Statistical Analysis Plan

Study Code:	LOKON002
Study Title:	Phase I/II Trial Investigating and Immunostimulatory Oncolytic Adenovirus for Cancer
Based on protocol version and date:	Version 5.1 2022-12-21
Biostatistician:	[redacted]
Clinical Data Manager:	[redacted]
Clinical Project Manager:	[redacted]
Sponsor:	Lokon Pharma AB

Reviewed and approved by Biostatistician (UCR);

[redacted]

Reviewed and approved by Biostatistician (UCR);

[redacted]

Approved by Sponsor's Representative;

[redacted]

SAP version: FINAL

Table of Contents

1. Introduction

The aim of this statistical analysis plan (SAP) is to describe details of planned presentation of the study data described to be performed by the UCR (Uppsala Clinical Research) Statistics section. The results will be presented according to the output specification (see Appendix 1 Output Shells). Study biostatistician is responsible for writing the plan with necessary input from other members of the study team.
A biostatistician not otherwise involved in the study together with the coordinating investigator will approve the final version.

The SAP is based on the study protocol: LOKON002 Study Protocol v5.1 2022-12-21.

2. Abbreviations

AE	Adverse Event
BMI	Body Mass Index
CI	Confidence Interval
CR	Complete Response
eCRF	electronic Case Report Form
CBR	Clinical Benefit Rate
[redacted]	[redacted]
CTCAE	