hours.

The interim study analysis performed on the subjects treated with CyPep-1 cream in Part 1 of the trial revealed no serious adverse events (SAEs). The majority of AEs were of mild nature and non-treatment emergent. Five AEs were classified as moderate, four of which were classified as non-treatment emergent (2 headaches, increase in transaminases and erysipelas) and one classified as treatment-emergent (headache). One AE was classified as severe: vasovagal collapse during blood sampling and considered as non-treatment emergent. A small number of AEs were classified as treatment emergent (coded as possibly related to the IMP) because they occurred within hours after IMP administration. The concomitant medication that was used post-dose was related to treatment of AEs. Besides a transient elevation of transaminases in one subject, there were no clinically significant changes observed in vital signs, laboratory values or electrocardiogram (ECG). In general, the IMP topical administration was well tolerated, and no systemic trends were noted in any of the parameters measured. No discontinuations due to AEs occurred and no treatment interruptions were necessary. Furthermore, since the randomization is 1:1, the AEs seemed rather evenly distributed and presumably comparable between the CyPep-1 cream and Placebo groups. Therefore, it can be concluded that seven consecutive days of topical application of CyPep-1 cream on the warts as well on an enlarged surface of 25cm^2 was safe and locally very well tolerated. Part 2 of the trial, assessing efficacy of CyPep-1, is ongoing.

In conclusion, the results of the preclinical studies with CyPep-1 and those of the interim analysis of the aforementioned clinical trial with CyPep-1 as topical formulation, including the favorable tolerability profile, support the initiation of clinical trials in subjects with solid tumors (CyPep-1 solution formulation).

1.7 Pre-clinical and Clinical Trials with Pembrolizumab

Therapeutic studies in mouse models have shown that administration of antibodies blocking PD-1/PD-L1 interaction enhances infiltration of tumor-specific CD8+ T cells and ultimately leads to tumor rejection, either as a monotherapy or in combination with other treatment modalities (Hirano, 2005; Blank, 2004; Weber, 2010; Strome, 2003; Spranger, 2014; Curran, 2010; Pilon, 2010). Anti-mouse PD-1 or anti-mouse PD-L1 antibodies have demonstrated antitumor responses in models of squamous cell carcinoma, pancreatic carcinoma, melanoma, acute myeloid leukemia and colorectal carcinoma (Strome, 2003; Curran, 2010; Pilon, 2010; Nomi, 2007; Zhang, 2004). In such studies, tumor infiltration by CD8+ T cells and increased IFN-γ, granzyme B and perforin expression were observed, indicating that the mechanism underlying the antitumor activity of PD-1 checkpoint inhibition involved local infiltration and activation of effector T cell function in vivo (Curran, 2010). Experiments have confirmed the in vivo efficacy of anti-mouse PD-1 antibody as a monotherapy, as well as in combination with chemotherapy, in syngeneic mouse tumor models (see the IB).

1.8 Justification for Pembrolizumab Dose

The planned dose of pembrolizumab for this trial is 400 mg every 6 weeks (Q6W).
A 400 mg Q6W dosing regimen of pembrolizumab is expected to have a similar benefit-risk profile as 200 mg Q3W, in all treatment settings in which 200 mg Q3W pembrolizumab is

currently appropriate (Lala et al., 2020). Specifically, the dosing regimen of 400 mg Q6W for pembrolizumab is considered adequate based on modeling and simulation (M&S) analyses, given the following rationale:

- Pharmacokinetic (PK) simulations demonstrating that in terms of pembrolizumab exposures –
    - Average concentration over the dosing interval (C_{avg}) (or area under the curve [AUC]) at 400 mg Q6W is similar to that at the approved 200 mg Q3W dose, thus bridging efficacy between dosing regimens.
    - Trough concentrations (C_{min}) at 400 mg Q6W are generally within the range of those achieved with 2 mg/kg or 200 mg Q3W in the majority (>99%) of patients.
    - Peak concentrations (C_{max}) at 400 mg Q6W are well below the C_{max} for the highest clinically tested dose of 10 mg/kg Q2W, supporting that the safety profile for 400 mg Q6W should be comparable to the established safety profile of pembrolizumab.
    - Exposure-response (E-R) for pembrolizumab has been demonstrated to be flat across indications, and OS predictions in melanoma and non-small cell lung cancer (NSCLC) demonstrate that efficacy at 400 mg Q6W is expected to be similar to that at 200 mg or 2 mg/kg Q3W, given the similar exposures; thus 400 mg Q6W is expected to be efficacious across indications.

1.9 Potential Risks and Benefits

1.9.1 Risks

In toxicology studies in mice, CyPep-1 administered as IV solution or topical cream was well tolerated for up to four weeks at doses higher that those planned to be used in this trial, with no clinical signs observed and minimal pathology findings. At single IV doses of 0.5, 1, or 3 mg/kg administered to rats, CyPep-1 had no effects on body weight, body temperature, pupil size, respiratory rate, and tidal and minute volume. In addition to the lack of effects listed above, in multiple *in vitro* (0.025 mg/mL) and *in vivo* (most commonly used: 5 mg/mL) studies, CyPep-1 had no mutagenic or cardiotoxic effects. Therefore, no specific adverse reactions are to be inferred at the clinical starting dose of 0.5 mg/mL of CyPep-1 or the subsequent proposed doses as “expected” based on preclinical experience.

Nevertheless, CyPep-1 is a soluble synthetic peptide with the potential risk of inducing allergic reactions. Possible AEs associated with an allergic reaction can include fever, chills, headache, weakness, nausea, vomiting, diarrhea, low blood pressure, respiratory symptoms, and rashes. Additionally, since the mode of action of CyPep-1 includes potential stimulation of the immune response, there is a likelihood of immune-related AEs. Generally, such AEs have been observed after specific blocking of inhibitory receptors expressed on T cells (e.g. PD-1) rather than after induction of tumor-specific immune responses. If CyPep-1 elicits a particularly strong T-cell immune response in a few subjects predisposed to develop autoimmunity, immune-related AEs may nonetheless be observed.

In addition to potential risks related to administration of CyPep-1 (e.g. leakage into circulation), there are also procedural risks related to blood sampling, other possible subcutaneous injections and diagnostic procedures. Blood sampling and subcutaneous injections may cause pain, bleeding, bruising, and/or infections at the site of cannula insertion. Rare complications due to blood sampling are syncope, thrombophlebitis, as well as accidental punctures of an artery or nerve.

The risks of pembrolizumab administration are detailed in the IB of the compound. Pembrolizumab has been documented to cause immune-related adverse reactions, for which a series of measures are recommended (see Section 3.1.2 and Section 5.3.6).

1.9.2 Benefits

This is the first clinical trial with the CyPep-1 injectable solution. The principal aim of this trial is to obtain safety and preliminary efficacy data when CyPep-1 is intratumorally administered to subjects with solid tumors for whom no other treatment options currently exist, as monotherapy and in combination with pembrolizumab. In addition to the minimal risk using the starting dose of 0.5 mg/mL of CyPep-1, preclinical experience as well as the safety profile of cohort 1 with the compound indicate a potential benefit that might be expected in terms of clinical efficacy. Preclinical assays describing the synergy of CyPep-1 with anti-PD-1 antibody treatment indicates the potential benefit of this therapeutic combination in patients with cancer.

The safety and efficacy data, together with the pharmacokinetic and pharmacodynamic data obtained from this trial, will help establish the treatment regimen and recommended doses suitable for subsequent clinical trials in the target population. Treatment of subjects with CyPep-1, as monotherapy and in combination with pembrolizumab, is hypothesized to result in improvement or stabilization of the disease state of the subjects.

1.9.3 Management of Risks

To minimize the risk to subjects and maximise safety, the following factors have been incorporated into the study design. Detailed safety and laboratory assessments will be performed.

- All clinical observations will be evaluated by the investigator on an ongoing basis.
- Each subject must stay on site for at least 4 hours after study drug administration on each treatment day (vital signs will be assessed on each treatment day pre-dose and at 15 minutes, 30 minutes, and 1, 2, and 4 hours post-dose during the first treatment cycle and on Day 1 of each additional treatment cycle). If only pembrolizumab is administered, subjects are not required to stay at the site for 4 hours for vital signs assessments. For Arm D, for subjects who have no clinically significant abnormal vital signs reported during the first four doses of CyPep-1 (in Week 1 to 4), the post-dosing safety monitoring at the clinic can be shortened to 1 h on all the subsequent dosing days.

- Premedication is mandatory prior to the first IT dose of CyPep-1. Premedication is started 30-60 minutes before the IT injection, and continues for up to 24-48 hours after the procedure was completed, if necessary. An antihistamine (e.g., 25 to 50 mg clemastine) is mandatory with optional anxiolytics if deemed appropriate by the investigator. Only for subjects experience pain during or after IT administration, it is recommended to provide celecoxib 400 mg as premedication every 6 hours for 24-48 hours. Premedication should be administered for subsequent CyPep-1 doses based upon clinical judgment and presence/severity of prior reactions. This regimen may be modified based on local treatment standards and guidelines as appropriate.
- For all subjects in Phase I, the first 3 subjects in Arm B, and the subjects in Arm C cohort 4, there must be a window of at least 24 hours between each subject's treatment start. This is not applicable for the subjects in Phase IIa Arm A, remaining subjects in Arm B, Arm C cohort 5, and Arm D.
- CyPep-1 will not be administered if at the time of the planned administration the subject is suffering from severe infection or receiving steroid-based immunosuppressive therapy (excluding topical steroids).
- The study agent must be administered in a clinical setting where emergency resuscitative equipment and personnel trained in the management of anaphylaxis are

immediately available to treat systemic reactions under the direct supervision of a physician.

To minimize the risks to subjects upon administration of pembrolizumab, the following measures are recommended, depending on the situation (Sections 5.3.6.2 and 5.3.6.3 for complete list of considerations on dose modification of pembrolizumab based on the occurrence and severity of immune- or infusion-related AEs)*:*

- Suspected Stevens-Johnson syndrome or toxic epidermal necrolysis: withhold until recovery to Grades 0-1; if this does not occur within 12 weeks after last dose of pembrolizumab or if corticosteroid dosing cannot be reduced to ≤10 mg prednisone or equivalent per day within 12 weeks, pembrolizumab should be permanently discontinued.
- Confirmed Stevens-Johnson syndrome or toxic epidermal necrolysis: permanent drug discontinuation.
- Grade 3 or 4 myocarditis, encephalitis, or Guillain-Barre syndrome: withhold until recovery to Grades 0-1 or, if above conditions apply, permanently discontinue.

Phase IIa Arm B:

CyPep-1 monotherapy can continue if there is unacceptable toxicity to pembrolizumab. Pembrolizumab monotherapy can continue if there is unacceptable toxicity to CyPep-1. Related dose modification rules for CyPep-1 and pembrolizumab is detailed in Section 5.3.6).

1.9.4 Benefit/Risk Ratio

The anticipated risks, based on the non-clinical experience with the CyPep-1 injectable solution and the clinical experience with the standard of care anti-PD-1 antibody pembrolizumab, are expected to be manageable. The importance of the objective of this trial is considered to outweigh the risks and burdens to the subjects. Measures are implemented to minimize burdens and risks for subjects. Subjects will be monitored closely for the occurrence of any significant clinical events and treatment will only continue if it is considered safe and appropriate to do so. The benefit/ risk assessment is favorable and justifies the planned trial in subjects with solid tumors at a situation with no therapeutic options available.

2. TRIAL OBJECTIVES AND ENDPOINTS

2.1 Objectives

2.1.1 Primary Objectives

The primary objectives are:

- To evaluate the safety and tolerability of intratumoral administration of CyPep-1, as monotherapy and in combination with pembrolizumab.
- To identify the recommended phase II dose (RP2D) of CyPep-1, as monotherapy and in combination with pembrolizumab.

2.1.2 Secondary Objectives

The secondary objectives are:

- To assess the preliminary anti-tumor efficacy of CyPep-1, as monotherapy and in combination with pembrolizumab.
- To characterize the pharmacokinetics (PK) of CyPep-1.

2.1.3 Exploratory Objectives

The exploratory objectives are:

- To assess the preliminary anti-tumor efficacy of CyPep-1, as monotherapy and in combination with pembrolizumab, in injected lesions and non-injected lesions, separately.
- To assess survival after treatment with CyPep-1, as monotherapy and in combination with pembrolizumab.
- To assess the immune mondulating properties of treatment with CyPep-1, as monotherapy and in combination with pembrolizumab.

2.2 Endpoints

2.2.1 Primary Endpoints

The primary endpoints are:

- Type and number of AEs according to National Cancer Institute (NCI) – Common Terminology Criteria for Adverse Events (CTCAE) criteria v5.0, and additional safety parameters of CyPep-1 as monotherapy and in combination with pembrolizumab.
- Dose limiting toxicities (DLTs) and the maximum tolerated dose (MTD) for determination of RP2D of CyPep-1 as monotherapy and treatment-limiting toxicities (TLTs) of CyPep-1 in combination with pembrolizumab.

2.2.2 Secondary Endpoints

The secondary endpoints are:

- Objective response rate (ORR), defined by complete and partial responses, according to immune Response Evaluation Criteria in Solid Tumors (iRECIST) based on the investigator's assessment.
- Time to and duration of response and duration of stable disease.
- The plasma concentration time profile of CyPep-1 and, if detectable, the derived PK parameters (i.e., area under the curve [AUC], peak plasma concentration [C_{max}], time to reach C_{max} [t_{max}], systemic clearance [CL], elimination half-life [$t_{1/2}$] and volume of distribution [VD]).

2.2.3 Exploratory Endpoints

The exploratory endpoints are:

- For all Phase IIa arms: ORR in injected lesions and non-injected lesions, separately, per itRECIST* [Goldmacher 2020]).
- Progression-free survival (PFS) per iRECIST based on the investigator's assessment.
- Overall survival (OS).
- The relative change in number of tumor infiltrating CD8+ T-cells in the injected and, whenever available, non-injected tumor biopsies.
- The association between the relative change in tumor infiltrating CD8+ T-cells and response rate in the injected lesions (per itRECIST) and all lesions (per iRECIST). The change in T-cell receptor (TCR) clonality levels in peripheral blood and (when available) biopsied lesions.
- Changes in the tumor microenvironment (injected and, whenever available, non-injected tumor biopsies) via expression of selected candidate immune markers: [redacted]
- For Arms A, B, and C: [redacted]
- Peripheral blood phenotyping of selected immune cell markers.

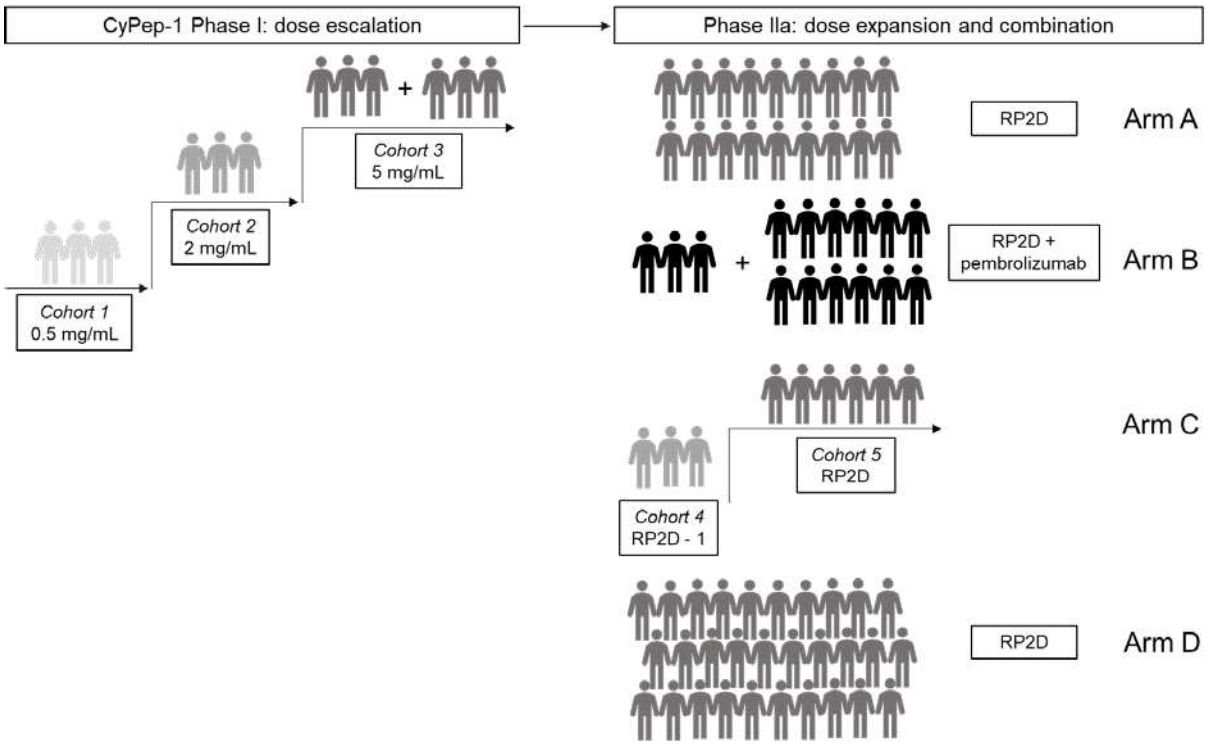
*itRECIST will be programatically calculated using data obtained from iRECIST assessments following the itRECIST principle summarized in Appendix F Figure 1.

3. TRIAL DESIGN

3.1 Overall Trial Design

This is a combined Phase I/IIa, open-label, dose escalation followed by dose expansion trial in subjects with advanced solid cancers. The trial consists of two phases and multiple arms, as shown in the diagram below (Figure 2).

Figure 2. Schematic trial design



Phase I (dose escalation, N=12) and Phase IIa (dose expansion Arm A N=9*; combination Arm B N=15 and dose expansion liver metastases Arm C, N=9; dose expansion melanoma Arm D, N= 30).

Abbreviations: RP2D: recommended phase 2 dose.

*In case in the first 12 subjects at RP2D, a responder per iRECIST (ORR based on best response or 2 subjects with stable disease per iRECIST are observed (SD based on duration >16 weeks from onset of SD), the number of subjects will be expanded to a total of 24 (CyPep-1 monotherapy; cohort 3 of Phase I and in Phase IIa Arm A).

Phase I– dose escalation:
In this phase of the trial, safety and tolerability will be documented and the MTD/RP2D will be determined. Cohorts of 3 subjects will receive intratumoral injections with CyPep-1. The DLT observation period for each dose level will be 6 weeks (5 weeks of trial treatment and 1 week of safety follow-up). The following CyPep-1 administrations are planned as continuous Q2W administrations.

Screening will occur during the 4 weeks prior to start of CyPep-1 treatment.
In three dose cohorts of 3 subjects each, subjects will receive CyPep-1 intratumorally at different concentrations (dose escalation) and volumes (depending on tumor size), i.e.,

0.5 mg/mL, 2 mg/mL, or 5 mg/mL, respectively. These dose concentrations have been selected based on previous preclinical experience with CyPep-1.

Dose escalation (Phase I of the trial) will be conducted to determine the MTD or RP2D using a standard "3+3" design, with at least three subjects treated at each dose-level. Arm C (from Phase IIa) will follow a similar dose escalation of two dose levels (RP2D and the dose immediately below). Dose escalation will proceed according to the following rules:

Number of subjects with DLT*	**Escalation decision rule**
0 out of 3 subjects	Enter 3 subjects at the next planned dose level.
1 out of 3 subjects	Enter 3 additional subjects at this dose level: • If 0 of these additional subjects experience a DLT, proceed to the next dose level. • If 1 or more of the additional 3 subjects experience a DLT, then dose escalation to higher dose levels is stopped. Three additional subjects may be entered at the second highest dose if only 3 subjects have been treated at that dose level. Alternatively, a new dose level may be introduced between the second highest dose and the MTD (3 additional subjects).
≤ 1 out of 6 subjects at the highest dose level below the MTD	This dose level is declared the MTD or RP2D.

*Assessed as related to the trial drug (CyPep-1) at any given dose level.

Each subject will receive IT injection(s) with CyPep-1 in Week 1, 3, and 5, respectively. After each CyPep-1 administration, subjects will be required to stay at the clinic for at least 4 hours for safety and, for those in which PK will be assessed, also for PK monitoring. The data from the additional three subjects in cohort 3 will be used for further confirmation of RP2D.

For each dose cohort, at least 24 hours must elapse between each subject to start treatment with CyPep-1. No intra-subject dose escalation is allowed.

In Phase I, subject replacement for subjects that drop out for any reason, except DLTs, will only occur before the DLT observation period is completed and is allowed if a subject does not receive all three CyPep-1 administrations, unless due to CyPep-1-related toxicity. No subject replacement will occur for subjects who withdraw later.

After completion of Phase I, all results will be evaluated by the DEC. This will include safety data and if available, other supportive clinical data (i.e. efficacy, pharmacokinetics, tumor biopsy analyses) from all subjects included in Phase I of the trial. The DEC will confirm the MTD or RP2D (in case the MTD is not reached).

All additional arms of the CICILIA trial can start once Phase I data has been evaluated by the DEC and an MTD or RP2D is confirmed for CyPep-1.

<u>Phase IIa – dose expansion (Arm A):</u>
In this phase, safety and tolerability will be further evaluated in an expanded cohort of 9 subjects at the RP2D of CyPep-1, determined in Phase I. Screening and CyPep-1

administration will be according to the schedule described for Phase I , except for the 24-h observation period before start of treatment of the next subject, which is not applicable.

In case in the first 12 subjects at RP2D, a responder per iRECIST (ORR as CR/PR as best response) or 2 subjects with stable disease (SD with duration >16 weeks from onset) per iRECIST are observed, the number of subjects will be expanded to a total of 24 (CyPep-1 monotherapy; subjects cohort 3 of Phase I and in Phase IIa Arm A).

Phase IIa – combination arm (Arm B):
The safety and tolerability of CyPep-1 in combination with pembrolizumab will be evaluated in a cohort of 15 subjects in total, using a staggered approach. Initially, 3 subjects will receive CyPep-1 at RP2D in combination with pembrolizumab and each subject will be observed for 24 h before the next subject can be administered with CyPep-1. After the first 3 subjects completed the TLT observation period of 6 weeks with no TLTs observed and after reviewing the safety data (including TLTs) by the DEC, the inclusion of all remaining subjects at RP2D of CyPep-1 in combination with pembrolizumab can be initiated. In the absence of any TLTs, the 24-h observation period before start treatment of the next subject is no longer applicable. Should any TLTs be observed in the first 3 subjects at the RP2D of CyPep-1 in combination with pembrolizumab, an additional 3 subjects will be included in this dose group. If ≤1 out of 6 subjects have a TLT, the remainder of the 15 subjects will be treated at the RP2D. In the unlikely case that 2 or more of the 6 subjects experience any TLTs at RP2D of CyPep-1, a recommendation to dose de-escalate and to what dose or to continue the combination part of the trial will be made by the DEC. The same screening is planned, and CyPep-1 will be administered according to the same schedule as in Phase I.

Scheduling of treatment of CyPep-1 and pembrolizumab (Arm B) will be as follows: CyPep-1 will be administered every 2 weeks (Q2W) and pembrolizumab will be administered following a Q6W schedule starting on Day 1, as per standard of care. Radiological assessment to determine occurrence of (un)confirmed PD will be performed Q8W from the start of treatment. Treatment will continue until confirmed PD, unacceptable toxicity or consent withdrawal, loss to follow up, or trial end, whichever occurs first.

Phase IIa – dose expansion liver metastases (Arm C):
The safety and tolerability of at least two dose levels of CyPep-1, namely the RP2D and the dose immediately below that, are planned to be evaluated when CyPep-1 is administered intratumorally using ultrasound guidance to one metastatic lesion in the liver. The two dose levels are planned to be investigated adhering to the 3+3 design and will follow the procedures as described for the dose escalation (Phase I) (Section 3.1). The first three subjects will receive CyPep-1 at the lower dose level and each subject will be observed for 24 h before CyPep-1 administration to a next subject (cohort 4). After the third subject at that dose finished the DLT observation period of 6 weeks (5 weeks CyPep-1 treatment plus 1 week safety follow-up), the DEC will decide on the start of the cohort to be administered at RP2D based on the review of the safety data (cohort 5). In the absence of any DLTs in the first 3 subjects, the 24-h observation period before start treatment of the next subject is not applicable. After the DLT observation period for the first three subjects at RP2D for CyPep-1 and the review of all safety data, the DEC will recommend on the inclusion of all remaining subjects. Should a DLT be observed in the first three subjects at the RP2D of CyPep-1, an additional three subjects in this dose group will be included. In case that two or more subjects experience DLTs at RP2D of CyPep-1, a recommendation to dose de-escalate and to what dose and how to continue Arm C will be made by the DEC.

Phase IIa – dose expansion melanoma subjects (Arm D):

The safety and tolerability of CyPep-1 at RP2D will be further evaluated with focus on assessing efficacy signals of CyPep-1 monotherapy in up to 30 subjects with melanoma. Although safety information will be collected, there will not be a formal DLT observation period. The dosing scheme starts with a once weekly (QW) CyPep-1 administration until the second iRECIST/itRECIST assessment at week 16, followed by a Q2W dosing scheme.

As part of the continuous safety monitoring of the trial, reports with cumulative safety data of Arm D subjects will be shared with all investigators, competent authorities, and ethical committees of countries where the trial is conducted at the following time points:

- After the first three subjects completed three weeks of treatment with CyPep-1.
- After the first three, six, and 12 subjects completed six weeks of treatment with CyPep-1.

In the occurrence of AEs fulfilling DLT criteria or other safety alters, the DEC will convene to review the safety data and provide recommendations on the continuation of dosing.

For both phases and all trial arms, subjects will stay in the trial until end of trial or until confirmed disease progression, unacceptable toxicity, death or discontinuation for any other reason. Information regarding subject replacement for each arm is detailed in Section 4.3.2.

3.1.1 Dose Escalation Committee

The decisions on dose escalation and CyPep-1 MTD/RP2D will be taken by the Dose Escalation Committee (DEC) after reviewing safety data (including DLTs) from all subjects who have entered Phase I of the trial and have completed the DLT observation period.

The DEC will review all data after all subjects in the highest dose cohort completed DLT observation period and before enrolment of subjects in the expansion cohort at RP2D in monotherapy and combination with pembrolizumab cohort can be initiated.

For Phase IIa of the trial, the decision to de-escalate the dose of CyPep-1 based on observed the severity and relatedness of safety events (DLT/TLT criteria for CyPep-1) and to what dose (either dose level of the next lowest dose level from Phase I or 50-70% of current dose level), will also be made by the DEC after reviewing available safety data (including TLTs). Dose modifications for pembrolizumab in the combination arm will also be taken into consideration (refer to Section 5.3.6).

Based on the review of this data, recommendations will be made regarding the further conduct and the scientific and ethical integrity of the trial.

The DEC is comprised of all the investigators or designees, as well as the medical monitor and representatives of the sponsor. The DEC, chaired by the medical monitor, will strive for a consensus opinion regarding the data reviewed. If DEC consensus is not possible, the DEC chair will assemble and present majority and dissembling opinions for all recommendations considered. If any of the DEC members cannot be present during the meeting due to the short notice, the DEC will be held (not postponed) provided that a minimum of 2 members are present. The DEC members may be allowed to provide their recommendations electronically to the DEC chair, prior to the meeting.

At the end of the DEC teleconference, the DEC will provide one of the following recommendations to the sponsor:

1. The trial is proceeding in line with the trial protocol.
2. The trial is not proceeding in line with the trial protocol and/or there is a possible change in the benefit/risk ratio.

The sponsor will act upon these recommendations as appropriate, i.e., the final decision will rest with the sponsor. The sponsor designee will notify the trial team (i.e., the responsible project manager at [redacted]) of the final decision regarding the DEC recommendations, including any actions to be taken. The [redacted] project manager will communicate the DEC recommendations and/or final decision of the sponsor to all investigators, Independent Ethics Committees (IECs) and Competent Authorities (CAs), if applicable.

3.1.2 Dose Limiting Toxicities / Treatment Limiting Toxicities

A DLT is defined by the occurrence of any of the following toxicities according to CTCAE v5.0 during treatment Cycle 1 and that are considered by the investigator to be possibly, probably, or definitely related to CyPep-1:

3. Any Grade ≥ 3 AEs of any etiology, except:
    - Nausea, vomiting, or diarrhea will be considered a DLT only if it persists at Grade ≥ 3 for > 3 days despite adequate supportive care measures. At the investigator's discretion, subjects who experience nausea, vomiting, or diarrhea after receiving CyPep-1 may receive antiemetic or anti-diarrheal medication prior to subsequent doses of CyPep-1.
    - Isolated laboratory abnormalities Grade ≥ 3 (not present at baseline) that resolve to Grade ≤ 1 in ≤ 7 days without clinical sequelae or need for therapeutic intervention.
    - Fatigue Grade ≥ 3 for ≤ 7 days.
    - For Phase I, Arms A and C:
        - Alanine amino transferase (ALAT) or Aspartate amino transferase (ASAT) toxicities:
        - ALAT or ASAT > 5 x upper limit of normal (ULN) (> 7.5 x ULN for subjects with liver metastases), unless greater than 14 days.
        - ALAT or ASAT > 5 x ULN (> 7.5 x ULN for subjects with liver metastases) accompanied by an elevation in total bilirubin of > 2.5 x ULN (not explained by obstruction), regardless of duration, will be considered a DLT.
4. Any other toxicity occurring at any time during the trial that in the view of the participating investigators and the medical monitor represents a clinically significant hazard to the subject.

All TLTs will be graded using NCI CTCAE v5.0 based on the investigator assessment. The TLT window of observation will be during Cycle 1.
The occurrence of any of the following toxicities during Cycle 1 will be considered a TLT, if assessed by the investigator to be possibly, probably, or definitely related to trial treatment administration.

1. Grade 4 nonhematologic toxicity (not laboratory).
2. Grade 4 hematologic toxicity lasting ≥7 days, except thrombocytopenia:
    - Grade 4 thrombocytopenia of any duration
    - Grade 3 thrombocytopenia associated with clinically significant bleeding
3. Any non-hematologic AE ≥Grade 3 in severity should be considered a TLT, with the following exceptions: Grade 3 fatigue lasting ≤ 3 days; Grade 3 diarrhea, nausea, or vomiting without use of anti-emetics or anti-diarrheal per standard of care; Grade 3 rash without use of corticosteroids or anti-inflammatory agents per standard of care.
4. Any Grade 3 or Grade 4 non-hematologic laboratory value if:
    - Clinically significant medical intervention is required to treat the participant or
    - The abnormality leads to hospitalization, or
    - The abnormality persists for >1 week.

- The abnormality results in a Drug-induced Liver Injury (DILI)
- Exceptions: Clinically nonsignificant, treatable, or reversible laboratory abnormalities including liver function tests, uric acid, etc.

5. Febrile neutropenia Grade 3 or Grade 4:
   - Grade 3 is defined as ANC <1000/mm^3 with a single temperature of >38.3 degrees C (101 degrees F) or a sustained temperature of ≥38 degrees C (100.4 degrees F) for more than 1 hour
   - Grade 4 is defined as ANC <1000/mm^3 with a single temperature of >38.3 degrees C (101 degrees F) or a sustained temperature of ≥38 degrees C (100.4 degrees F) for more than 1 hour, with life-threatening consequences and urgent intervention indicated.
6. Prolonged delay (>2 weeks) in initiating Cycle 2 due to treatment-related toxicity.
7. Any treatment-related toxicity that causes the participant to discontinue treatment during Cycle 1.
8. Missing >25% of CyPep-1 doses as a result of drug-related AE(s) during the first cycle.
9. Grade 5 toxicity.

3.2 Measures to Minimize Bias

This is an open-label non-randomized trial; blinding and randomization are not applicable.

3.3 Duration of Subject Participation

Each subject will undergo up to 4 weeks of screening, followed by a DLT/TLT observation period of 6 weeks (5 weeks of CyPep-1 treatment plus 1 week observation). In absence of DLT/TLTs, treatment continues with Q2W intratumoral injections of CyPep-1. For the combination arm (Arm B), plus Q6W administration of pembrolizumab for up to 24 months (18 cycles). For Arm D, after screening, each subject will undergo QW CyPep-1 treatment until the second iRECIST/itRECIST assessment at week 16, followed by a Q2W dosing scheme. Continuous treatment cycles of CyPep-1 and/or pembrolizumab are planned and should follow the Q2W administration schedule until confirmed PD, unacceptable toxicity, death, or consent withdrawal, loss to follow up, or trial end, whichever occurs first.

Hereafter, there will be efficacy (PFS) follow-up every 8 weeks, or until confirmed PD, death, withdrawal of consent, loss of subject to follow-up, or trial end, whichever occurs first.

- Start of inclusion: Q1 2020
- Planned last subject last follow-up visit: last subject's last visit for follow-up

For both phases and all trial arms, subjects will stay in the trial until end of trial or until confirmed disease progression, unacceptable toxicity, death or discontinuation for any other reason.

<u>Phase IIa Arm B</u>:

The maximum number of cycles of pembrolizumab is 18 with an approximately duration of 2 years. Then pembrolizumab will be stopped. CyPep-1 treatment may continue.

3.4 Trial Stopping Rules

The investigator or the sponsor may terminate this trial prematurely for any reasonable cause. The IECs and CAs should be informed promptly.

Conditions that may warrant termination include, but are not limited to:

- The discovery of an unexpected, significant, or unacceptable risk to the subjects enrolled in the trial, or potential trial subjects

- The incidence or severity of AEs in this trial posing an unacceptable risk to the subjects enrolled in the trial.
- A decision by the sponsor to suspend or discontinue testing, evaluation, or development of the product.

The identification of a trial-stopping criterion in a subject will result in the permanent discontinuation of CyPep-1 as monotherapy and in combination with pembrolizumab in this subject and the enrolment will be temporarily held until an appropriate evaluation of the cause of the toxicity has been determined and a correction action plan is established if needed.

The Medical Monitor should be notified immediately i.e. within 24 hours of learning of their occurrence, and the subject should be followed for safety as clinically indicated until the toxicity resolves and, in the opinion of the investigator, no further follow-up regarding the toxicity is needed (minimum of at least 14 days after last dose of CyPep-1 as monotherapy and in combination with pembrolizumab).

If the competent authority obtains information that raises doubts about the safety or scientific validity of the clinical trial, the competent authority can suspend or prohibit the trial. Before the competent authority reaches its decision, it shall, except where there is imminent risk, ask the sponsor and/or the investigator for their opinion, to be delivered within one week (Directive 2001/20/EC, Article 12, Section 1).

The investigator will be notified by the sponsor if the trial is terminated or placed on hold. The relevant IECs and CAs will also be informed according to appropriate regulatory requirements.

If the trial is prematurely terminated or suspended for any reason, the investigator/Institution should promptly inform the trial subjects and should assure appropriate therapy and follow-up for the subjects.

3.5 End of Trial

The end of trial is defined as the last subject's last visit.

For the purposes of data summarization, data analyses will be performed after the last enrolled subject has completed 3 months of trial participation. Subjects may still be in the trial at the time of data summarization, as all subjects may continue to participate until confirmed disease progression, unacceptable toxicity, or discontinuation for any other reason. Subjects who continue will follow the protocol as per SoA.

Phase IIa Arm B:

The maximum number of cycles of pembrolizumab is 18 with an approximately duration of 2 years. Then pembrolizumab will be stopped. CyPep-1 treatment may continue.

4. SELECTION OF TRIAL POPULATION

4.1 Inclusion Criteria

For Phase I and Phase IIa Arms A and C:

1. Histologically or cytologically confirmed locally advanced (unresectable) or metastatic tumors (solid tumor or lymphoma) with an accessible tumor lesion for intratumoral injection of CyPep-1 malignancy (including lymphomas) that is either:
    a. Refractory to standard-of-care treatment
    b. Have a disease for which there is no standard therapy considered appropriate.
    Metastatic deposits (including cutaneous/subcutaneous lesions and metastatic deposits in lymph nodes) of tumors for which IT injections may be performed are eligible. Pure cutaneous infiltrations (e.g., breast cancer cutaneous carcinomatosis) are ineligible.

2. 1 to 3 non-ulcerated transcutaneously accessible lesion(s) for injection and measurable as defined by iRECIST. All other tumor lesion(s) may be selected for efficacy follow-up, but will not be subjected to treatment with CyPep-1.
3. Presence of tumor lesion(s) (that have not been previously irradiated) suitable for biopsy at screening and at Week 6.

For Arm C:

4. Confirmation of the presence of at least one liver metastasis by imaging.
5. Subjects must have measurable disease which is equal to one or more metastatic liver lesions that can be accurately and serially measured; that are greater than 1 cm dimension andfor which the longest diameter is greater or equal to 1 cm as measured by CT (computed tomography) scan or magnetic resonance imaging (MRI). The metastatic liver lesion(s) must not be in an area that received prior localized therapies.
6. Metastatic liver lesion(s) for injection with >50% radiological visible necrosis must be avoided and the leasion must be located where any tumor swelling will not lead to gall bladder tract obstruction or lead to bleeding risk.

For Arm D:

7. Histologically or cytologically confirmed diagnosis of advanced (unresectable Stage III) or metastatic (Stage IV: M1a and/or M1b) melanoma considered incurable by the standard of care. For metastatic melanoma, only cutaneous, subcutaneous, lymph node, or lung metastases are allowed.
8. Previously exposed to ICI(s) and be categorized following the SITC Immunotherapy Resistance Taskforce (Kluger 2020) meeting one of the following:
    a. Have primary resistance: PD-(L)-1 inhibitor exposure ≥6 weeks and have the best response as one of the following:
        i. PD,
        ii. SD for <6 months.
    b. Have secondary resistance: PD-(L)-1 inhibitor exposure ≥6 weeks and best response CR, PR, or SD >6 months.
    c. Have adjuvant therapy resistance: recurrence subcategorized into primary resistance/early relapse occurred <12 weeks after the last dose, and late relapse occurred ≥12 weeks after the last dose. If BRAF mutated, patients must have progressed to treatment with BRAF inhibitors.
    d. Have neoadjuvant therapy resistance including subjects with or without major pathologic response and subsequent PD that fulfills criteria for primary or secondary resistance

e. Discontinued from ICI(s) therapy due to immune-related adverse events grade 3 or 4 other than endocrine insufficiencies treatable with hormonal replacement therapy, and meet one of the following:
    i. Remain on SD at discontinuation of PD-(L)1 inhibitor in combination with ipilimumab or show regrowth after <12 weeks of the last dose
    ii. Have not achieved a CR with single-agent PD-(L)1 inhibitor or combination of PD-(L)1 with LAG-3 inhibitor

9. At least 1 non-ulcerated lesion, not exceeding 5 cm in (the longest) diameter, for intratumoral injection(s) as a target lesion as defined by iRECIST.
10. Resolution of toxicity due to prior therapy returned to baseline or < Grade 2, except for alopecia or other irreversible immune-mediated AEs, as defined by CTCAE v5.0. and SITC ICI-related AEs (Brahmer et al, 2021).
11. Prior treatment(s) delivered by IT injection to the to-be injected lesion(s), including investigational agents, is allowed.

For Phase I and Phase IIa Arms A, C, and D in addition:

12. Age ≥ 18 years.
13. Estimated life expectancy of at least 3 months.
14. Eastern Cooperative Oncology Group (ECOG) Performance Status of 0 or 1 (Appendix B).
15. Resolution of toxicity due to prior therapy to Grade < 2 (except for alopecia and transaminases in case of liver metastases) as defined by CTCAE v5.0 (Appendix C).
16. Ability to give written informed consent and to comply with the protocol.
17. All subjects of childbearing potential (defined as < 2 years after last menstruation or not surgically sterile) must have a negative highly sensitive pregnancy test at screening (urine/serum) and agree to use highly effective method for contraception according to the EU Clinical Trial Facilitation Group guidance from time of signing the informed consent form (ICF) until at least 120 days after the last administration of CyPep-1. The partners of subjects with childbearing potential must also apply contraceptive methods and are recommended not to donate sperm.
18. A male participant must agree to use contraception and refrain from sperm donation during the treatment period and for at least 120 days after the last dose of trial medication.
19. Adequate bone marrow, liver, and renal function:
    a. Platelet count ≥ 100 x 10^9/L
    b. Hemoglobin ≥ 6.0 mmol/L or 9.67 g/dL
    c. Absolute Neutrophil Count (ANC) ≥ 1.5 x 10^9/L
    d. Total bilirubin ≤ 1.5 x ULN, except for subjects with familial bilirubinemia (Gilbert's disease)
    e. Serum ASAT and ALAT ≤ 2.5 x ULN (≤ 5 x ULN in case of liver metastases)
    f. Creatinine clearance ≥ 30 mL/min (Glomerular Filtration Rate [GFR] to be calculated by CKD-EPI formula)

For Phase IIa Arm B:
Participants are eligible to be included in Arm B of the trial only if all of the following criteria apply:

Type of Participant and Disease Characteristics

20. The participant provides written informed consent for the trial.
21. Be ≥ 18 years of age on day of signing informed consent.

22. Participant with histologically or cytologically confirmed diagnosis of advanced (unresectable Stage III) or metastatic (Stage IV) solid tumor malignancy (including lymphomas) that is refractory to standard-of-care treatment or for which there is no standard therapy considered appropriate. Metastatic deposits (including cutaneous/subcutaneous lesions and metastatic deposits in lymph nodes) of tumors for which IT injections may be performed are eligible. Pure cutaneous infiltrations (e.g., breast cancer cutaneous carcinomatosis) are ineligible.
23. Subjects must have progressed on treatment with an anti-PD1/L1 monoclonal antibody (mAb) administered either as monotherapy, or in combination with other checkpoint inhibitors or other therapies. Treatment progression is defined by meeting all of the following criteria:
    a. Has received at least 2 doses of an approved anti-PD-1/L1 mAb.
    b. Has demonstrated disease progression (PD) after PD-1/L1 based on clinical progression and / or based on RECIST v1.1 as defined by the Investigator/treating physician.
24. A male participant must agree to use contraception and refrain from sperm donation during the treatment period and for at least 120 days after the last dose of trial medication.
25. A female participant is eligible to participate if she is not pregnant, not breastfeeding, and at least one of the following conditions applies:
    a. Not a woman of childbearing potential (WOCBP)
    b. A WOCBP (defined as < 2 years after last menstruation or not surgically sterile) must have a negative highly sensitive pregnancy test at screening (urine/serum) and must follow contraceptive guidance (highly effective method for contraception according to the EU Clinical Trial Facilitation Group guidance) from time of signing the ICF until at least 120 days after the last administration of trial medication. The partners of subjects with childbearing potential must also apply contraceptive methods and are recommended not to donate sperm.
26. 1 to 3 non-ulcerated transcutaneously accessible lesion(s) for injection and measurable as defined by iRECIST. All other tumor lesion(s) may be selected for efficacy follow-up but will not be subjected to treatment with CyPep-1.
27. Presence of tumor lesion(s) (that have not been previously irradiated) suitable for biopsy at screening and at Week 6.
28. Have an ECOG performance status of 0 to 1 (Appendix B).
29. Have adequate organ function as defined in the following table. Specimens must be collected within 10 days (or less) prior to the start of trial treatment.

Adequate Organ Function Laboratory Values:

System	Laboratory Value
Hematological	
Absolute neutrophil count (ANC)	≥1500/µL
Platelets	≥100 000/µL
Hemoglobin	≥9.0 g/dL or ≥5.6 mmol/L[1]
Renal	
Creatinine OR Measured or calculated[2] creatinine clearance (GFR can also be used in place of creatinine or CrCl)	≤1.5 × ULN OR ≥30 mL/min for participant with creatinine levels >1.5 × institutional ULN
Hepatic	
Total bilirubin	≤1.5 ×ULN OR direct bilirubin ≤ULN for participants with total bilirubin levels >1.5 × ULN
AST (SGOT) and ALT (SGPT)	≤2.5 × ULN (≤5 × ULN for participants with liver metastases)

ALT (SGPT)=alanine aminotransferase (serum glutamic pyruvic transaminase); AST (SGOT) =aspartate aminotransferase (serum glutamic oxaloacetic transaminase); GFR=glomerular filtration rate; ULN=upper limit of normal.

[1] Criteria must be met without packed red blood cell (pRBC) transfusion within the prior 2 weeks. Participants can be on stable dose of erythropoietin (≥ approximately 3 months).

[2] Creatinine clearance (CrCl) should be calculated per institutional standard.

Note: This table includes eligibility-defining laboratory value requirements for treatment; laboratory value requirements should be adapted according to local regulations and guidelines for the administration of specific chemotherapies.

30. Only for subjects with lymphoma: have measurable disease defined as at least one lesion that can be accurately measured in at least two dimensions with spiral CT scan. Minimum measurement must be > 15 mm in the longest diameter by > 10 mm in the short axis.
31. Estimated life expectancy of at least 3 months.
32. HIV infected participants must be on anti-retroviral therapy (ART) and have a well-controlled HIV infection/disease defined as:

    a. Participants on ART must have a CD4+ T-cell count ≥ 350 cells/mm^3 at time of screening.
    b. Participants on ART must have achieved and maintained virologic suppression defined as confirmed HIV RNA level below 50 copies/mL or the lower limit of qualification (below the limit of detection) using the locally available assay at the time of screening and for at least 12 weeks prior to screening.
    c. Participants on ART must have been on a stable regimen, without changes in drugs or dose modification, for at least 4 weeks prior to study entry (Day 1).

4.2 Exclusion Criteria

For Phase I and Phase IIa Arms A, C, and D: Subjects who meet ANY of the following criteria at screening will be excluded from trial entry:

1. There is no limit to the number of prior treatment regimens, but prior treatment(s) should not include compounds delivered by IT injection to the to-be injected lesion(s), including investigational agents. Subjects with prior IT therapies are allowed in Arm D.
2. Participation in another clinical trial within 4 weeks prior to first dose of CyPep-1.
3. Anti-cancer therapy within 4 weeks prior to the first dose of CyPep-1 (within 2 weeks for palliative radiotherapy, within 1 week for endocrine therapy).
4. Major surgical procedure within 14 days prior to the first dose of CyPep-1.
5. Live vaccine within 30 days prior to first dose of CyPep-1.
6. Expected to require any other form of systemic or localized antineoplastic therapy while in this trial. Localized palliative radiotherapy for pain relief is allowed on tumor lesions that are not selected for evaluation of treatment response.
7. Clinical evidence of an active second malignancy that is progressing or requires active treatment, except for curatively treated early stage (carcinoma *in situ* or stage 1) carcinomas or non-melanoma skin cancer.
8. Active autoimmune disease requiring immunosuppressive therapy.
9. Any condition requiring continuous systemic treatment with either corticosteroids (> 10 mg daily prednisone equivalents) or other immunosuppressive agents within 2 weeks prior to first dose of CyPep-1. Inhaled, intranasal or topical (only on areas

outside the injected lesion[s]) and physiological replacement doses of up to 10 mg daily prednisone equivalent are permitted in the absence of active auto-immune disease.

10. Abnormal or clinically significant coagulation parameters.:
    - Prothrombin Time – International Normalized Ratio (PT-INR) ≥ 1.5 x ULN
    - Activated Partial Thromboplastin Time (APTT) ≥ 1.5 x ULN

    Subjects being treated with anticoagulants are excluded if the coagulation parameters are outside the therapeutic intervals as described in the SmPC for the administered treatment.
11. Subjects on anticoagulants with temporarily stop and start, supported by low molecular weight heparin (or other anticoagulation therapy at the discretion of the investigator and/or per local standard of care) during treatment period.
12. Known hypersensitivity to any component of CyPep-1.
13. Prior allogeneic tissue/solid organ transplant, stem cell or bone marrow transplant.
14. Known active human immunodeficiency virus (HIV). Subject is eligible when normal levels of CD4 are present.
15. Central nervous system (CNS) metastasis that is symptomatic or progressing or that requires current therapy (e.g., evidence of new or enlarging CNS metastasis, carcinomatous meningitis or new neurological symptoms attributable to CNS metastasis).
16. QTcF > 480 ms, history of long or short QT syndrome, Brugada syndrome, or known history of QTc prolongation, or Torsade de Pointes.
17. Women who are pregnant or breastfeeding.
18. Any serious and/or unstable pre-existing medical, psychiatric or other condition which in the investigator's opinion could interfere with subject safety, obtaining written informed consent, or compliance with the trial protocol.
19. Has an active acute or chronic infection requiring systemic therapy at the time of CyPep-1 injection. Note: Subjects treated for mild/moderate infection with oral antibiotics only may be included based on consultation with the study medical monitor and the sponsor.

<u>Additional exclusion criteria for Phase IIa Arm C</u>:

20. Subject is a candidate for hepatic surgery or local regional therapy of liver metastases with curative intent.
21. More than one third of the liver is estimated to be involved with metastases.
22. There is invasion by cancer into the main blood vessels such as the portal vein, hepatic vein or the vena cava.
23. Subject is currently receiving or has received liver metastatic-directed therapy (e.g. radiation, ablation, embolization) less than 4 weeks prior to enrolment or hepatic surgery.

<u>Exclusion criteria specific for Phase IIa Arm B</u>:

Participants are excluded from the trial if ANY of the following criteria apply at screening:

Pregnancy Exclusion

24. A WOCBP who has a positive urine pregnancy test (within 72 hours) prior to trial treatment. If the urine test is positive or cannot be confirmed as negative, a serum pregnancy test will be required.

Prior/Concomitant Therapy

25. Has received prior therapy with an anti-PD-1, anti-PD-L1, or anti PD-L2 agent or with an agent directed to another stimulatory or co-inhibitory T-cell receptor (e.g., CTLA-4, OX 40, CD137), and was discontinued from that treatment due to a Grade 3 or higher irAE.

26. Has received prior systemic anti-cancer therapy including investigational agents within 4 weeks (within 1 week for endocrine therapy) prior to first dose of CyPep-1.
    Note: Participants must have recovered from all AEs due to previous therapies to ≤Grade 1 or baseline as defined by CTCAE v5.0 (Appendix C). Participants with ≤Grade 2 neuropathy may be eligible. Participants with endocrine-related AEs Grade ≤2 requiring treatment or hormone replacement may be eligible.
    Note: If the participant had major surgery, this should not have been within 14 days prior to the first dose of CyPep-1 and the participant must have recovered adequately from the procedure and/or any complications from the surgery prior to starting study intervention.
27. Has received prior (palliative) radiotherapy within 2 weeks of start of trial treatment. Participants must have recovered from all radiation-related toxicities, not require corticosteroids, and not have had radiation pneumonitis. A 1-week washout is permitted for palliative radiation (≤2 weeks of radiotherapy) to non-CNS disease.
28. Has received a live or live-attenuated vaccine within 30 days prior to the first dose of CyPep-1. Note: Administration of killed vaccines are allowed.
29. Has received prior compounds delivered by IT injection to the to-be injected lesion(s), including investigational agents.
30. Expected to require any other form of systemic or localized antineoplastic therapy while in this trial. Localized palliative radiotherapy for pain relief is allowed on tumor lesions that are not selected for evaluation of treatment response.
31. Ongoing pembrolizumab-related toxicity event(s) as per TLT definition.

Prior/Concurrent Clinical Trial Experience

32. Is currently participating in or has participated in a trial of an investigational agent or has used an investigational device within 4 weeks prior to the first dose of trial treatment.

Note: Participants who have entered the follow-up phase of an investigational trial may participate as long as it has been 4 weeks after the last dose of the previous investigational agent.

33. Has had an allogeneic tissue/solid organ transplant.

Diagnostic assessments

34. Has a diagnosis of immunodeficiency or is receiving chronic systemic steroid therapy (in dosing exceeding 10 mg daily of prednisone equivalent) or any other form of immunosuppressive therapy within 7 days prior the first dose of CyPep-1.
35. Has a known additional malignancy that is progressing or has required active treatment within the past 3 years, except for curatively treated early stage (carcinoma *in situ* or stage 1) carcinomas or non-melanoma skin cancer.

Note: Participants with basal cell carcinoma of the skin, squamous cell carcinoma of the skin or carcinoma in situ (e.g., breast carcinoma, cervical cancer in situ) that have undergone potentially curative therapy are not excluded.

36. Has known active CNS metastases and/or carcinomatous meningitis. Participants with previously treated brain metastases may participate provided they are radiologically stable, i.e., without evidence of progression for at least 4 weeks by repeat imaging (note that the repeat imaging should be performed during study screening), clinically stable and without requirement of steroid treatment for at least 14 days prior to first dose of study treatment.
37. Has severe hypersensitivity (≥Grade 3) to pembrolizumab and/or any of its excipients or to another mAb, as well as any known hypersensitivity to any component of CyPep-1.
38. Has an active autoimmune disease that has required systemic treatment in past 2 years (i.e., with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (e.g., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency) is not considered a form of systemic treatment and is allowed.

39. Has a history of (non-infectious) pneumonitis / interstitial lung disease that required steroids or has current pneumonitis / interstitial lung disease.
40. Has an active infection requiring systemic therapy.
41. HIV-infected participants with a history of Kaposi sarcoma and/or Multicentric Castleman Disease.
42. Has a known history of Hepatitis B (defined as HBsAg reactive) or known active Hepatitis C virus (defined as HCV RNA [qualitative] is detected) infection.
    Note: No testing for Hepatitis B and Hepatitis C is required unless mandated by a local health authority.
43. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the participant's participation for the full duration of the trial, or is not in the best interest of the participant to participate, in the opinion of the treating investigator.
44. Has a known psychiatric or substance abuse disorder that would interfere with the participant's ability to cooperate with the requirements of the study.
45. Has received radiation therapy to the lung that is >30 Gy within 6 months of the first dose of trial treatment (applicable only for subjects with NSCLC, mesothelioma and small cell lung cancer [SCLC]).

4.3 Withdrawal, Discontinuation, and Replacement of Subjects

4.3.1 Withdrawal and Discontinuation

Subjects are free to withdraw consent from participation in the trial at any time and for any reason upon request. Withdrawal from the trial will not affect or prejudice the subjects' further care or treatment.

Subjects may be withdrawn from trial treatment and/or assessments at any time, if deemed necessary by the investigator.

Examples of reasons for withdrawal/discontinuation of subjects from this trial are:

- Screening failure;
- The decision of a subject to withdraw from the trial (i.e. if the subject withdraws informed consent);
- Pregnancy;
- Failure to comply with the trial visits and procedures;
- Subject is lost to follow-up (defined as a subject who has failed to return for two consecutive scheduled visits and cannot be contacted by the study site staff);
- Subject fulfils any other criteria that, in the opinion of the investigator, justifies discontinuation of the subject from the trial.
- Subjects experience side effects and/or require concomitant medications for treatment of HIV infection and/or its complications that are incompatible with continued study treatment (exceptions are permissible but should be discussed with the Sponsor and the medical monitor).

Subjects who withdraw from trial due to withdrawal of consent, inability or unwillingness of the subject to comply with trial procedures or are lost to follow-up are considered as trial dropouts and will not perform the follow-up visits.

Subjects who discontinue treatment with CyPep-1 for safety reasons (Section 7.2.1) or due to concomitant medication or therapy (Section 5.6) will not necessarily be considered withdrawn from the trial unless there are other reasons for withdrawal. They should return for all following visits and assessments until the last follow-up visit. For Arm B, treatment with pembrolizumab may continue at the discretion of the investigator.

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

- Discontinuation of treatment may be considered for subjects who have attained a confirmed complete response (CR) and have been treated for at least 8 cycles (at least 24 weeks), receiving for combination treatment: 2 cycles of the combination including 2 doses of pembrolizumab beyond the date when the initial CR was declared.
- Completion of 18 (for Q6W dosing) administrations (approximately 2 years) with pembrolizumab

(Note: The number of administrations is calculated starting with the first dose of pembrolizumab.)

- Any study intervention-related toxicity specified as a reason for permanent discontinuation as defined in the guidelines for dose modification due to AEs in Section 5.3.6. If CyPep-1 treatment is delayed > 7 days, that dose will be omitted. If the delay of > 7 days is during the TLT observation period, the subject will be replaced.

For Phase I, Phase IIa Arms A and C, if CyPep-1 treatment is delayed for > 6 weeks during the DLT period (first 3 injections), the subject should be discontinued from the trial. For Arm B, treatment with pembrolizumab may continue at the discretion of the investigator. For Arm D, in case CyPep-1 treatment is delayed for > 3 weeks (first 3 injections), the investigator and the Medical Monitor must assess on a case by case basis the decision whether the subject should be discontinued from the trial. Treatment delay due to TEAEs will be considered for treatment discontinuation.

For any subject who is withdrawn or discontinued from the trial, the reason, date and subsequent planned replacement therapy (if applicable), must be recorded in the electronic case report form (eCRF) and in the medical record. (S)AEs ongoing at trial withdrawal will be followed up for outcome information until resolution or stabilization (see Section 7.2.1).

When a subject is withdrawn or discontinued from active trial follow-up after having received at least one intratumoral CyPep-1 injection, and if the subject agreed on the ICF, every effort will be made to report serious adverse events (SAEs) until at least 3 months after the last intra-tumoral CyPep-1 injection administered for both trial phases.

If a subject withdraws consent from specific assessment(s) following intratumoral CyPep-1 injection (i.e. tumor biopsy, radiological or hematological assessments, etc.), all other assessments should be completed as per protocol and a protocol deviation should be recorded for both trial phases.

If a subject is withdrawn or discontinued from the trial, all data collected until the time of withdrawal will be used in the analyses.

4.3.2 Replacement of Subjects

In Phase I, replacement for subjects who drop out for any reason, except DLTs, will only occur before the DLT observation period is completed and is allowed if a subject does not receive all three CyPep-1 administrations, unless due to CyPep-1-related toxicity.

No subject replacement will occur for subjects who withdraw later or for subjects who are part of the Phase IIa expansion monotherapy Arms A and D.

For Arm B, replacement of the first 3 subjects is allowed if a subject drops out for any reason, and does not receive all three CyPep-1 injections of the first cycle, unless due to CyPep-1 and/or pembrolizumab related toxicity (TLT) during the TLT observation period. In case of pembrolizumab-related toxicity, treatment with CyPep-1 will be delayed and planned after a review by the investigator and medical monitor. If CyPep-1 treatment is delayed > 7 days, that dose will be omitted. If the delay is > 7 days during the TLT observation period, the subject will be replaced. No subject replacement will occur for subjects who discontinue later.

For Arm C, replacement for subjects who drop out for any reason, except DLTs, before the DLT observation period is completed is allowed when a subject did not receive all three CyPep-1 administrations. No subject replacement will occur for subjects who discontinue later.

5. TRIAL TREATMENT

5.1 Manufacturing and Labelling of Investigational Medical Product

5.1.1 Identity

CyPep-1 is a synthetic linear 27-amino acid peptide with an acetylated amino group at the N-terminus and an amido group at the C-terminus. All optically active amino acid residues are in D-configuration with the exception of achiral glycine. CyPep-1 is formulated in a clear and colorless aqueous solution (5 mg/mL).

5.1.2 Packaging and Labelling

The intratumoral formulation of CyPep-1 is a liquid dosage form for subcutaneous administration supplied in single-use vials. The CyPep-1 5 mg/mL solution consists of the drug substance CyPep-1 dissolved in 0.9% NaCl solution. Each vial contains a deliverable volume of 2 mL of CyPep-1 solution corresponding to 10 mg of the drug substance CyPep-1, present as acetate salt. An additional overfill is included in each vial to ensure that the labelled amount of 10 mg of CyPep-1 (corresponding to 14 mg of the acetate salt) can be delivered.

5.2 Instructions for Use, Handling, and Storage

The preliminary proposed shelf life of the CyPep-1 injectable solution is 18 months upon the date of manufacture, when stored at 5 ± 3°C (protected from light). The product may be kept outside the proposed storage condition at temperatures up to 25°C for up to 3 days to cover short-term temperature excursions (e.g. during shipping, labelling, and handling).

Pembrolizumab should be used, handled and stored in line with institutional guidelines.

5.3 Treatment Regimen

The overall study treatment regimen is defined as intratumoral CyPep-1 as monotherapy (Phase I and Phase IIa Arms A, C and D) and in combination with pembrolizumab (Phase IIa Arm B).

The interventions to be used in this trial are outlined below in Table 2.

Table 2. Trial Interventions

Intervention Name	Dosage Formulation	Unit Dose Strength(s)	Dosage Level(s)	Route of Administration	Regimen/Treatment Period	Sourcing
Pembrolizumab (MK-3475)	Solution for infusion	100 mg/vial	400 mg Q6W	IV infusion	Until end of trial or confirmed PD, unacceptable toxicity, death or discontinuation for any other reason for a maximum of 18 treatment cycles.	As provided by sponsor
CyPep-1	Solution for injection	See Section 5.3.1		Intratumoral	See Section 5.3.1	Provided centrally by the Sponsor

5.3.1 Administration of IMP (CyPep-1)

Administration will be through a needle inserted trans-dermally. The needle should be redirected along multiple tracks to ensure even dispersion of CyPep-1 throughout the lesion. For Arm C, CyPep-1 will be administered via intralesional injection with ultrasound guidance into one metastatic liver lesion.

A detailed guidance for intratumoral administration of CyPep-1 is presented in the Study Procedural Manual.

5.3.1.1 CyPep-1 Dose Levels

For Phase I, in three dose cohorts of 3 subjects each, subjects received CyPep-1 intratumorally at different concentrations (dose escalation) and volumes (depending on tumor size), i.e., 0.5 mg/mL, 2 mg/mL, or 5 mg/mL, respectively.

The CyPep-1 concentration for each dose cohort of the dose escalation (Phase I) was as follows:

Dose cohort	CyPep-1 concentration
1	0.5 mg/mL
2	2 mg/mL
3	5 mg/mL

Subjects in Phase IIa expansion arms (Arms A, B and D) of the trial will be treated at the CyPep-1 RP2D dose determined in Phase I. Based on the review of all safety data for the three dose cohorts and recommendation by the DEC (dated 09 Sep 2021), a RP2D for CyPep-1 is 5.0 mg/mL to be taken forward in Phase IIa.

During the dose expansion in liver metastases (Arm C), the safety and tolerability of at least two dose levels of CyPep-1, the RP2D (cohort 5) and the dose immediately below that (cohort 4), are planned to be evaluated when CyPep-1 is administered intratumorally into 1 single lesion in the liver.

5.3.1.2 CyPep-1 Injection Volume

The total amount of CyPep-1 injected will depend on the tumor volume(s). The volume delivered to each lesion selected for injection will be determined based on the longest diameter measured by the most accurate measurement technique available for each lesion (e.g. caliper, CT, or MRI) at screening and every 8 weeks during the iRECIST/itRECIST assessments. A chosen technique used at screening will be consistently used throughout the study. Lesion sizes need to be checked before each injection by either caliper (mandatory for Arm D and to-be-injected cutaneous/subcutaneous lesions for Phase IIa Arms A and B) or other imaging modalities used to guide CyPep-1 injections, i.e., ultrasound, CT or MRI, . For Arm C, , the lesion size measurement will be performed by the same imaging modality used to guide CyPep-1 injections. To ensure consistency of the measurements, the sites need to strive for having the same operator to perform all measurement for a chosen measuring technique. If there are major changes in the lesion size, the injection volume may be adjusted based on the ultrasound measurements per investigator's opinion and consultation with the Medical Monitor is possible.

For both phases of the trial, the cumulative maximal injected volume will be 4 mL per treatment day, corresponding to a cumulative maximal dose of 20 mg of CyPep-1 (with the maximal concentration of 5 mg/mL of CyPep-1, which is the RP2D). Effort should be made to administer the maximum planned injection volume of CyPep-1 as planned per lesion size (see tables below).

For Phase I, Phase IIa Arms A and B, the to-be-injected volume may be divided for injection over
1-3 tumor lesions (e.g., one large lesion or 2-3 relatively small lesions). In case more than 3 lesions are good candidates for injection, the to be injected lesions selection can be discussed with the sponsor and the medical monitor.

For Phase IIa Arm C, CyPep-1 will be administered with ultrasound guidance into one metastatic liver lesion. The same single metastatic lesion is to be injected with CyPep-1 based on lesion size.

For Phase IIa Arms A and B, for a lesion > 7 cm, the same lesion should be considered to be injected weekly with up to 4 mL of CyPep-1 for the first 6 weeks (on Day 1, 8, 15, 22, 29 and 36), independent of the potential change in lesion size, followed by Q2W 4 mL administrations of CyPep-1. The visits on Days 1, 15, 29 and 36 will follow the assessments as per schedule. The visits on Days 8 and 22 will follow the assessments as per D15 (C1V2; excluding central lab sampling for cytokine analysis and blood sampling for immune cell phenotyping).

For Phase IIa Arm D, the to-be-injected volume for injection is preferably divided over as many lesions as possible. The longest diameter of the lesions for injection is ideally smaller than 2 cm and should not exceed 5 cm.

For subjects in Arms A, B and C, the injection volume will be:

Measured Lesion Diameter (cm)	Injected volume (mL)
≤ 1	1
> 1 and ≤ 2	2
> 2	4

For subjects in Arm D, the injection volume will be:

Measured Lesion Diameter (cm)	Injected volume (mL)
≤ 0.79	0.1
0.80 to 0.99	0.2
1.00 to 1.24	0.5
1.25 to 1.50	1.5
1.51 to 2.49	3.0
≥ 2.50	4.0

5.3.1.3 Sequence of CyPep-1 Administration for Phase IIa Arm D Only

Lesion Map
A lesion map that will guide the preferred sequence of injection of the identified Lesion Sets must be created prior to the first CyPep-1 injection (detailed in Study Procedural Manual). A lesion map will contain the following information:
For superficial lesions:

- Digital color photography of the lesions must be taken and the mark-ups of individual lesions (or lesion groups) with lesion ID are overlayed on top of the digital photography.
- Silhouette of body parts must be used to present the relative location of the lesions.
- All digital color photography containing a ruler held next to the longest diameter of the lesion to indicate the size of the lesion, and the lesions marked-up with lesion IDs, together with the silhouette of body parts indicating the location of the lesions, need to be submitted to eCRF with inspection readiness for the reported responses.
- In the case of bilateral lesions, the laterality of the lesions must be documented on the lesion map.

For deep lesions:

- CT or MRI scan will be used for iRECIST/itRECIST assessment at baseline, and the location of lesions provided by the CT/MRI scan will be used.

- In the case of bilateral lesions, the laterality of the lesions must be documented on the lesion map.

Once the lesion map is created, Lesion Sets for sequential CyPep-1 injections will be planned and documented in the source. The size of the injected lesions and the lesion map should be updated when assessments of local measurements of tumor lesions, iRECIST and itRECIST assessments are performed during the treatment period.

Lesion Sets
A Lesion Set will be composed of multiple lesions, and will be administered with CyPep-1 during the same treatment visit for three consecutive doses. Unless there are no other Lesion Sets to be injected, each Lesion Set will not receive more than three consecutive doses of CyPep-1. In the case of bilateral lesions, both of the bilateral lesions should be included in the same lesion set if feasible considering the maximum injected volume allowed per treatment visit (4 mL).

The sequence of CyPep-1 Administration
After three doses of CyPep-1, the next Lesion Set will be administered with CyPep-1. The ideal next to-be-injected lesion set should be located as far as possible from the last injected Lesion Set on the lesion map to activate different draining lymph nodes and thus optimize a systemic immune response against the tumor. Once all injectable lesions have been injected and the subject remains on treatment, a new lesion map that permits reprioritizing of the to-be-injected lesion sets will be created, taking into account size changes of individual lesions, e.g., an individual lesion enlarged or regressed after initial 3 doses of CyPep-1 injections.

5.3.2 Administration of Pembrolizumab

The dose of pembrolizumab in combination with CyPep-1 in the combination arm (Arm B) will be 400 mg Q6W. Pembrolizumab will be administered using IV infusion on Day 1 of each 6-week treatment cycle after all procedures and assessments have been completed.

Dosing of CyPep-1 will be aligned such that subjects will receive both pembrolizumab and the first dose of CyPep-1 on the same day (C1V1). After C1V1, pembrolizumab dosing will continue following a Q6W dosing scheme until 18 cycles, confirmed PD, unacceptable toxicity, death, or consent withdrawal. Trial treatment of pembrolizumab may be administered up to 3 days before or after the scheduled Day 1 of each cycle due to administrative reasons after the first administration (C1D1).

On the visits that CyPep-1 and pembrolizumab are administered on the same day, CyPep-1 is planned to be administered 30 to 60 min after pembrolizumab is administered. In case of pembrolizumab-related toxicity, treatment with CyPep-1 will be delayed and planned after a review by the investigator and medical monitor.

Pembrolizumab will be administered as a dose of 400 mg using a 30-minute IV infusion. Sites should make every effort to target infusion timing to be as close to 30 minutes as possible. However, given the variability of infusion pumps from site to site, a window between -5 minutes and +10 minutes is permitted (i.e., infusion time is 30 minutes [-5 min/+10 min]).

The Pharmacy Manual contains specific instructions for the preparation of the pembrolizumab infusion and administration of infusion solution.

5.3.3 Meals and Dietary Restrictions

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

5.3.4 Selection of CyPep-1 Doses in the Trial

In Phase I of the trial, CyPep-1 will be administered intratumorally in escalating doses as follows:

1. Cohort 1: 0.5 mg/mL on Day 1 of Weeks 1, 3, and 5 (Days 1, 15, and 29)
2. Cohort 2: 2 mg/mL on Day 1 of Weeks 1, 3, and 5 (Days 1, 15, and 29)
3. Cohort 3: 5 mg/mL on Day 1 of Weeks 1, 3, and 5 (Days 1, 15, and 29)

Continuous Q2W administration of CyPep-1 is planned after Day 29.

Selection of these doses was based on previous preclinical experience with CyPep-1 (Szczepanski et al, 2014). The total amount of CyPep-1 injected will depend on the tumor volume. The volume delivered to each lesion selected for injection will be determined based on the longest diameter measured by the most accurate measurement technique available for each lesion (e.g. caliper, CT, or MRI) at screening and every 8 weeks during the iRECIST/itRECIST assessments (see Section 5.3.1.2). Before each injection, the longest diameter of the lesion will be measured. The approximate injected volume per lesion will be 1 - 4 mL for lesions <1.5 cm, 2 - 4 mL for lesions ≤ 4 cm, 3 - 4 mL for lesions > 4 cm. Efforts should be made to administer the maximum volume of CyPep-1 per administration as per dose level.

For Phase IIa Arms A, B and C, weekly administration schedule for the first 6 weeks should be considered for a lesion > 7 cm followed by Q2W administration.

The proposed CyPep-1 starting dose for the clinical trial is obtained from the therapeutic range identified in the nonclinical in vitro (0.025 mg/mL) and *in vivo* intratumoral studies (1-5 mg/mL (refer to IB). The data suggests that the starting dose of 0.5 mg/mL in the tumor microenvironment is within the identified range to achieve an initial and meaningful biological effect. Since intravascular administration can occur during intratumoral injection, the NOEL from IV administration studies was considered relevant to identify the first dose. Based on the 7 days toxicology study of repeat IV bolus with CyPep-1, the NOEL of 3,0 mg/kg was identified in rats. The corresponding HED is 0,48 mg/kg (0.16 x 3,0 mg/kg). For a 70 kg person this equals 33.6 mg. As the maximal cumulative starting dose in trial is 2 mg, a safety factor of 16.8 has been taken into account, considering that the route of administration is intratumoral instead of IV with limited systemic exposure anticipated.

Planned doses in the trial:

- 2 mg/mL (cumulative 8 mg) and 5 mg/mL (cumulative 20 mg)
- CICILIA Phase I dose escalation used IT administered CyPep-1 (comparable to subcutaneous administration study showing ~100-fold lower Cmax / ~20-fold lower AUC compared to IV with an ~ 5% bioavailability).
- AE summary of RP2D determined during Phase I: 5 mg/mL dose (20 mg CyPep-1) resulted in related TEAEs of injection site pain Grade 1 and 2 (AE number = 12) without DLTs, SAEs or AEs leading to discontinuation.

In Phase IIa of the trial, all subjects will receive intratumoral CyPep-1 at the doses specified in Section 5.3.1.

Phase I, the dose escalation part of the CICILIA trial, has been completed. The determined RP2D of IT administered CyPep-1 was 5 mg/mL. The MTD was not reached. The CyPep-1 dose to be applied in Phase IIa Arms A, B and D is 5 mg/mL.

For cohorts 4 and 5 of Phase IIa Arm C, the doses of CyPep-1 to be investigated are 2 mg/mL and 5 mg/mL, respectively.

5.3.5 Duration of the Treatment Regimen

Intratumoral CyPep-1

Each subject will undergo up to 4 weeks of screening, followed by a DLT/TLT observation period of 6 weeks (5 weeks of CyPep-1 treatment plus 1 week observation). In absence of DLT/TLTs, treatment continues with Q2W intratumoral injections of CyPep-1 until confirmed PD, unacceptable toxicity, death, withdrawal of consent, loss to follow-up, or trial end, whichever occurs first. For Arm D, after screening, each subject will undergo QW CyPep-1 treatment until the second iRECIST/itRECIST assessment at week 16, followed by a Q2W dosing scheme until confirmed PD, unacceptable toxicity, death or consent withdrawal, loss to follow-up, or trial end, whichever occurs first.

Pembrolizumab (intravenous)

Pembrolizumab will be administered as specified in Section 5.3.2.

In case a subject discontinues treatment with CyPep-1 in the combination arm (Arm B) and the subject remains eligible to participate in the trial after review by the Investigator and medical monitor, pembrolizumab treatment is planned to continue at Q6W as per institutional guidelines until PD, unacceptable toxicity, death, or consent withdrawal for a maximum of 18 treatment cycles. All assessments continue to be reported as per the schedule of assessments.

In case a subject discontinues with pembrolizumab in the combination arm (Arm B) and the subject remains eligible to participate in the trial after review by the Investigator and medical monitor, CyPep-1 treatment is planned to continue at Q2W until PD, unacceptable toxicity, death, or consent withdrawal. All assessments continue to be reported as per the schedule of assessments.

5.3.6 Dose Modification

CyPep-1 dose modifications are not planned. In case of CyPep-1 related toxicity, a dose delay may be considered after review by the investigator and medical monitor.

5.3.6.1 Dose Modifications and Management of CyPep-1 associated AEs

CyPep-1 associated hematological and non-hematological adverse effects according to CTCAE v5.0 toxicity grade may occur. Toxicity management and dose modifications are listed below:

Dose adjustments according to CTCAE toxicity grade for drug-related AEs

Occurrence	Grade 1	Grade 2	Grade 3	Grade 4
First appearance	No action with study drug.	No action with study drug.	Interrupt treatment until resolved to Grade 0-1 (or according to inclusion criteria). If interruption is < 14 days, restart at next lower dose level. If interruption is ≥14 days, check with Medical Monitor.	Discontinue study drug.

Second appearance			Discontinue study drug.	

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

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

Table 3. Dose Modification and Toxicity Management Guidelines for Immune-related AEs Associated with Pembrolizumab

General instructions:

1. Severe and life-threatening irAEs should be treated with IV corticosteroids followed by oral steroids. Other immunosuppressive treatment should begin if the irAEs are not controlled by corticosteroids.
2. Pembrolizumab must be permanently discontinued if the irAE does not resolve or the corticosteroid dose is not ≤10 mg/day within 12 weeks of the last pembrolizumab-treatment.
3. The corticosteroid taper should begin when the irAE is ≤ Grade 1 and continue at least 4 weeks.
4. If pembrolizumab has been withheld, pembrolizumab may resume after the irAE decreased to ≤ Grade 1 after corticosteroid taper.

irAEs	Toxicity grade (CTCAE V5.0)	Action with pembrolizumab	Corticosteroid and/or other therapies	Monitoring and follow-up
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper • Add prophylactic antibiotics for opportunistic infections	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment
	Recurrent Grade 2, Grade 3 or 4	Permanently discontinue		
Diarrhea / Colitis	Grade 2 or 3	Withhold	Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus

	Recurrent Grade 3 or Grade 4	Permanently discontinue		in stool with or without fever) and of bowel perforation (ie. peritoneal signs and ileus) • Participants with ≥Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis • Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion
AST or ALT elevation or Increased Bilirubin	Grade 2 [a]	Withhold	Administer corticosteroids (initial dose of 0.5 to 1 mg/kg prednisone or equivalent) followed by taper	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme value returned to baseline or is stable)
	Grade 3 [b] or 4 [c]	Permanently discontinue	Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper	
Type 1 diabetes mellitus (T1DM) or Hyperglycemia	New onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold [d]	• Initiate insulin replacement therapy for participants with T1DM • Administer antihyperglycemc in participants with hyperglycemia	Monitor participants for hyperglycemia or other signs and symptoms of diabetes
Hypophysitis	Grade 2	Withhold	Administer corticosteroids and initiate hormonal replacements as clinically indicated	Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue [d]		
Hyperthyroidism	Grade 2	Continue	Treat with nonselective beta-blockers (eg, propranolol) or thionamides as appropriate	Monitor for signs and symptoms of thyroid disorders
	Grade 3 or 4	Withhold or permanently discontinue [d]		
Hypothyroidism	Grade 2, 3 or 4	Continue	Initiate thyroid replacement hormones (eg, levothyroxine or	Monitor for signs and symptoms of thyroid disorders

			liothyronine) per standard of care	
Nephritis: grading according to increased creatinine or acute kidney injury	Grade 2	Withhold	Administer corticosteroids (prednisone 1 to 2 mg/kg or equivalent) followed by taper	Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Neurological Toxicities	Grade 2	Withhold	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 2, 3 or 4	Permanently discontinue	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology and/or exclude other causes
Exfoliative Dermatologic Conditions	Suspected SJS, TEN, or DRESS	Withhold	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology or exclude other causes
	Confirmed SJS, TEN, or DRESS	Permanently discontinue		
All Other irAEs	Persistent Grade 2	Withhold	Based on severity of AE administer corticosteroids	Ensure adequate evaluation to confirm etiology or exclude other causes
	Grade 3	Withhold or discontinue based on the event [e]		
	Recurrent Grade 3 or Grade 4	Permanently discontinue		

AE(s)=adverse event(s); ALT= alanine aminotransferase; AST=aspartate aminotransferase; CTCAE=Common Terminology Criteria for Adverse Events; DRESS=Drug Rash with Eosinophilia and Systemic Symptom; GI=gastrointestinal; IO=immuno-oncology; ir=immune related; IV=intravenous; SJS=Stevens-Johnson Syndrome; T1DM=type 1 diabetes mellitus; TEN=Toxic Epidermal Necrolysis; ULN=upper limit of normal.

Note: Non-irAE will be managed as appropriate, following clinical practice recommendations.

[a] AST/ALT: >3.0 to 5.0 x ULN if baseline normal; >3.0 to 5.0 x baseline, if baseline abnormal; bilirubin:>1.5 to 3.0 x ULN if baseline normal; >1.5 to 3.0 x baseline if baseline abnormal

[b] AST/ALT: >5.0 to 20.0 x ULN, if baseline normal; >5.0 to 20.0 x baseline, if baseline abnormal; bilirubin:>3.0 to 10.0 x ULN if baseline normal; >3.0 to 10.0 x baseline if baseline abnormal

[c] AST/ALT: >20.0 x ULN, if baseline normal; >20.0 x baseline, if baseline abnormal; bilirubin: >10.0 x ULN if baseline normal; >10.0 x baseline if baseline abnormal

[d] The decision to withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician. If control achieved or ≤ Grade 2, pembrolizumab may be resumed.

[e] Events that require discontinuation include but are not limited to: encephalitis and other clinically important irAEs (eg. vasculitis and sclerosing cholangitis).

5.3.6.3 Dose modification and toxicity management of infusion-reactions related to pembrolizumab

Pembrolizumab may cause severe or life-threatening infusion-reactions including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Dose modification and toxicity management guidelines on pembrolizumab associated infusion reaction are provided in Table 4.

Table 4. Pembrolizumab Infusion Reaction Dose Modification and Treatment Guidelines

NCI CTCAE Grade	Treatment	Premedication at Subsequent Dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	• Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires therapy or infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤24 hrs.	• **Stop Infusion.** • Additional appropriate medical therapy may include but is not limited to: • IV fluids • Antihistamines • NSAIDs • Acetaminophen • Narcotics • Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. • If symptoms resolve within 1 hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g. from 100 mL/hr. to 50 mL/hr.). Otherwise dosing will be held until symptoms resolve and the participant should be premedicated for the next scheduled dose. **Participants who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further study drug treatment**	Participant may be premedicated 1.5h (± 30 minutes) prior to infusion of study intervention with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of analgesic).
Grades 3 or 4 **Grade 3:** Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) **Grade 4:** Life-threatening; pressor or ventilator support indicated	• **Stop Infusion.** • Additional appropriate medical therapy may include but is not limited to: • Epinephrine** • IV fluids • Antihistamines • NSAIDs • Acetaminophen • Narcotics • Oxygen • Pressors • Corticosteroids • Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. • Hospitalization may be indicated. **In cases of anaphylaxis, epinephrine should be used immediately. Participant is permanently discontinued from further study drug treatment.	No subsequent dosing
Appropriate resuscitation equipment should be available at the bedside and a physician readily available during the period of drug administration. For further information, please refer to the Common Terminology Criteria for Adverse Events v5.0 (CTCAE) at http://ctep.cancer.gov.		

5.3.7 Other allowed dose interruption for pembrolizumab

Pembrolizumab may be interrupted for situations other than treatment-related AEs such as medical or surgical events and/or unforeseen circumstances not related to study intervention. However, study intervention is to be restarted within 6 weeks or 42 days (for Q6W dosing) of the originally scheduled dose and within 84 days (for Q6W dosing) of the previously administered dose, unless otherwise discussed with the Sponsor. The reason for interruption is to be documented in the patient's study record. If pembrolizumab is interrupted after the TLT observation period, CyPep-1 can continue to be administered at a Q2W schedule as per schedule.

5.3.8 Blinding

Blinding is not applicable, as this is an open label trial.

5.4 Method of Assigning Subjects to Treatment

Eligible subjects (see Section 4) will receive IT CyPep-1 in this trial, as monotherapy and in combination with pembrolizumab. Subjects will be assigned to treatment cohorts based on order of enrolment.

5.5 End of Treatment

In this trial, end of treatment will vary per subject, in view of their participation in additional treatment cycles with CyPep-1, for both trial phases.

5.6 Prior and Concomitant Medication and Therapy

Medications and therapies taken specifically for the management of solid tumors during 6 months prior to ICF signature will be recorded on the Medication History page of the eCRF.

All medications (prescription and over-the-counter drugs, vitamins and minerals) and therapies used during the trial, in addition to the trial intervention (CyPep-1 or pembrolizumab) will be considered concomitant.

All concomitant medications and therapies will be recorded in the eCRF from time of ICF signature until the FU visit (or until EoT, if it occurs more than 30 days after the last CyPep-1 administration). All concomitant medications administered during SAEs or Events of Clinical Interest (ECIs) are to be recorded.

The following concomitant medications or therapies are of special interest:

- Palliative medications
- Medication for the treatment of infections, including prophylactic or pre-emptive use of anti-infective medication
- Medication for the treatment of disease relapse
- Medication for the treatment of other reported AEs
- Prophylactic use of immunosuppressive medication

5.6.1 Permitted Medication and Other Treatments

If a subject uses anticoagulants, they should be stopped per Investigator discretion, according to local practice for invasive examinations.

Premedication is mandatory prior to the first IT dose of CyPep-1. Premedication is started 30-60 minutes before the IT injection, and continues for up to 24-48 hours after the procedure was completed, if necessary. An antihistamine (e.g., 25 to 50 mg clemastine) is mandatory with optional anxiolytics if deemed appropriate by the investigator. Only for subjects experience pain during or after IT administration, it is recommended to provide celecoxib 400 mg as premedication every 6 hours for 24-48 hours. Premedication should be administered for subsequent CyPep-1 doses based upon clinical judgment and presence/severity of prior reactions. This regimen may be modified based on local treatment standards and guidelines as appropriate.

However, the prophylactic use of systemic corticosteroids is not permitted. Administration of steroids through a route known to result in a minimal systemic exposure (topical [only on areas outside the injected lesions], intranasal, intro-ocular, or inhalation) is acceptable at the lowest possible dose. Following CyPep-1 administration, subjects must be observed for 4 hours post-injection for potential immediate injection-related reactions.

Medications may be administered due to anticipated adverse reactions or anticipated emergency situations (see Appendix E: Medication for Hypersensitivity Type Reactions Anticipated to be Possibly Occurring Due to CyPep-1 Administration).

At the discretion of the Investigator, any concomitant medication or therapy deemed necessary for the welfare of the subject during the trial, the treatment of AEs, and the treatment of disease relapse may be given. It is the responsibility of the Investigator to ensure that these medications or therapies are documented in the Concomitant Medication page of the eCRF.

5.6.2 Prohibited Medication and Other Treatments

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

The following treatments must not be administered during the trial:

- Immunotherapy not specified in this protocol, immunosuppressive drugs (that is, chemotherapy or systemic corticosteroids except for short-term treatment [≤1 week] of allergic reactions or for the treatment of immune-related AEs), or other investigational agents other than CyPep-1 or pembrolizumab, with the exception that COVID-19 treatments are allowed after consultation with the Sponsor and the Medical Monitor. Steroids with no or minimal systemic effect are allowed.
- Chronic concurrent therapy with antibiotics is prohibited within 2 weeks before and during the treatment period.
- Herbal remedies with immunostimulating properties (for example, mistletoe extract) or known to potentially interfere with major organ function (for example, hypericin).
- Any other anti-neoplastic systemic chemotherapy, biological treatment or concurrent anticancer treatment or chemotherapy not specified in this protocol
- Radiation therapy, with the exception of palliative short course, limited-field radiotherapy (ie, ≤ 10 fractions and ≤ 30% bone marrow involvement or per institutional standard), which may be administered during the trial.
- Live or live attenuated vaccines, including live or live attenuated COVID-19 vaccines.

- Systemic glucocorticoids are permitted only for the following purposes:
    - To modulate symptoms of an AE that is suspected to have an immunologic etiology
    - As needed for the prevention of emesis
    - Premedication for IV contrast allergies
    - Short-term oral or IV use in doses >10mg/day prednisone equivalent for COPD exacerbations
    - For chronic systemic replacement not to exceed 10 mg/day prednisone equivalent
    - In addition, the following glucocorticoid use is allowed:
        - For topical use or ocular use
        - Intraarticular joint use
        - For inhalation in the management of asthma or chronic obstructive pulmonary disease.
- Concomitant medications for treatment of HIV infection and/or its complications, except stable ART regimen maintained for at least 4 weeks prior to study entry (exceptions are permissible but should be discussed with the Sponsor and medical monitor).

Note: Inhaled steroids are allowed for management of asthma.

If the investigator determines that a subject determines that a participant requires any of the aforementioned treatments for any reason, study intervention CyPep-1 + pembrolizumab must be discontinued.

5.6.3 Rescue Medications & Supportive Care

Subjects should receive appropriate supportive care measures as deemed necessary by the treating investigator. Suggested supportive care measures for the management of AEs with potential immunologic etiology are outlined along with the dose modification guidelines in Section 5.3.6 for pembrolizumab.

Note: If after the evaluation of the event, it is determined not to be related to pembrolizumab, the investigator does not need to follow the treatment guidance. Refer to Table 3 in Section 5.3.6.3 for guidelines regarding dose modification and supportive care.

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

5.7 Treatment Compliance

Administration of intratumoral CyPep-1 and administration of IV pembrolizumab will be done in a hospitalized environment by trained trial personnel. Treatment compliance will be accomplished by documenting in record (i.e. the drug accountability, preparation, administration logs, the subjects' eCRF and medical records) information on, but not limited to: the batch number of CyPep-1 and of pembrolizumab used, the time point for preparation, the time point for administration, and signatures of designated site staff preparing and administering intratumoral CyPep-1 and pembrolizumab. Any deviations will be documented on the appropriate eCRF page.

5.8 Pregnancy and Contraception

CyPep-1 and pembrolizumab may have adverse effects on a fetus in utero. Furthermore, it is not known if pembrolizumab has transient adverse effects on the composition of sperm.

Participants should be informed that taking the trial medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the trial.

Pregnant or breastfeeding women are not allowed to participate in this trial. In women of childbearing potential (defined as <2 years after last menstruation or not surgically sterile), a serum pregnancy test has to be performed at screening and prior to trial treatment. This test has to be negative for a subject to participate in this trial.

Subjects must be willing and be able to adhere to the following prohibitions and restrictions during the course of the trial:

- All male subjects with partners who are women of childbearing potential and female subjects of childbearing potential (as defined above) must consistently and correctly use a contraceptive method in accordance with the recommendation of the EU Clinical Trial Facilitation Group. Examples include female oral contraceptives, female intrauterine contraceptive device, female bilateral tubal occlusion, male vasectomy, or sexual abstinence. Additionally, the partners of subjects with childbearing potential are recommended not to donate sperm.
- Contraception is to be used from screening until at least 120 days after the last dose of CyPep-1 or pembrolizumab. If there is any question that a participant of childbearing potential will not reliably comply with the requirements for contraception, that participant should not be entered into the trial.
- Female subjects will be instructed to notify the investigator immediately if they become pregnant during the trial. Male subjects will be instructed to notify the investigator immediately if their partner becomes pregnant. If a participant inadvertently becomes pregnant while trial on treatment, the participant will be immediately discontinued from further trial treatment. The subjects will also be instructed to report pregnancies discovered after the last visit, if they believe that conception occurred during their participation in the trial. The outcome of the pregnancy will be reported to the Sponsor without delay and within 24 hours if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or new born). The study investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or new born to the Sponsor.
    - A pregnancy, as such, is not an adverse event (AE) unless there is a possibility that the IMP and / or pembrolizumab have interfered with the efficiency of any contraceptive measures. However, the investigator should report pregnancies according to the procedures and timelines described for reporting of SAEs (see Section 7.2.3). The pregnancy report form should be used instead of the SAE form.
    - The site will contact the pregnant subject or partner at least monthly and document the participant's status until the pregnancy has been completed or terminated. Any complication during the pregnancy should preferably be reported as an AE. The outcome of the pregnancy must be reported (on the pregnancy report form) to the Sponsor without delay and within 24 hours if the outcome is a serious adverse experience (e.g., death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or new born). The investigator will make every effort to obtain permission to follow the outcome of the pregnancy and report the condition of the fetus or new born to the Sponsor. Any spontaneous abortion, stillbirth, birth defect/congenital

anomaly, death, or other serious infant condition must be reported and followed up as a SAE.

5.8.1 Use in Nursing Women

It is unknown whether CyPep-1 or pembrolizumab are excreted in human milk. Since many drugs are excreted in human milk, and because of the potential for serious adverse reactions in the nursing infant, participants who are breastfeeding are not eligible for enrollment.

5.9 Post-trial Treatment

After participation, subjects will continue treatment in accordance with clinical practice, i.e. as per local guidelines and Standard of Care.

In case of early closure or termination of the trial, eligible and benefitting patient, will have the option to continue CyPep-1 treatment. Continued CyPep-1 treatment for eligible and benefitting patients will be allowed after a decision by the investigator, medical monitor and sponsor.

5.9.1 Continued Access pembrolizumab

Subjects who are still on study intervention at the time of study completion/termination may continue to receive study intervention if they are experiencing clinical benefit. The continued access to study intervention will end when a criterion for discontinuation is met or 18 (for Q6W dosing) doses of pembrolizumab have been administered.

5.10 Accountability Procedures

Cytovation will ensure that the IMP is characterized and manufactured in accordance with any applicable requirements of Good Manufacturing Practice (GMP) and regulatory requirements.

After the trial protocol was approved by the regulatory authorities, the trial medicinal product will be supplied to the investigators and trial sites together with all relevant documentation including a description of the storage conditions.

Cytovation will maintain records of document shipment, receipt, dispensation, return, and destruction of the IMP in the Trial Master File. Cytovation is responsible for the storage of retention samples of the IMP according to applicable laws and guidelines.

The Investigator will maintain records of IMP accountability in the ISF for the IMP provided by Cytovation.

After the end of the trial, the Investigator will return all used and unused IMPs to Cytovation or destroy used and unused IMP according to written agreement with Cytovation.

6. INVESTIGATIONAL PLAN

6.1 Assessment Overview

An overview of the assessments performed in the trial is provided in Table 5 below.

Eligibility and safety of CyPep-1 (plus pembrolizumab in combination arm) will be assessed by means of demographics, medical and oncology history, vital signs, physical examination, cardiovascular function evaluation, ECOG performance status, routine laboratory tests (pregnancy test, hematology, clinical chemistry, urinalysis), disease assessment, concomitant medications, and collection of AEs.

Efficacy of CyPep-1 (plus pembrolizumab in combination arm) will be assessed by means of radiological assessment, local measurements of injected tumor lesions, and survival data (PFS based on iRECIST per investigator assessments).

Additional assessments include PK, changes in the tumor microenvironment (tumor biopsy), , and levels of cytokines (cytokine analysis not applicable for Arm D) and selected immune cell markers in the peripheral blood.

Table 5. Overview of Assessments

Objective	Assessment	Specification, if any
Eligibility	Informed consent	Confirmation of written informed consent prior to any trial specific procedure
	Eligibility check	Based on inclusion/exclusion criteria
	Demographics	Recording of subjects' demographics
	Medical and surgical history	Recording of subjects' complete medical history including details of cancer onset, prior surgery, cancer-related and other treatments, and known HIV, HCV, and HBV exposure
	Tumor biopsy	Cycle 1: should be available for at least one injected lesion(s) at screening and at Week 6; may be taken from non-injected lesions. Subsequent cycles: from any new injected lesion(s) before the first treatment and after 6 weeks (not mandatory). A biopsy from lesions that were injected in previous cycles can be taken (not mandatory) after 6 weeks.
Safety	Physical examination	Physical examinations per institutional guideline.
	Concomitant medications	To be assessed at screening and then at each visit. Will include concurrent illness.
	ECG	Single 12-lead ECG recordings will be taken. Subjects must be supine for approximately 10 minutes before ECG collection and remain supine but awake during the ECG collection.
	Vital signs	The following will be assessed: 1. Pulse rate 2. Systolic and diastolic blood pressure 3. Body temperature

		Blood pressure and pulse rate should always be measured on the same arm. A time window of ± 5 min is allowed.
	Biochemistry	The following will be assessed: creatinine, alkaline phosphatase, phosphate, total bilirubin, direct bilirubin (only applicable if total bilirubin levels > 1.5 x ULN), ALAT, ASAT, gamma-glutamyl transferase, total protein, albumin, uric acid, urea, sodium, potassium, calcium, chloride, glucose, lactate dehydrogenase, amylase and lipase.
	Hematology	The following will be assessed Hb, hematocrit, RBC count, WBC count with differential count (neutrophils, eosinophils, basophils, lymphocytes, monocytes), and platelet count.
	Coagulation	The following will be assessed: prothrombine time- international normalized ratio (PT-INR) and activated partial thromboplastin time (APTT)
	Urinalysis	The following will be assessed: leukocytes, nitrite, pH, protein, glucose, ketone, urobilinogen, bilirubin, blood (Hb and erythrocytes) (dipstick). If abnormal, sediment will be performed.
	ECOG performance status	Using the ECOG performance status scale (Appendix B)
	Recording (S)AEs and hospitalizations	To be assessed at screening and then at each visit, according to NCI-CTCAE v5.0 (Appendix C)
	Pregnancy test	A serum pregnancy test will be performed at screening. At all other occasions, a urine test before any study treatment is administered is sufficient. For the assessment of childbearing potential, FSH and estradiol tests may be required at screening.
	Thyroid function	To be evaluated at the timepoints specified in the schedule of assessments.
Efficacy	Tumor biopsy	Arrangements to be made for any tumor material to be sent for analysis are described in the laboratory manual.
	iRECIST (and itRECIST) assessment	A CT/MRI scan is the recommended modality, which should include chest, abdomen, pelvis and all other known sites of disease. For the case of skin lesions, measurement by clinical examination using calipers and by color photography should be performed. Tumor response will be evaluated according to the iRECIST using modified RECIST 1.1 (Appendix D) based on investigator assessment. itRECIST will be programmatically calculated using data obtained from iRECIST assessments following the itRECIST principle summarized in Appendix F. itRECIST tumor response for

		Phase I Cohort 3, Phase IIa Arms A, B and C will be performed retrospectively.
	Local measurement of (to-be) injected tumor lesion(s)	For Phase I and Arms A, B, and D, local measurements of accessible (to-be) injected cutaneous/subcutaneous tumor lesion(s) must be done prior to each CyPep-1 injection by caliper and color digital photography. If needed, guidance with ultrasound is permitted. The (to-be) injected lesions under ultrasound control must be measured with ultrasound guidance before each CyPep-1 injection and only on dates of CyPep-1 injection.
	Lesion mapping (Arm D only)	Will be performed during screening; a lesion map will be created prior to the first CyPep-1 injection (detailed in Study Procedural Manual). Once all injectable lesions have been injected and the subject remains on treatment, a new lesion map that permits reprioritizing of the to-be-injected lesion sets will be created (details see Section 5.3.1.3)
	Administration of CyPep-1	For Arms A, B and C, administration of CyPep-1 will be performed at Weeks 1, 3, and 5 (Day 1 of each week). All subjects are planned for additional treatment cycles (Q2W CyPep-1 administration). For Arm D, it will be performed QW until the second iRECIST/itRECIST assessment at week 16, followed by a Q2W dosing scheme.
	Administration of pembrolizumab	Will be performed as specified in the schedule of assessments (at Q6W administration).
Other	PK	A time window of ± 5 min is allowed for the PK blood sampling.
	Levels of cytokines (Arms A, B, and C)	[redacted]
	Blood sampling for immune cell phenotyping	An additional blood sample will be taken for peripheral blood phenotyping of selected immune cell markers.

Note: no more peripheral blood sampling required for TCR clonality analysis (See Sections 6.2 and 8.1.6 for details).
Abbreviations: ALAT: alanine transaminase; ANC: absolute neutrophil count; APTT: activated partial thromboplastin time; ASAT: aspartate transaminase; CT: computed tomography; CTCAE: Common terminology Criteria for Adverse Events; ECG: electrocardiogram; ECOG: Eastern Cooperative Oncology Group; FSH: follicle-stimulating hormone; HBV: hepatitis B virus; HCV: hepatitis C virus; HIV: human immunodeficiency virus; IL: interleukin; IFN: interferon; iRECIST: immune Response Evaluation Criteria in Solid Tumors; itRECIST: Response Criteria for Intratumoral Immunotherapy in Solid Tumors ; MRI: magnetic resonance imaging; NCI: National Cancer Institute; OS: overall survival; PFS: progression-free survival; PK: pharmacokinetic; PT-INR: prothrombin time – international normalized ratio; RBC: red blood cell; TCR: T cell receptor; TGF: transforming growth factor; TNF: tumor necrosis factor; WBC: white blood cell.

6.2 Trial Assessment Specifications

Obtaining of written informed consent prior to any trial specific procedure (including screening procedures) will be performed at screening. Eligibility check, recording of subject demographics, past medical and surgical history (including disease under trial), and recording of concurrent illness and treatments will also be performed at screening or at Day 1 of Cycle 1 prior to treatment.

Eligibility

Subjects who do not meet all inclusion criteria or who meet any of the exclusion criteria are not eligible for trial entry. As soon as a subject is deemed ineligible during screening, all further trial evaluations will be cancelled for this subject.

Subjects considered suitable for the trial may only enter the trial after they have been properly informed and have signed the ICF.

Each subject will receive a 5-digit subject number (e.g. 01-001), which is assigned by the site during the screening visit and will be used throughout the trial.

- Digits 1 / 2: [redacted] trial site code
- Digits 3 / 4 / 5: Individual subject number (consecutively in the order of trial entry per site

All subjects, including screening failures, are added to the Electronic Data Capture (EDC) system by site staff.

Demography and Medical and Surgical History

Demographic characteristics, obtained during screening, will include age (in years at screening), sex, and date of birth as described by the subject.

Each subject's relevant medical history will focus on current or past abnormalities or diseases of the following systems: special senses, cardiovascular, respiratory, gastrointestinal, hepatic biliary, genitourinary/reproductive, renal, endocrine/metabolic, musculoskeletal, hematologic/lymphatic, neurologic/psychiatric, dermatologic, immunologic, and infectious disease, bleeding tendency, and allergy/drug sensitivity.

Disease History and Treatment

Special attention will be paid to the subject's disease history and treatments. The disease information that will be documented and verified at the screening visit for each subject includes:

1. Detailed history of the malignancy, including onset, histopathological diagnosis, grading, and staging at diagnosis/trial entry, and date of recurrence
2. All therapy used for prior treatment (prior surgery, and past treatment including chemotherapy and/or radiation therapy) in the past two years (for therapy used for prior treatment > 2 years before trial initiation, only name of compound and treatment year need to be documented)
3. Any other conditions that were treated with chemotherapy, radiation therapy, or immunotherapy
4. Current signs and symptoms of the malignancy and AEs from current and previous anticancer treatments
5. Tumor localization at trial entry

Furthermore, medical history will include details of prior HIV, HCV, and HBV exposure.

For Arms A, C, and D: known HIV and HBV/HCV history results in ineligibility. Testing for either is required if mandated by local health authority. HIV+ subject is eligible when normal levels of CD4 are present.

For Arm B: HIV+ subjects are eligible if having controlled disease and not immune compromised. Details see inclusion criteria 32 (Section 4.1) and exclusion criteria 41 (Section 4.2).

Height, Weight, Physical Examinations

Physical examinations will be performed at several time points throughout the trial (excluding height determination, which will only be performed at screening; see Table 1) and may include as per institutional guidelines the following: head, eyes, ears, nose and throat; respiratory system/ chest; cardiovascular system/ heart; abdomen (including liver and spleen); skin, lymph nodes; extremities and (at the Investigator's discretion) genitourinary system/ pelvis.

The assessments are to be performed prior to CyPep-1 administration or pembrolizumab (if pembrolizumab administration day is different from CyPep-1 scheduled day). For height and body weight measurements, preferably the same equipment should be used throughout the trial. To obtain the actual body weight, subjects must be weighed lightly clothed. Body height will be recorded in centimeters and body weight in kilograms.

If the investigator (or qualified designee) encounters an abnormal finding, the investigator should indicate whether the finding is clinically significant or not.

Information about the physical examination must be present in the source documentation at the investigational site. Significant findings that are present prior to the signature of the ICF must be included on the Medical History eCRF. Significant findings that meet the definition of an AE, must be recorded on the AE eCRF.

Vital Signs

Vital signs will be assessed at regular intervals as indicated in the Table 1. The following variables will be measured:

1. Systolic and diastolic blood pressure (mm/Hg)
2. Pulse rate (beats per minute)
3. Temperature

All blood pressure measurements require the use of completely automated device on the same arm. The same type of automated device is to be used at least for the same patient. The automated device must be calibrated annually. Body temperature will be measured in a consistent manner throughout the trial. Continuous oxygen monitoring will be performed if the subject has respiratory problems.

ECOG Performance Status

The ECOG performance status score, determined at the time points indicated in the Table 1 will be used to quantify the general well-being and activities of daily life in cancer subjects and uses scores as described in Appendix B. Only subjects with an ECOG Performance Status score of 0 or 1 are allowed to enter the trial.

The assessment is to be performed prior to CyPep-1 or pembrolizumab administration.

Cardiovascular function (ECG)

Single standard 12-lead ECGs (i.e. according to Einthoven and Goldberger as well as 6 precordial leads according to Wilson) will be recorded using a computerized ECG device after at least 10 min rest in supine position (lying horizontally with the face and torso facing

up). ECGs should be performed prior to CyPep-1 or pembrolizumab administration and any scheduled vital signs measurements (see time points for ECG recordings in the Table 1).

The following parameters will be automatically calculated by the ECG device: HR, PR/PQ interval, QRS interval, QT interval (uncorrected).

All ECGs will be evaluated by a physician to provide immediate safety monitoring offering an ECG diagnosis and overall assessment (including clinical relevance). Corrected QT intervals will be derived in addition for data evaluation.

For all safety laboratory assessments

If the time between screening and the first CyPep-1 administration is <72 hours, safety laboratory tests do not need to be repeated.

Hematology

At the time points indicated in the Table 1 (Table 1 a – e), blood sampling for all safety laboratory tests should be performed and results should be available prior to administration of CyPep-1 or pembrolizumab (if pembrolizumab administration day is different from CyPep-1 scheduled day).

Hematology tests will be performed at the local (institute's) hospital.

Parameters to be assessed include: hemoglobin, hematocrit, RBC count, WBC count with differential count (neutrophils, eosinophils, basophils, lymphocytes, monocytes), and platelet count. Further assessments may be performed up to one day prior to the visit in order to have the results (of at least hemoglobin, white blood cells, platelets, PT-INR, and APTT) available on the visit day prior to CyPep-1 administration.

The Investigator (or qualified designee) should evaluate all blood parameters either as normal or abnormal. If an abnormal finding is encountered, the investigator should indicate whether the finding is clinically significant or not. Any hematological abnormalities that are considered to be clinically significant need to be followed until resolution.

Biochemistry

At the time points indicated in Table 1, blood sampling for all safety laboratory tests should be performed and results should be available prior to administration of CyPep-1 or pembrolizumab (if pembrolizumab administration day is different from CyPep-1 scheduled day).

Biochemistry tests will be performed at the local (institute's) hospital.

Parameters to be assessed include: creatinine, total bilirubin, direct bilirubin (only applicable if total bilirubin levels > 1.5 x ULN), alkaline phosphatase, phosphate, ALAT, ASAT, GGT, total protein, albumin, uric acid, urea, sodium, potassium, calcium, chloride, glucose, LDH, amylase and lipase. Creatinine clearance will be estimated according to the CKD-EPI formula.

The Investigator (or qualified designee) should evaluate all clinical chemistry parameters either as normal or abnormal. If an abnormal finding is encountered, the Investigator should indicate whether the finding is clinically significant or not. Any clinical chemistry abnormalities that are considered to be clinically significant need to be followed until resolution.

Coagulation

Coagulation parameters will be assessed at several different time points throughout the trial (Table 1) and include PT-INR-, and activated partial thromboplastin time (APPT) prior to dosing. In case there are no normal ranges available for the PT test, the international normalized ratio (INR) test may be used instead of PT. At screening, subjects should have an INR <1.5 x ULN.

Urinalysis

At the time points indicated in Table 1a to 1e of Table 1 urinalysis is to be performed by gross (dipstick) urine examination. Midstream, clean-catch urine specimens will be collected for dipstick analysis. Parameters to be assessed include: leukocytes, nitrite, pH, protein, glucose, ketone, urobilinogen, bilirubin, blood (hemoglobin and erythrocytes).

If dipstick findings are abnormal according to local reference ranges, then a microscopic evaluation may be performed to assess the abnormal findings. Any urinalysis abnormalities that are considered to be clinically significant need to be followed until resolution.

Adverse Events

AEs need to be collected as soon as the subject has consented to the trial (ICF signature) until the FU visit (or until EoT, if it occurs more than 30 days after the last CyPep-1 or pembrolizumab administration). After the FU visit (or EoT visit, if it occurs more than 30 days after the last CyPep-1 administration or last administration of pembrolizumab in combination arm), only ongoing AEs or SAEs related to CyPep-1 and / or pembrolizumab administration will be collected.

For Arm B*:* All AEs meeting serious criteria, from start of treatment through 90 days following cessation of study treatment, or 30 days following cessation of study treatment if the participant initiates new anticancer therapy, whichever is earlier must be reported by the investigator.

Subjects who dropped out of the trial with (S)AEs that are ongoing at the time treatment was withdrawn, need to visit the hospital 30 days after the last CyPep-1 or pembrolizumab administration for monitoring of these (S)AEs, if possible.

AEs will be assessed using CTCAE v5.0, including start and stop dates, severity, relationship to CyPep-1 and / or pembrolizumab, outcome and action taken. Section 7 of the protocol provides detailed instructions on AE reporting and definitions related to it. For more information on AE monitoring, please refer to Section 7.2.

Pregnancy

Women of childbearing potential must have a highly sensitive negative pregnancy test at screening (serum) and throughout the treatment period (Table 1a to 1e, Table 1; highly sensitive urine/serum). In this trial, pregnancies occurring during participation (including pregnancies of partners of male subjects) are not considered as an AE per se, but they must be reported to the sponsor using the trial Pregnancy Reporting Form, according to the rules described in Section 7.2.3 (Reporting of SAEs).

Concomitant and Prior Medications

All concomitant medication should be reported from ICF signature during the treatment period until the FU visit (or until EoT, if it occurs more than 30 days after the last CyPep-1 or pembrolizumab administration) in the CRF. Non-drug interventions and any changes to a concomitant medication or other intervention should also be recorded in the CRF. At screening, prior medications taken by the subject beginning 28 days prior to ICF signature are to be recorded in the CRF, noting the name, dose, duration and indication of each drug.

Thyroid function

In clinical trials using pembrolizumab, the thyroid function needs to be evaluated at regular time intervals. For this trial, this evaluation applies for subjects in Phase IIa Arm B and should be performed at the timepoints specified in Table 1.

Clinical Response based on iRECIST and itRECIST assessments

Radiological assessments are to be performed at the time points given in Table 1a to Table 1e (depending on trial phase and treatment arm). The visit window (where applicable) is specified in footnote #22 of the schedule of assessments. The first on-study scan assessment should be performed at 8 weeks ±3 days from the date of treatment. Subsequent tumor scans should be performed every 8 weeks ±3 days or more frequently if clinically indicated. Scan timing should follow calendar days and should not be adjusted for delays in cycle starts. Scans should continue to be performed until disease progression is identified by the investigator. All skin lesions must be assessed by caliper measurement during clinical exam on the dates corresponding to iRECIST assessment. Documentation by color photography including a ruler to estimate the size of the lesion should be stored in the patient file (SoA, footnote #22).

Once a tumor lesion is documented, the target area should be recorded at every subsequent time point for the duration of the trial. The same type of scan should be used in a subject throughout the study to optimize the reproducibility of the assessment of existing and new tumor burden and improve the accuracy of the assessment of response or progression based on imaging.

In order to account for the possibly delayed onset of CyPep-1 action and for subjects who are of acceptable clinical performance status (as deemed by the Investigator), if PD is recorded and provided that the tumor enlargement is smaller than 50% compared to the previous measurement, the subject will remain in the trial until confirmed PD and will be reassessed radiologically after 4 weeks. A confirmatory radiological assessment only has to be done if at the previous visit PD was observed with radiological assessment. If the lesion(s) continue(s) to enlarge, the original PD will be considered as the “first documented PD”. If lesion(s) remain(s) unchanged or decrease(s) in size, assessments should continue as per protocol (i.e., the next radiological assessment will be performed after an 8-week interval). In this case, the first measured PD will be disregarded. In case of unconfirmed PD, the subject should stay on treatment with CyPep-1 (plus pembrolizumab in the combination arm). If PD is confirmed 4 weeks later, then the subject will be taken off-trial and move to the end of treatment visit.

Tumor response or progression on CT/MRI will be determined according to iRECIST (modified RECIST1.1 using up to 10 target lesions) and itRECIST [Goldmacher 2020]). A summary of itRECIST in comparison to iRECIST is in Appendix F.

ORR in all lesions will be evaluated based on the investigator’s assessments per iRECIST. ORR in injected and non-injected lesions, separately, per itRECIST will be programmatically calculated using data obtained from iRECIST assessments.

Local measurement of tumor lesion(s)

For Phase I, and Phase IIa Arms A, B and D: Local measurements of (to-be) accessible injected cutaneous/subcutaneous tumor lesion(s) must be done within one day (<24h) prior to each CyPep-1 injection by caliper and color digital photography, using a ruler held flush to the skin next to the longest diameter of the lesion to indicate the size of the lesion. If needed, guidance with ultrasound is permitted. The (to-be) injected lesions under ultrasound control, must be measured with ultrasound guidance before each CyPep-1 injection only on dates of CyPep-1 injection. All ultrasound and color digital photography image files should be stored and available in the patient file). Lesion(s) that disappeared will be checked on the days of CyPep-1 injection.

Lesion mapping

A lesion map that will guide the preferred sequence of injection of the identified Lesion Sets must be created prior to the first CyPep-1 injection (detailed in Study Procedural Manual). A lesion map will contain the information as described in Section 5.3.1.3.

Once the lesion map is created, lesion sets for sequential CyPep-1 injections will be planned and documented in the source. The size of the injected lesions and the lesion map should be updated when assessments of local measurements of tumor lesions and iRECIST and itRECIST assessments are performed during the treatment period.

End of Treatment and Follow-up Tumor Imaging (Arm B)

If subjects discontinue study treatment, tumor scans should be performed at the time of discontinuation (±4-week window) unless previous scans were obtained within 4 weeks of discontinuation. Subjects who are clinically stable and treated past radiographic progression may continue to be assessed until progression is confirmed according to the rules of iRECIST, when clinically appropriate.

If subjects discontinue study treatment without documented disease progression, every effort should be made to monitor disease status by acquiring tumor scans using the same schedule used while on treatment (every 8 weeks in Year 1 or every 12 weeks after Year 1). Scans are to be continued until one of the following conditions are met:

- Disease progression as defined by iRECIST
- The start of a new anticancer treatment
- Pregnancy
- Death
- Withdrawal of consent
- The end of the study

Survival

PFS

PFS is defined as the time from enrolment until disease relapse or disease progression (based on iRECIST per the investigator's assessments), death due to any cause, withdrawal of consent, loss to follow-up, or until the end of the trial whichever occurs first and will be evaluated at the time points defined in Table 1.

Once a tumor lesion is documented, the target area should be recorded at every subsequent time point for the duration of the trial. For subjects who leave the trial early and do not receive any other anti-cancer therapy or any other investigational therapy, PFS visits should continue to be performed every 8 weeks (from C1D1) until occurrence of confirmed progressive disease or until start of any further line of anti-cancer treatment. In the case of PD or start of any further line of anticancer treatment, the next FU will be OS-FU (Schedule of Assessments).

OS

Every three months after the confirmation of PD and until the end of the trial, a phone call for survival status (OS) will be done. Additionally, any next line of anti-cancer therapies, ORR and duration of response to next line anti-cancer therapies, and additional relevant information will be recorded. If necessary, patients may be contacted occasionally outside of this FU window, and data regarding next line anti-cancer therapies can be collected retrospectively.

Additional efficacy assessments

Pharmacokinetics

Blood samples for PK determination will be collected from the first 9 subjects of Phase I, all subjects of Arm C, and the first 12 subjects of Arm D receiving CyPep-1 at the time points given in Table 1a. All relevant sample collection and analysis will be performed according to qualified and/ or Good Laboratory Practice (GLP) methods, as appropriate. Details of the sampling and processing procedures, storage, and transportation will be provided in a separate laboratory manual.

Peripheral Cytokines (Arms A, B, and C)

Levels of blood cytokines will be determined at a central laboratory at the time points specified in Table 1 by enzyme-linked immunosorbent assay (ELISA).

Changes in the Tumor Microenvironment (Tumor Biopsy)

Primary (archival only for screening) tumor material of all subjects will be used for analysis (see Table 1a to Table 1e for time points of when tumor biopsies will be taken). Immunohistochemistry/immunofluorescent staining of paraffin-embedded samples will be conducted at a central laboratory. Tumor samples will be processed according to laboratory standards. Tumor tissue will be stained using immunohistochemistry/immunofluorescence in order to determine T-cell infiltration and characteristics of tumor tissue.

No biopsy is to be taken if tumor is inaccessible, biopsy not in subject's best interest or thought to be dangerous or if subject declines to consent to biopsy.

Tumor tissue staining will be performed provided there is access to sufficient tissue (formalin-fixed paraffin-embedded), post-hoc, batch-wise during the trial or at the end of the trial and from the most recent available tissue.

Additional analysis (as for example tumor mutational burden) might be performed according to the investigator's or sponsor's decision.

Stainings will be performed for the following:

1. Immune cell infiltrates:
    - CD3, CD4, CD8 (T cells)
    - CD80, CD86 (dendritic cells)
2. Immune-suppressive cell markers:
    - [redacted]
    - [redacted]
    - [redacted]
    - [redacted]
    - [redacted])
3. Immune markers:
    - PD-1
    - PD-L1

Additional stainings for other factors based on emerging scientific understanding of the intratumoral CyPep-1 therapy may also be performed.

TCR Clonality

The change in T-cell receptor (TCR) clonality levels will be assessed in the available peripheral blood samples. To assess the hypothesis that CyPep-1 increases the TCR clonality

systematically, which suggests that T-cells are targeting the tumor, the clonality metric will be computed for all subjects providing peripheral blood samples at screening and/or Week 6 of every CyPep-1 treatment cycle.

TCR sequencing might be performed on the available remaining tumor biopsy material from the analyses of changes in the tumor microenvironment. Overlaps of T cell clones in respect to expansion and breadth of T cell clonality before and after intratumoral injection with CyPep-1, as monotherapy and in combination with pembrolizumab (central analysis) will be analyzed.

Peripheral blood phenotyping

A peripheral blood sample will be collected at the time points specified in the Table 1.

PBMCs will be isolated, stored and analyzed at a central laboratory. Subjects will be asked to provide consent for these additional samples. [redacted]

7. ADVERSE EVENTS

All AEs in this trial will be collected and processed in accordance with ICH-GCP and national and local laws and regulations. In line with these regulations, the investigator is responsible for reporting SAEs to Allucent, the sponsor's designee, within the appropriate timelines (see below). If required, the investigator is also responsible for notifying the appropriate local ethics committee of SAEs per the guidelines of the Institution and in accordance with aforementioned laws and regulations.

All AEs involving injection site reactions and dermatological reactions must be documented with color digital photography in addition to standard documentation methods.

7.1 Definitions

7.1.1 Adverse Event (AE)

An AE is defined as any untoward medical occurrence in a clinical trial subject administered with a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an IMP, whether or not considered related to the IMP. All AEs reported by the subject or observed by the investigator or his staff will be recorded.

7.1.2 Treatment-Emergent Adverse Event (TEAE)

All AEs reported from the start of the trial (Day 1) are considered TEAEs.

7.1.3 Adverse Drug Reaction (ADR)

All noxious and unintended responses to the CyPep-1 and / or to the pembrolizumab medicinal product, related to any dose, should be considered as adverse drug reactions (ADRs). The phrase "responses to medicinal product" means that a causal relationship between the medicinal product and an AE is at least a reasonable possibility, i.e., a relationship cannot be ruled out. ADRs are also referred to as toxicity.

7.1.3.1 Tumor lysis syndrome (TLS)

A possible risk associated with CyPep-1 intratumoral administration, considering the mode of action of CyPep-1, or of pembrolizumab, is TLS, which needs to be mitigated, especially for the frail elderly patients. TLS can be mitigated by starting therapy at a low dose of CyPep-1 (as planned for the first cohort of subjects enrolled in Phase I of the trial), by slow dose ramp-up (as planned for cohorts in Phase I of the trial), and by staggered approach in Phase II combination arm (initial dosing of 3 subjects at RP2D in combination with pembrolizumab before enrolling all subjects) and by appropriate preventive measures. Measures to prevent TLS include TLS risk assessment, prophylactic hydration and medication prior to initial dosing with careful monitoring during the first 4 hours after dosing for metabolic or clinical signs of impending TLS, according to institutional standards.

To ensure prophylactic hydration, subjects should consume ≥2 L of fluid intake per day, starting ≥48 hours prior to first dose/each dose escalation and for ≥24 hours thereafter. For patients who have difficulty to maintain adequate oral hydration, intravenous hydration is recommended, at an infusion rate of 150-200 mL/hr (i.e., 2-3 L per day) for initial dosing; for following dose escalations, an intravenous hydration with 1.5-2 L per day is advisable.

Prophylactic medications for TLS include uric acid reducers such as allopurinol (300 mg orally daily, starting ≥72 hours prior to first dose and continued for ≥1 week after the last dose escalation).

7.1.4 Unexpected Adverse Drug Reaction

An unexpected adverse drug reaction is an adverse drug reaction of which the nature or severity is not consistent with the available product information, e.g. the IB for an unapproved investigational product. ADRs that are more specific or more severe than described in the investigator's brochure should also be considered unexpected.

7.1.5 Serious Adverse Event (SAE)

Any untoward medicinal occurrence or effect that at any dose:

- Results in death (not due to disease progression);
- Is life-threatening (the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe);
- Requires hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability or incapacity;
- Is a congenital anomaly or birth defect.

The following are not considered (and do not need to be reported) as SAEs:

- Hospitalization due to a social indication
- Elective pre-planned hospitalizations for treatment of an existing condition prior to entering the trial
- Hospitalizations due to tumor-related symptoms or tumor progression

Any event that does not meet the above criteria may also be considered by the investigator to be a SAE, based on appropriate medical and scientific judgment. This includes important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. For example, allergic bronchospasm requiring intensive treatment in an emergency room or at home; convulsions that may not result in hospitalization; development of drug abuse or drug dependency.

7.1.6 Suspected Unexpected Serious Adverse Reaction (SUSAR)

SUSARs are SAEs that are at a minimum possibly related to the study agent and are unexpected (i.e., not listed in the investigator brochure). SUSARs will be collected and expeditiously reported to CAs and IECs according to applicable regulatory requirements.

7.1.7 Toxic Death

Any death to which drug toxicity is thought to have made a contribution should be notified to [redacted] at once. [redacted] will notify the DEC and sponsor immediately.

7.1.8 Pregnancy

In this trial, pregnancies occurring during participation (including pregnancies of partners of male subjects) are not considered as an AE per se, but they must be reported to the sponsor using the trial Pregnancy Reporting Form, according to the rules described in Section 7.2.3.

The CA and IEC will be informed on these pregnancies.

Pregnancies must be followed-up to determine outcome (including spontaneous miscarriage, elective termination, normal birth, or congenital abnormality) and status of mother and child, even if the subject was discontinued from the trial. Pregnancy complications, elective terminations for medical reasons and spontaneous miscarriages must be reported as SAEs.

7.2 Monitoring, Reporting, and Documentation of Adverse Events (AEs)

7.2.1 Monitoring of AEs

Subjects will be monitored for AEs and Events of Clinical Interest (ECIs) from the time of ICF signature until the FU visit (or until EoT, if it occurs more than 30 days after the last CyPep-1 administration for Phase I and Arms A and C and D; for Arm B refer to Section 7.2.1.3). After the FU visit (or EoT visit, if it occurs more than 30 days after the last CyPep-1 administration), only ongoing AEs or SAEs related to CyPep-1 administration will be collected.

For all phases and arms, AEs will be assessed using CTCAE v5.0 (Appendix C), assessments will include start and stop dates, severity, relationship to CyPep-1 and / or pembrolizumab, outcome and action taken.

At every trial visit, subjects will be asked whether they have experienced or are experiencing any medically related changes in their health. They will also be asked if they have been hospitalized, had any accidents, used any new medications/therapies, or changed concomitant medication regimens (both prescription and over the counter medications).

In addition, for all subjects with any remaining CyPep-1 and / or pembrolizumab-related AEs at the last follow-up visit after the last CyPep-1 intratumoral administration, AE information needs to be collected until:

- The symptom subsides or stabilizes;
- Any clinically relevant abnormal laboratory value has returned to baseline;
- There is a satisfactory explanation other than the trial medication for the change(s) observed; *or*
- Death, in which case an autopsy report should be supplied to [redacted], if performed.

7.2.1.1 Events of Clinical Interest (Arm B)

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported to the Sponsor within 24 h of awareness.
Events of clinical interest for this trial include:

1. an overdose of Sponsor's product, as defined in Section 7.2.1.2
2. an elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

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

7.2.1.2 Treatment of Overdose (Arm B)

For this study, an overdose of pembrolizumab will be defined as any dose of 1000 mg or greater.

No specific information is available on the treatment of overdose of pembrolizumab. In the event of overdose, the subject should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

7.2.1.3 Time Period and Frequency for Collecting AE, SAE and Other Reportable Safety Event Information (Arm B)

Specific considerations on safety reporting for Arm B are summarized below:

- All AEs, SAEs and other reportable safety events that occur after the consent form is signed but before treatment /allocation must be reported by the investigator if the participant is receiving placebo run-in or other run-in treatment, if the event cause the participant to be excluded from the study, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, or a procedure.
- All AEs or ECIs (Events of Clinical Interest) from the time of treatment/ allocation through 30 days following cessation of study treatment must be reported by the investigator.
- All AEs meeting serious criteria, from the time of treatment/ allocation through 90 days following cessation of study treatment, or 30 days following cessation of study treatment if the participant initiates new anticancer therapy, whichever is earlier must be reported by the investigator.
- All pregnancies and exposure during breastfeeding, from the time of treatment/ allocation through 120 days following cessation of study treatment, or 30 days following cessation of study treatment if the participant initiates new anticancer therapy must be reported by the investigator.
- Additionally, any SAE brought to the attention of an investigator at any time outside of the time period specified above must be reported immediately to the Sponsor if the event is considered to be drug-related.

7.2.2 Documentation of AEs

All AEs are to be evaluated for duration, intensity, and relationship to (associated with) the trial treatment or due to other causes including underlying disease.

All AEs (problems, complaints, signs, and symptoms and clinically relevant laboratory abnormalities), both those observed by trial site personnel and those spontaneously reported by the trial subjects, must be recorded on the AE page in the eCRF, as well as in the subject's medical record using standard medical terminology **regardless of causality**. If an AE occurred after signing the ICF, but the subject withdraws from the trial before receiving any trial treatment, the AE should be recorded in the eCRF.

Each event should be recorded as a single diagnosis. Accompanying signs (including abnormal laboratory values, physical examination findings, pulmonary or cardiovascular function tests) or symptoms should not be recorded as additional AEs. However, if the diagnosis is unknown or uncertain, signs and symptoms must be recorded. As soon as the diagnosis causing the signs and symptoms is known, the event terms will be adjusted to the final diagnosis.

For each AE, a minimum required information should be reported, which includes:

- The type of AE,
- An estimate of its severity (using NCI-CTCAE version 5.0, see Appendix C),
- Date and time of occurrence,
- Date of resolution,
- Actions required,
- An assessment of its causal relationship to trial medication (using the WHO system for standardized case causality assessment, provided separately in the investigator File).

AEs resulting from disease relapse/ progression themselves are not considered to be an AE. Likewise, any medical condition that is present at the Screening Visit will be considered as baseline and will not be reported as an AE but as medical history. However, if the condition changes at any time during the trial, it will be recorded as an AE. Investigators should ensure that the event term recorded captures the change in the condition (e.g., “worsening of...”).

7.2.2.1 Severity of AEs

Each AE is to be classified and graded according to CTCAE v5.0 criteria (see Appendix C). Dates of onset and resolution, including dates at which the grade of an AE changes, are to be recorded in the subject file.

If no CTCAE grading is available, the severity of an AE is to be graded as follows:

- **Mild (Grade 1):** the event causes discomfort without disruption of normal daily activities.
- **Moderate (Grade 2):** the event causes discomfort that affects normal daily activities.
- **Severe (Grade 3):** the event makes the subject unable to perform normal daily activities or significantly affects his/her clinical status.
- **Life-threatening (Grade 4):** the subject was at risk of death at the time of the event or the event caused death.
- **Death (Grade 5)**: death related to an AE.

7.2.2.2 Relationship to Trial Treatment

The investigator should use medical judgment to determine whether there is a reasonable causal relationship, including all relevant factors such as temporal course and latency, pattern of the reaction, known pharmacological properties of the product, and alternative explanations (e.g., other drugs, medical history, and concomitant diseases). The expression “reasonable causal relationship” means to convey in general that there is evidence or argument to suggest a causal relationship. The relationship of an AE to trial medication will be recorded on the eCRF and defined as follows:

- **Not Related:** An AE that does not follow a reasonable temporal sequence related to IMP and / or pembrolizumab and is likely to have been produced by the patient’s clinical state, other modes of therapy or other known etiology.
- **Unlikely Related:** An AE that follows such a temporal sequence from administration of the study medication (IMP and / or pembrolizumab) that a relationship is not likely, and is likely

to be due to a cause such as (known characteristics of) the patient's clinical state or other treatment.

- **Possibly Related:** AE that has a reasonable possibility that the event may have been caused by IMP and / or pembrolizumab. The AE has a timely relationship to the IMP and / or pembrolizumab; however, the pattern of response is untypical, and an alternative cause seems more likely or there is significant uncertainty about the cause of the event.
- **Probably Related:** An AE that has a reasonable possibility that the event is likely to have been caused by IMP and / or pembrolizumab. The AE has a timely relationship and follows a known pattern of response, but a potential alternative cause may be present.
- **Definitively Related:** There is a reasonable possibility that the event may have been caused by IMP and / or pembrolizumab. A certain event has a strong temporal relationship and an alternative cause is unlikely.

Ultimately, at the time of data analysis, the relationship to the AE will be categorized as either "**unrelated**" (including, unlikely or not related) or "**related**" (including definitely, probably or possibly related).

7.2.3 Reporting of SAEs

Information on SAEs will be collected from the time of ICF signature until the FU visit (or until EoT, if it occurs more than 30 days after the last CyPep-1 or pembrolizumab administration). After the FU visit (or EoT visit, if it occurs more than 30 days after the last CyPep-1 or pembrolizumab administration), only ongoing AEs or SAEs related to CyPep-1 and / or pembrolizumab administration will be collected.
Specific timing for Arm B is provided in Section 7.2.1.3.

All AEs that meet the criteria for SAE require the completion of a trial specific SAE Form (or Additional Safety Information [ASI]) Form), including an assessment of the relationship to the trial drug. This applies to all SAEs, whether or not they were considered to be related to the trial treatment.

The investigator must report all SAEs to [redacted] immediately, i.e. within 24 hours (by fax or e-mail) of learning of its occurrence. For this reporting, a SAE Form (provided in the investigator File) needs to be completed in English. The original SAE report form, together with the fax confirmation sheet (if applicable) or e-mail, must be kept at the study site. Follow-up information about a previously reported SAE must also be reported to [redacted] within 24 hours of receiving the information. This follow-up data must be provided on an ASI Form or an updated SAE form. The SAE report should provide a detailed description of the AE and should include anonymized copies of hospital records and other relevant documents. Autopsy results, if applicable, should also be sent to [redacted] as soon as they become available. Copies of each report will be kept in the investigator File.

All SAEs will need to be followed actively until resolution or stabilization. The above is also applicable to follow-up SAE information.

7.2.4 SUSARs

[redacted] on behalf of Cytovation, is required by law to report to the health authorities in a written safety report: 1) all fatal or life-threatening SUSARs within seven (7) calendar days of initial notification; and 2) all other SUSARs within fifteen (15) calendar days of initial notification.

All investigators in the trial will be informed about SUSARs that have occurred [redacted] on behalf of Cytovation.

7.2.5 Pharmacovigilance Contact for Reporting SAEs

[redacted]
Stationsplein Noord-Oost 438 1117 CL Schiphol
The Netherlands
Tel: 31 (0)20 4350581
Fax: 31 (0)20 4350589
[redacted]

8. STATISTICAL METHODS

8.1 Statistical Analytical Plan

A statistical analysis plan (SAP) will be written in which the details of the statistical methods will be described. The SAP will be finalized before database lock. Any deviations from the originally planned statistical analysis or SAP will be described and justified in the clinical trial report. Changes known at the time of SAP preparation will also be described in the SAP.

A data review meeting will be held before database hard lock. Protocol deviations will be reviewed during the data review meeting. Furthermore, assignment of subjects to the analysis sets will be performed.

Data of subjects who did not meet the entry criteria (screening failures) and data of subjects who received no investigational product (e.g., due to withdrawal) entered in the clinical database will only be listed. Minimal information will include date of ICF signed, demography, screen failure details and eligibility criteria, end of study form, SAEs if applicable. These data will be entered into the eCRF. These data will not be included in the evaluation of any analysis population.

8.1.1 Data Sets to be Analyzed

The analysis population sets are defined as follows:

All Screened Set (SCR): All subjects who were screened for the study and signed informed consent. This analysis set will be used for subject disposition table and for creation of listings.

Safety Analysis Set (SAF): All subjects who received at least one dose of trial drug (CyPep-1 or pembrolizumab in the combination arm). All analyses (except for PK) will be based on this analysis set.

CyPep-1 PK Analysis Set: The first 9 subjects of Phase I, all subjects of Phase IIa Arm C, and the first 12 subjects of Phase IIa Arm D who received at least one dose of CyPep-1, have no clinically important protocol deviations or important events considered affecting PK, and have provided at least one evaluable pre-dose and post-dose PK blood sample. All PK analyses will be based on this analysis set.

8.1.2 Summary Statistics

In general, data will be summarized by means of summary statistics. All data collected in this trial will be documented with the help of subject data listings, summary tables, and figures. Data will be summarized with respect to population for analysis, sub-grouped by dose level of CyPep-1 and extension arms, regarding demographic and baseline characteristics, PK measurements, clinical efficacy observations and measurements, safety observations and measurements. Continuous variables will be summarised using descriptive statistics (arithmetic mean and/or geometric mean, standard deviation and/or coefficient of variation, median, minimum, and maximum). Categorical variables will be summarised using counts and percentages. All individual subject data will be listed.

8.1.3 Adverse Events

Analyses of AEs will be based on the SAF set. AEs will be coded according to CTCAE v5.0 (for severity) and the Medical Dictionary for Regulatory Activities (MedDRA).

The total number/incidence of AEs will be summarized, including the number of subjects with at least one AE and the number of unique AEs per cohort and overall. The number of AEs per

severity (CTCAE) and relation to trial drug will also be included and summarized per cohort and overall. Additionally, listings of AEs leading to treatment discontinuation, resulting in death, and according to maximum severity will be tabulated. The incidence DLT/TLTs will also be evaluated for each dose level, and for all subjects combined. SAEs will be summarized in a similar manner.

The number of subjects and the number of AEs will be tabulated by MedDRA system organ class and preferred term. AEs will also be tabulated versus worst severity and worst relationship to treatment. In this table, subjects with AEs will be identified by their subject number.

Vital signs and body weight, 12-lead ECG parameters, laboratory data (hematology, biochemistry, coagulation, urinalysis) will be summarized using descriptive statistics. Shift tables showing changes from baseline will be generated where appropriate.

Pre-treatment events occurring between first assessment at screening and first dose of CyPep-1 will be summarized separately.

8.1.4 Efficacy Analyses

The efficacy endpoints are defined in relation to the trial objectives. Efficacy endpoints will be evaluated using the SAF. Further analyses will be defined in the SAP.

Efficacy will be determined from the number of subjects with CR, PR, SD or PD, according to iRECIST based on the investigator's assessments. PFS (percentage of subjects alive and progression-free after trial treatment start) will also be determined. These clinical response data will be summarized.

ORR (proportion of subjects with a best overall response of PR or, CR) will be evaluated based on iRECIST.

For Phase I Cohort 3 and all Phase IIa arms, ORR (proportion of subjects with a best overall response of PR or, CR) will be evaluated by two additional approaches based on itRECIST:

- Injected response: considering only the targeted-injected lesions.
- Non-injected response: considering only the targeted-non-injected lesions.

8.1.5 Pharmacokinetic Analysis (PK)

The first 9 subjects of Phase I,all subjects of Arm C, and the first 12 subjects of Arm D who have received CyPep-1 and have provided one evaluable pre-dose and at least one post-dose PK blood sample will be evaluable for PK (CyPep-1 PK Analysis Set).

PK analysis is planned via the determination of the plasma concentration time profile of CyPep-1 and, if detectable plasma drug levels are identified, the derived PK parameters will be assessed. These include area under the curve (AUC), peak plasma concentration (C_{max}), time to reach C_{max} (t_{max}), systemic clearance (CL), elimination half-life ($t_{1/2}$) and volume of distribution (VD).

8.1.6 Immunological Analysis

This analysis will be performed on the SAF.

The relative change in intratumoral CD8+ T-cell infiltration (CD8+ expression) in the biopsied injected, and whenever available, non-injected lesions between screening and Week 6 will be assessed.

The association between the relative changes in CD8+ T-cell infiltration and response rate in the injected lesions (per itRECIST) and all lesions (per iRECIST) will be assessed.

The change in T-cell receptor (TCR) clonality levels will be assessed in the available peripheral blood samples. To assess the hypothesis that CyPep-1 increases TCR clonality systematically, which suggests that T-cells are targeting the tumor, the clonality diversity metric will be computed for all subjects providing peripheral blood samples at screening and/or Week 6 of every CyPep-1 treatment cycle. Based on preliminary on file data of TCR clonality analysis in peripheral blood dated Nov 2022, the dataset collected is sufficient for the intended analysis and further sampling will provide no additional insights. Uncertainty about the representativeness of data generated with blood samples regarding TCR changes in the tumor microenvironment has lead Cytovation to reconsider this scientific relevance of this assay. Therefore, Cytovation decided to stop collecting blood samples for TCR clonality analysis. TCR sequencing might be performed on the available remaining tumor biopsy material from the analyses of changes in the tumor microenvironment. Overlaps of T cell clones in respect to expansion and breadth of T cell clonality before and after intratumoral injection with CyPep-1, as monotherapy and in combination with pembrolizumab (central analysis) will be analyzed.

The change in tumor microenvironment will be examined via the expression of selected candidate immune markers by immunohistochemistry: [redacted] For each marker, a difference in expression will be explored.

Changes in cytokine levels (only for Arms A, B, and C) and in frequency of various immune cell types in peripheral blood (immunophenotyping) will be explored.

8.1.7 Concomitant Treatment

Concomitant medication and concomitant therapy will be summarized as number of subjects being treated with each type of medication/therapy classified according to Anatomical Therapeutic Chemical (ATC) level 2 and World Health Organization (WHO) Drug Dictionary preferred term. The SAF will be used for this presentation.

8.1.8 Tabulation of Individual Participant Data

Individual participant data will be listed by measure and time point.

8.1.9 Exploratory Analyses

Exploratory analyses will be specified in the SAP. A separate analysis of subjects having received weekly vs Q2W CyPep-1 administration is planned as sub-group analysis.

8.2 Determination of Sample Size

This is a proof-of-concept trial and the number of subjects chosen is based on clinical considerations and not on a formal statistical power calculation.

The exact number will be dependent on the number of dose cohorts investigated and the number of subjects enrolled in each dose cohort in Phase I (dose escalation), based on the toxicity encountered.

Phase I (dose escalation): It is anticipated that 12 subjects will be included in this phase of the trial (Figure 2) at 3 dose levels, with the last dose level including three additional subjects for further confirmation of RP2D.

Phase IIa (dose expansion Arm A): It is planned that 9 subjects will be enrolled in this phase of the trial for further evaluation of CyPep-1 in monotherapy arm (Figure 2).

Formal assessment of response will be based on the first 12 evaluable subjects as follows: in case of no responders in 12 subjects, the likelihood of a 20% ORR is 6.9%; in case of one responder in 12 subjects, the likelihood of a 20% ORR is 26.5%. Assuming stable disease to occur in 30% of subjects, in case no subject has stable disease in the first 12 evaluated subjects, the likelihood is 1.4%; in case one subject has stable disease in the first 12 evaluated subjects, this becomes 8.5%, while with two subjects having stable disease, this becomes 25.2%. Therefore, in case of either one of 12 subjects having an objective response or two of 12 subjects having stable disease, the number of subjects in Arm A may be increased to a total of 24 subjects (meaning 18 subjects in Arm A; based on the total number of subjects at RP2D during dose escalation plus the number of subjects at RP2D in Arm A). In case, these outcomes are not observed, Arm A will enrol the planned 9 subjects.

In the current trial, the ORR assessment is based on sum of diamter of all target lesions and SD decision is based on the duration >16 weeks of SD from onset.

Phase IIa (combination Arm B): The safety and tolerability of CyPep-1 in combination with pembrolizumab will be evaluated in an arm of 15 subjects in total (Figure 2).

Phase IIa (liver metastasis, Arm C): For Arm C, a total of 9 subjects is planned to be enrolled to evaluate at least two dose levels of CyPep-1 based on results of Phase I.

Phase IIa (dose expansion melanoma, Arm D): The sample size for Phase IIa Arm D is based on a desired precision by which preliminary clinical efficacy (ORR for injected lesions) may be evaluated. Using 90% 1-sided Clopper-Pearson exact confidence intervals, lower bounds identified in the Table 6 would apply.

Table 6. 90% 1-sided Confidence Intervals for the Number of Responders in N=30 Subjects

Number or Responders	Observed ORR	One-sided 90% Lower Bound
1	3.3%	0.4%
2	6.7%	1.8%
3	10.0%	3.7%
4	13.3%	5.9%
5	16.7%	8.3%
6	20.0%	10.9%
7	23.3%	13.5%
8	26.7%	16.2%
9	30.0%	19.0%

For example, the lower bound for an observed ORR of 13.3% when there are 4 responders would be higher than 5% (i.e. 5.9%); the lower bound if 6 responders are observed would be 10.9%, which is greater than 10%, allowing for a sufficient sample to review preliminary effectiveness of CyPep-1 monotherapy for Arm D subjects.

8.3 Disposition, Demographic and Other Baseline Characteristics

A subject disposition will be made including the number of subjects included, exposed to trial drug, and completing or withdrawing from trial (including reason for withdrawal). The number of subjects in each analysis set will be included. The subject disposition will be made separately by cohort and overall.

Demographic, medical history, and other baseline data will be presented using summary statistics based on the SAF overall and split by cohort.

8.4 Procedures for Reporting any Deviation(s) from the Original Statistical Analysis Plan

Any deviation(s) from the original SAP will be described and justified in a protocol amendment and/or in a revised SAP and/or in the final report, as appropriate.

8.5 Exposure to Treatment

All details collected in relation to CyPep-1, as monotherapy and in combination with pembrolizumab, will be listed and tabulated if applicable. Exposure data for CyPep-1 and / or pembrolizumab use will be listed per cohort and overall. Number of doses and total treatment length will be summarized using descriptive statistics.

8.6 Safety Analysis for Dose Selection

Following completion of Phase I of the trial, a safety analysis (dose escalation analysis) will be performed for RP2D dose selection. All subjects having participated in Phase I of the trial will be part of this analysis to evaluate treatment safety. The DEC will issue a recommendation for the continuation of the trial based on safety and will confirm the MTD or RP2D (in case the MTD is not reached).

8.7 Follow-up Analysis of Post-Trial Survival Data

Survival data will be analyzed post-treatment end as described in Section 8.1.4 and presented as addendum(s) to the final trial report. Time-point(s) for analysis will be confirmed when considered justified by Cytovation and the investigator.

9. DATA MANAGEMENT AND MONITORING

9.1 Data Collection, Validation, and Handling

Data management and handling of data will be conducted according to the trial specific Data Management Plan and [redacted] standard operating procedures (SOPs).

Data for this trial will be captured using eCRFs. Data collection and entry into the eCRF is the responsibility of the clinical trial staff at the investigational site under the supervision of the investigator. The data will be subjected to validation according to the Data Validation and Medical Review Plans in order to ensure the information in the eCRF is complete, consistent, and accurate. The clinical trial site staff are responsible for resolving data queries issued by Clinical Research Associates (CRAs), Medical Monitor(s) and Data Management team. A system audit trail will maintain a record of initial entries and changes made; reasons for change; time and date of entry; and username of trial personnel authorizing entry or change.

Prior to the database lock, the eCRF must be completed and electronically signed by the principal investigator or authorized delegate from the clinical trial site staff. A complete eCRF package will be transferred to sponsor and the investigator will receive a copy at the end of the trial.

All external data will be electronically transferred to [redacted] for further data handling.

9.2 Data Review

This trial will be monitored by appropriate staff from [redacted] and may be also audited/inspected by [redacted] the sponsor, or by an independent body and/or authority. By agreeing to this Protocol, the investigator agrees to cooperate with compliance checks by allowing access for authorized individuals to all relevant trial documents.

CRAs will conduct regular monitoring visits. A representative of the sponsor may accompany the CRA on that occasion. Amongst others, the following will be reviewed at those visits:

- Trial progress;
- Compliance with the Protocol;
- Consent procedures, including date of consent and signatures;
- Completion of eCRFs and verification of data against the source data;
- Adverse events reporting;
- Storage, dispensing and accountability of trial medication;
- Archiving of trial documentation.

It is the investigator’s responsibility to assure that adequate time for these visits will be made available by him/her and other study personnel.

It is a prerequisite of the investigator’s participation in this trial that the CRA has direct access to source data for data verification. All information on eCRFs must be traceable to these source documents in the subject's file (permission will be sought from the subject as part of the consent process). Direct access to source documents will also be required for representatives of the sponsor and for competent authorities.

In addition, participation and personal information is treated as strictly confidential to the extent the applicable law permits and not publicly available. The audit or inspection may include, for example, a review of all source documents, drug records, original clinical medical notes, some or all of the facilities used in the trial.

9.3 Medical Coding

Coding of prior and concomitant medications will be performed using WHO Drug Dictionary. AEs and medical history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA, version to be specified in the data management plan) and CTCAE, version 5.0: Appendix C).

9.4 Health Economics Data Collection

Not applicable.

10. ADMINISTRATIVE ASPECTS

10.1 Quality Control and Quality Assurance

The sponsor or its designee will perform the quality assurance and quality control activities of this trial. However, responsibility for the accuracy, completeness, and reliability of the trial data presented to the sponsor lies with the principal or qualified investigator generating the data.

10.2 Maintenance of Subject Records

The investigator will maintain adequate records, including flow sheets, laboratory reports, signed subject consent forms, drug disposition records, and information on AEs, subject treatment discontinuation and reasons for treatment discontinuation. All records will be signed and dated by the investigators. All records are to be retained for a period of 25 years following the date the entire clinical investigation is completed, terminated or discontinued.

10.3 Investigator Site File (ISF)

At trial initiation, each investigator will be provided with an Investigator Site File (ISF) containing information as specified in Section 8.2-4 of ICH GCP.
Among others, the following documents will be provided in that file:

- Trial Protocol
- Site-specific ICF
- IB for the trial medication
- Subject registration forms and instructions
- Subject screening and enrolment logs
- Curricula Vitae (CVs)
- Template institute signature sheet
- Template training log
- Medical/ laboratory/ technical documentation
- Documents regarding trial medicinal products shipment, receipt, preparation, administration, and accountability
- Safety Handling Manual and SAE Forms
- NCI-CTCAE criteria (version 5.0, see Appendix C)
- Subject insurance
- Synopsis Clinical Trial Protocol

In addition, the investigator will be instructed to keep in the file institute-specific documents, among others those regarding:

- Agreements
- Site Insurance
- Protocol deviations/ notes to file
- Site submission and approval
- The original subject informed consent (a copy is provided to the subject)
- Site personnel qualifications (CVs)
- Laboratory accreditation/quality assurance and normal ranges
- Correspondence with [redacted] and Cytovation

It is the investigator's responsibility to keep the ISF up to date.

10.4 Handling of Investigational Product(s)

The trial medication must be received by a designated person at the trial site, handled and stored safely and properly, and kept in a secured location to which only the investigator, Pharmacist, and designated assistants have access. Upon receipt of the trial medication, all supplies should be stored according to the instructions specified on the drug labels.

Medication labels will comply with the legal requirements of the country. In addition, they will include storage conditions for the drug but no information about the trial. For drug accountability, the investigator must maintain an accurate record of the shipment and dispensing of trial drug. The trial medication must be used only as directed in the Protocol and for subjects enrolled in this trial only. For more information, see Sections 5.1 to 5.4.

10.5 Drug Accountability

Each time the trial medication is dispensed to a subject this must be recorded on a drug dispensing/accountability log. Copies of this form will be supplied in the ISF.

At regular intervals, the CRA(s) will perform a 'drug reconciliation visit', verifying if all trial medication that has been shipped to the institute can be accounted for by records of receipt, dispensing, and destruction.

Unused IMP that is not dispensed may only be destroyed following authorisation by a representative of [redacted] and destruction is fully documented. Alternatively, the IMP may be returned to the sponsor.

At the end of the trial, it must be possible to reconcile delivery records with records of usage and destroyed or returned stock. It is essential that the investigator or institute account for all trial treatment, and that any discrepancies are explained and documented.

10.6 Procedures for Protocol Amendments

Should any change be required to the approved Protocol, a Protocol amendment must be prepared. Any amendment is to be approved by the same persons approving the original Protocol. Upon approval, the final Protocol amendment will be incorporated into the Protocol. In case of a substantial Protocol amendment, this must be submitted to the IEC and competent authorities, detailing the reasons necessitating the amendment. Approval by an IEC and competent authorities is required for all substantial amendments, in accordance with international and local regulations. Notwithstanding the need for approval before implementation of formal Protocol amendments, the investigator is expected to take any immediate action required for the safety of any subject included in this trial, even if this action represents a deviation from the Protocol.

10.7 Protocol Deviations

A protocol deviation is any non-compliance with the clinical trial protocol, GCP, or requirements in other procedures. The non-compliance may be either on the part of the participant, the investigator, or the study site staff. Because of deviations, corrective actions are to be developed by the site and implemented promptly. These practices are consistent with ICH E6:

- 4.5 Compliance with Protocol, Sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, Section 5.1.1
- 5.20 Non-compliance, Sections 5.20.1 and 5.20.2.

Major protocol deviations are any deviations that might significantly affect the completeness, accuracy, and/or reliability of the trial data or that might significantly affect a subject's rights, safety, or well-being. This includes deviations related to subject eligibility, informed consent, IMP dosing errors, or failing to perform assessments required to interpret the primary endpoint. Additional categories may be identified as deemed necessary by the Medical Monitor.

All protocol deviations will be reported by the CRAs or other trial-involved personnel, such as data manager and statisticians. The protocol deviations will be reviewed by the Medical Monitor. The Medical Monitor determines whether a deviation is major or not. Major deviations are reported to the sponsor as part of the regular reporting. Important protocol deviations will be summarized in the clinical trial report. In accordance with applicable competent authority mandates, the investigator is responsible for reporting protocol deviations to the IEC.

In case of a deviation, the investigator enters a comment in the source documents and the non-compliance will be documented in a Monitoring Visit Report by the CRA. All non-compliance will be followed up and reported to CA and IEC as per local regulations. In parallel, corrective and/or preventive actions will be undertaken and documented, including any retraining of the investigator and site staff. No waivers for inclusion or exclusion criteria will be given.

10.8 Clinical Trial Report

A clinical trial report according to ICH Guideline "Note for Guidance on Structure and Content of Clinical Trial Reports" will be issued. A summary of the trial results will be provided to the IEC and the competent authority within one year after end of trial.

11. ETHICAL AND LEGAL CONSIDERATIONS

The investigator will ensure that this trial is conducted in full conformance with the Protocol, the principles of the "Declaration of Helsinki" (64th WMA General Assembly, Fortaleza, Brazil, October 2013) or with the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual. The trial must fully adhere to the principles outlined in "Guideline for Good Clinical Practice" ICH Tripartite Guidelines for Good Clinical Practice or with local law if it affords greater protection to the subject. As this trial is conducted in the EU/ European Economic Area (EEA) countries, the investigator will ensure compliance with the EU Clinical Trial Directive [2001/20/EC].

11.1 Competent Authority / Independent Ethics Committees

The protocol and any accompanying material provided to the subject (such as subject information sheets or descriptions of the trial used to obtain informed consent as well as any recruitment materials or compensation given to the subject) will be submitted to the CA and IEC.

Approval from the committee must be obtained before starting the trial and should be documented in a letter to the investigator specifying the date on which the committee met and granted the approval.

Any modifications made to the protocol after receipt of the CA/IEC approval must be re-submitted in the EEA member states in accordance with local procedures and regulatory requirements.

11.2 Site Review

The investigator will submit this protocol, the site-specific ICF, and any required documents for site review and approval. A letter confirming approval must be forwarded to [redacted] prior to initiation of this trial at each Investigational site.

Prior to trial start, the investigator is required to sign a Protocol Signature Page confirming his/her agreement to conduct the trial in accordance with these documents and all of the instructions and procedures found in this protocol and to give access to all relevant data and records to CRAs, auditors, and competent authorities as required. Investigators ascertain they will apply due diligence to avoid protocol deviations.

The investigator will make appropriate reports on the progress of this trial to [redacted] in accordance with applicable government regulations and their agreement with [redacted].

11.3 Informed Consent

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH-GCP, the Data Protection Directive (Directive 95/46/EC), and local regulations. The investigator will prepare the ICF and provide the documents to the RA and IEC for approval. Informed consent must be obtained before any trial specific procedures (including screening procedures) are performed. The process of obtaining informed consent should be documented in the subject source documents.

Before enrolment in the trial, the investigator or an authorized member of the investigational staff must explain to potential trial subjects and/or his/her legal representative the aims, methods, reasonably anticipated benefits, and potential hazards of the trial, and any discomfort participation in the trial may entail. Subjects will be informed that their participation is voluntary,

and they may withdraw consent to participate at any time. They will be informed that choosing not to participate will not affect the care that the subject will receive for the treatment of his or her disease. Subjects will be told that alternative treatments are available if they refuse to take part and that such refusal will not prejudice future treatments. By signing the ICF, the subject agrees to allow his or her trial physician to re-contact the subject for obtaining consent for additional safety evaluations if needed, or to obtain information about his or her vital status.

The subject will be given sufficient time to read the ICF and the opportunity to ask questions. After this explanation and before entry into the trial, consent should be appropriately recorded by means of the subject's personally dated signature and authorized trial staff's personally dated signature. After obtaining the consent, a copy of the ICF must be given to the subject.
If the subject is unable to read or write, an impartial witness should be present for the entire ICF process (which includes reading and explaining all written information) and should personally date and sign the ICF after the oral consent of the subject is obtained.

The investigator shall provide a copy of the signed ICF to the subject and the signed original shall be maintained in the ISF. The original of the signed ICF must be filed in the subject's source file. If new safety information results in significant changes in the risk/benefit assessment, the ICF should be reviewed and updated if necessary. All subjects should be informed of the new information and give their consent to continue the trial.

All changes to the consent form will be IEC-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

11.4 Trial Discontinuation and Closure

This trial may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for trial suspension/termination, will be provided by the sponsor to the investigators and CAs. If the trial is prematurely terminated/suspended, the principal investigator will promptly inform the IEC and will provide the reason(s) for termination/suspension.

Circumstances that may warrant termination/suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination of futility

The trial may resume once concerns about safety, protocol compliance and/or data quality are addressed and satisfy the sponsor, IEC and/or the CAs.

11.5 Confidentiality

By conducting this trial, the investigator affirms to [redacted] that all information regarding this trial and CyPep-1 will be maintained in strict confidence. Such information can be communicated to the investigator's local review and approval authority under an appropriate understanding of confidentiality. Updated General Data Protection Regulations (GDPR) procedures are covered in the ICF and in site agreements.

Every effort will be made to maintain the anonymity and confidentiality of subjects during this clinical trial. However, because of the experimental nature of this treatment, the investigator agrees to allow representatives of [redacted] the sponsor, and authorized employees of the CAs

to inspect the facilities used in this trial as well as to review, for purposes of verification, the hospital or clinic records of all subjects enrolled into this trial. A statement to this effect is to be included in the subject consent form.

11.6 Financing and Insurance

Financial aspects of the trial are addressed in a separate clinical trial agreement.

The investigator/ institution is required to have adequate current insurance to cover claims for negligence and/ or malpractice according to applicable national regulations. The sponsor will provide insurance coverage for the clinical trial as required by applicable national regulations.

Trial subjects will not be paid for their participation. Any trial-related travel expenses made by the subject or the accompanying person will be reimbursed based on actual cost as proven by original or copies of receipts, or an allowance per kilometre travelled.

11.7 Statement of Compliance

This study will be conducted in accordance with International Council for Harmonisation GCP (ICH GCP E6[R2]), and applicable local laws and regulations. The principal investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the IEC, except where necessary to eliminate an immediate hazard to the study participants. All relevant personnel involved in the conduct of this study have completed ICH-GCP training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IEC for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled.

11.8 Publication Policy

The sites and the principal investigator shall have the right to publish and present results of the study following submission to Cytovation for review of the manuscript, abstract or presentation intended for publication or presentation at least 70 days prior to the date of submission for publication or presentation. Regard shall be given to the sponsor's legitimate interests, e.g., containing optimal patent protection, coordination of submissions to health authorities or with other ongoing studies in the same therapeutic field, protection of confidential data, and information, etc. Cytovation shall complete its review within 60 days of receipt of the submitted manuscript, abstract or presentation. Cytovation may request that the principal investigator or the sites delete from the manuscript, abstract or presentation any confidential information. At the end of the 60-day period, the sites and the principal investigator will have the right to publish and present the material, abstract or presentation.

The above-described procedure also applies to information on prematurely discontinued and other non-completed studies.

Results from investigations shall not be made available to any third parties by the investigating team outside the publication procedure as set out above.

The sponsor will not quote from publications by investigators in its scientific information and/or promotional material without full acknowledgement of the source (i.e., author and reference).

11.9 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this study will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the study.

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13. APPENDICES

Appendix A: List of Non-clinical Studies Mentioned in the Protocol

Study number	Study name
506066	A Combined Single Dose and 7 Day Dose Range Finding Study of CyPep-1 by Intravenous Injection (Bolus) in Rats
506092	A 4 Week Study of CyPep-1 by Intravenous Injection (Bolus) in Rats with a 4 Week Recovery Period
507379	A Combined Single Dose Maximum Tolerated Dose and 14 Day Dose Range Finding Study of CyPep-1 by Intravenous Injection (Bolus) in Dogs
614716	Respiratory Effects following Single Intravenous Injection (Bolus) Administration in Male Han Wistar Rats
614721	Effects in the Irwin Screen after Single Intravenous Injection (Bolus) Administration in Male Han Wistar Rats
01027002	*Salmonella-E. Coli* Mammalian Microsome Reverse Mutation Assay
01027003	*In Vitro* Micronucleus Assay in TK6 Cells
AB22310	A 14-Day Range-Finding Toxicity Study of CyPep-1 by the Dermal Route in the Göttingen Minipig
AB22311	A 04-week Toxicity Study of 1% CyPep-1 Cream by the Dermal Route in the Göttingen Minipig with a 04-week Recovery Period
614700	Effect of CyPep-1 by Intravenous Injection (Bolus) on Cardiovascular Parameters in Conscious Telemetered Beagle Dogs
B-02852	Pharmacokinetic profile of CyPep-1 after a single intravenous (bolus) or subcutaneous administration in Sprague-Dawley rats

Appendix B: Eastern Cooperative Oncology Group Performance Status Scoring (Oken MM et al, 1982)

Grade	ECOG
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all 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

Appendix C: Common Terminology Criteria for Adverse Events (CTCAE) version 5.0

CTCAE term (AE description) and grade: The descriptions and grading scales found in the revised NCI-CTCAE version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

Quick Reference

The NCI-CTCAE is a descriptive terminology, which can be utilized for Adverse Event (AE) reporting. A grading (severity) scale is provided for each AE term.

Components and Organization

System Organ Class (SOC), the highest level of the Medical Dictionary for Regulatory Activities (MedDRA) hierarchy, is identified by anatomical or physiological system, etiology, or purpose (e.g. SOC Investigations for laboratory test results). CTCAE terms are grouped by MedDRA Primary SOCs. Within each SOC, AEs are listed and accompanied by descriptions of severity (Grade).

CTCAE Terms

An AE is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure that may or may not be considered related to the medical treatment or procedure. An AE is a term that is a unique representation of a specific event used for medical documentation and scientific analyses. Each CTCAE v 5.0 term is a MedDRA LLT (Lowest Level Term).

Definitions

A brief definition is provided to clarify the meaning of each AE term.

Grades

Grade refers to the severity of the AE. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline:

- Grade 1 Mild: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2 Moderate: minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living.
- Grade 3 Severe or medically significant but not immediately life-threatening: hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
- Grade 4 Life-threatening consequences: urgent intervention indicated.
- Grade 5 Death: related to AE.

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

iRECIST is based on RECIST 1.1 but adapted to account for the unique tumor response seen with immunotherapeutic drugs. iRECIST will be used by the investigator to assess tumor response and progression, and to guide decisions about changes in management.

Assessment at Screening and Prior to RECIST 1.1 Progression

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

Assessment and Decision at RECIST 1.1 Progression

For participants who show radiological PD by RECIST 1.1, the Investigator will decide whether to continue a participant on study treatment until repeat scans are obtained, as described in Section 14.

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

- Increase in the sum of diameters of target lesion(s) identified at baseline to ≥20% and ≥5 mm from nadir
    - Note: The iRECIST publication uses the terminology “sum of measurements”, but “sum of diameters” will be used in this protocol, consistent with the original RECIST 1.1 terminology.
- Unequivocal progression of non-target lesion(s) identified at baseline
- Development of new lesion(s)

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

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

New lesions will be classified as measurable or non-measurable, using the same size thresholds and rules as for baseline lesion assessment in RECIST 1.1. From measurable new lesions, up to 10 lesions total (up to 2 per organ), may be selected as New Lesions – Target. The sum of diameters of these lesions will be calculated and kept distinct from the sum of diameters for target lesions at baseline. All other new lesions will be followed qualitatively as New Lesions – Non-target.

Assessment at the Confirmatory Scans

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

Confirmation of Progression

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

- Any of the factors that were the basis for the initial iUPD show worsening
  - For target lesions, worsening is a further increase in the sum of diameters of ≥5 mm, compared to any prior iUPD time point
  - For non-target lesions, worsening is any significant growth in lesions overall, compared to a prior iUPD time point; this does not have to meet the “unequivocal” standard of RECIST 1.1
  - For new lesions, worsening is any of these:
    - An increase in the new lesion sum of diameters by ≥5 mm from a prior iUPD time point
    - Visible growth of new non-target lesions
    - The appearance of additional new lesions
- Any new factor appears that would have triggered PD by RECIST 1.1

Persistent iUPD

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

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

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

Resolution of iUPD

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

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

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

In this case, the initial iUPD is considered to be pseudoprogression, and the level of suspicion for progression is “reset”. This means that the next visit that shows radiographic progression, whenever it occurs, is again classified as iUPD by iRECIST, and the confirmation process is repeated before a response of iCPD can be assigned.

Management Following the Confirmatory Scan

If repeat scans do not confirm PD per iRECIST, as assessed by the Investigator, and the participant continues to be clinically stable, study intervention is to continue and the regular scan schedule is to be followed. If PD is confirmed, participants may be discontinued from study intervention.

NOTE: If a participant has confirmed radiographic progression (iCPD) and clinically meaningful study intervention may be continued after consultation with the Sponsor. In this case, if study treatment is continued, tumor imaging should continue to be performed following the intervals as outlined in Section 14.

Detection of Progression at Visits After Pseudoprogression Resolves

After resolution of pseudoprogression (ie., after iSD/iPR/iCR), another instance of progression (another iUPD) is indicated by any of the following events:

- Target lesions
    - Sum of diameters reaches the PD threshold (≥20% and ≥5 mm increase from nadir) either for the first time, or after resolution of previous pseudoprogression. The nadir is always the smallest sum of diameters seen during the entire trial, either before or after an instance of pseudoprogression.
- Non-target lesions
    - If non-target lesions have never shown unequivocal progression, their doing so for the first time results in iUPD.
    - If non-target lesions have shown previous unequivocal progression, and this progression has not resolved, iUPD results from any significant further growth of non-target lesions, taken as a whole.
- New lesions
    - New lesions appear for the first time
    - Additional new lesions appear
    - Previously identified new target lesions show an increase of ≥ 5 mm in the new lesion sum of diameters, from the nadir value of that sum
    - Previously identified non-target lesions show any significant growth

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

The decision process on the subsequent iUPD is identical to the iUPD confirmation process for the initial PD, with one exception, which can occur if new lesions had occurred at a prior instance of iUPD, had not resolved, then worsened (increase in size or number) leading to the second iUPD. If new lesion worsening has not resolved at the confirmatory scan then iUPD cannot resolve to iSD or iPR. It will remain iUPD until either a decrease in the new lesion burden allows resolution to iSD or iPR, or until new or worsening causes of progression indicates iCPD.

Additional details about iRECIST are provided in the iRECIST publication (Seymour 2017).

Appendix E: Medication for Hypersensitivity Type Reactions Anticipated to be Possibly Occurring Due to CyPep-1 Administration

Anaphylactic reactions are usually categorized into the following stages:

Stage	Extent and type of reactions
0	Locally confined skin reaction
I	Mild systemic reaction: - Disseminated skin reactions: flushing, pruritus, generalized urticaria - Mucosal reactions (nose, conjunctiva) - General reactions: restlessness, headache
II	Profound systemic reactions: - Cardiovascular dysregulation: changes in blood pressure and pulse rate - Difficulty in breathing: mild dyspnea, slight bronchospasm - Urinary and fecal urgency
III	Life threatening systemic reaction: s - Shock - Dyspnea, bronchospasm - Loss of consciousness (possibly with urinary and fecal incontinence)
IV	Vital insufficiency: - Cardiac and/or respiratory arrest

Depending on severity and type of the AEs, the following treatments are suggested and may be changed at the discretion of the investigator and local standards:

Fever, anaphylaxis
Paracetamol (acetaminophen) 1000 mg orally and/or metamizole (dipyrone) 1000 mg slowly i.v. infusion of physiological electrolyte solutions, adrenaline, anti-histamine H1- and H2-receptor antagonists, parenteral glucocorticoids.

Blood pressure instability, hypotension
Therapy according to the local best standard treatment options (e.g., catecholamines, plasma expanders [colloids, cristalloids], oxygen, etc.).

Bronchospasm, dyspnea
Bronchodilators: e.g., inhalatory β2-sympathomimetics such as fenoterol by inhalation of two puffs as often as required, parenteral glucocorticoids (theophylline 200–400 mg i.v. additionally, if necessary).

Antibiotic therapy

Infection with *Salmonella typhi* can be treated effectively with fluoroquinolones, including ciprofloxacin or ofloxacin.

Appendix F: High-level Summary of itRECIST and the Comparison of itRECIST with iRECIST

Current oncology response criteria, such as Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1) and guidelines for immunotherapeutic trials (iRECIST), were designed to assess response to systemic therapy and does not allow separate response assessment in injected and non-injected lesions. As intratumoral (IT) immunotherapy applies focal intervention, the ability to assess treatment response of injected and non-injected lesions is critical for IT immunotherapy trials.

Goldmacher et al proposed a modified RECIST 1.1 for intratumoral immunotherapy (itRECIST) with the goal to create guidelines for capturing data and assessing response in IT immunotherapy trials (Goldmacher 2020).

itRECIST is designed to address the unique needs of IT immunotherapy trials but, where possible, aligns with RECIST 1.1 and iRECIST. It does not dictate which lesions to inject at each visit, but rather provides guidelines for assessing responses as treatment evolves.
A high level summary of the difference between itRECIST and iRECIST are provided below: Table 1 presents the general difference between itRECIST and iRECIST, while Table 2 presents the difference in response assessment between the two.

Table 1. General difference between itRECIST and iRECIST

General Difference	itRECIST	iRECIST
Capture of systemic and/or local effects of immunotherapy	Capture both systemic and local effects	Only capture systemic effects
Lesion Measurement	Should be performed per RECIST 1.1, with the except of permitting ultrasound measurement for subcutaneous if no other lesions are available for quantitative assessment. Recommendations for imaging procedures: 1. Same operator should perform the ultrasound at all visits using the same equipment and acquisition parameters, capturing lesion images in a similar orientation, with anatomic landmarks to align with preceding scans. 2. The same imaging technique is used for a given target lesion at each assessment to evaluate changes over time.	Does not allow ultrasound for lesion measurement due to operator dependence and difficulty with standardization
Evaluable lesions	1. Treatment response in injected lesions can be evaluate separately from non-injected lesions 2. Injected lesions remain evaluable for overall response assessment even after termination of local procedures. 3. Excisional tumor biopsy renders a lesion non-evaluable. (Core needle biopsy would not render a lesion non-evaluable but its use is discouraged for target lesions). When feasible, biopsies should be restricted to non-target lesions.	Treatment response is evaluated as a combination of injected and non-injected lesions.

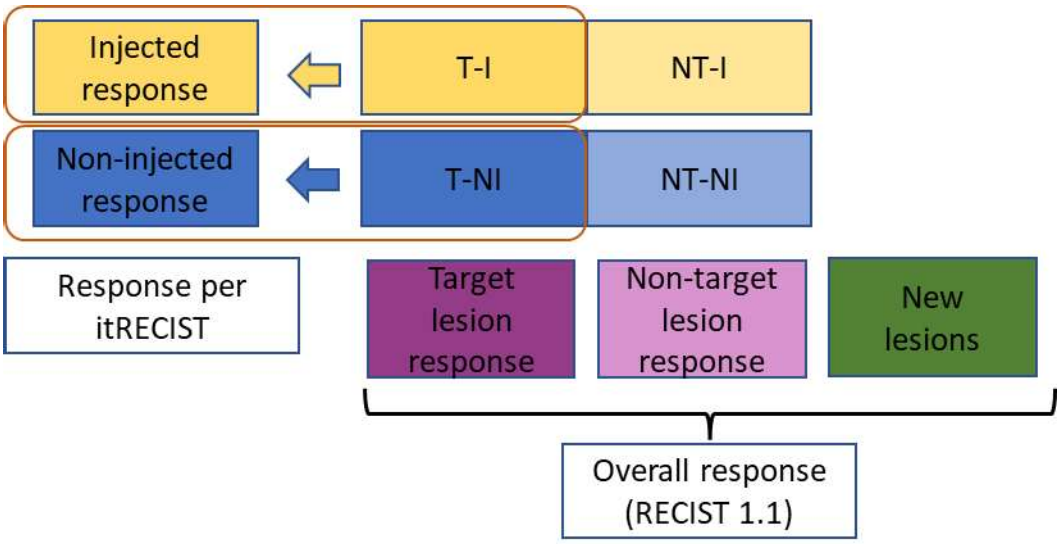
Reclassification of Lesions After Baseline	Injected lesions may change to non-injected lesions and vice versa based on individual circumstances judged per investigators, as long as target lesions always remain target and nontarget lesions remain non-target. However, once any T-NI lesion is injected, the non-injected response becomes non-evaluable. Note: Lesions designated T-NI at baseline should remain non-injected for as long as possible to allow assessment of maximal systemic response to IT therapy in non-injected lesions.	Reclassification not allowed.

Table 2. Difference in Response Assessment between itRECIST and iRECIST

Difference in Response Assessment	itRECIST	iRECIST
Overall Response	Similar to RECIST 1.1, with the difference only in allowing more target lesions, in injected lesions nob becoming non-evaluable, and allowing ulatrasound assessment.	Per RECIST 1.1
Injected Response and Non-injected Response	• Injected response is based entirely on the changes in the sum of diameters (SOD) of the targeted-injected (T-I) lesions • Non-injected response is based entirely on the changes in the SOD of the targeted-non-injected (T-NI) lesions. • Response from non-targeted lesions are not taken into account (See Figure 1)	Per RECIST 1.1, Target lesion response is based on the combination response of T-I and T-NI lesions, while nontarget lesion response is based on the combination response of NT-I and NT-NI.
Response Definition until disease progression	• Target lesion response is based on the changes in the SOD of all target lesions together. • Nontarget lesion response is the qualitative assessment of all target lesions together. • The evaluation for possible new lesions • The Overall response is the sum of the target lesion response, nontarget lesion response and new lesion response per RECIST 1.1 • Response by lesion category is summarized in Table 3.	Per RECIST 1.1
Decisions at REICIST progression	Same as per iRECIST (Appendix D)	(Appendix D)
Response Assessment After RECIST Progression	Similar as per iRECIST (Appendix D), except injected response and non-injected response should follow the rule of “Injected Response and Non-injected Response”	(Appendix D)
Interval to Confirmatory Reassessment	4 to 12 weeks	4 to 8 weeks

Treatment response calculation per itRECIST is summarized in Figure 1.
Definition of response by lesion category per itRECIST is presented in Table 3.

Figure 1. Calculation of Responses in itRECIST



Adopted from Figure 2 in Goldmacher 2020.
Abbreviations: NT-I = Non-Target-Injected; NT-NT = Non-Target-Non-Injected; T-I = Target-Injected; T-NI = Target Non-Injected.

Table 3. Definition of Response by Lesion Category in itRECIST

Response	Definition
T-I lesions	
CR	All nonmodal lesions gone, nodal lesions <10 mm
PR	≥ 30% decrease in SOD from last imaging assessment
PD	≥ 20% increase in SOD from last imaging assessment (≥ 5 mm absolute)
SD	Not enough growth for PD & Not enough shrinkage for PR
NE	≥ 1 lesion cannot be measured
T-NI lesions	
CR	All nonmodal lesions gone, nodal lesions <10 mm
PR	≥ 30% decrease in SOD from baseline
PD	≥ 20% increase in SOD from nadir (≥ 5 mm absolute)
SD	Not enough growth for PD & Not enough shrinkage for PR
NE	≥ 1 lesion cannot be measured or has been injected

Adopted from Table 1 in Goldmacher 2020.

Abbreviations: CR = complete response; NE = non-evaluable; PD = progressive disease ; PR = partial response ; SD = stable disease ; SOD = sum of diameters; T-I = Target-Injected; T-NI = Target Non-Injected.

An simplified example of iterative assessment of injected lesion response during treatment can be found in Figure 3 of Goldmacher 2020. More detailed guidance on the lesion mapping and sequence of CyPep-1 injection are detailed in the Study Procedural Manual.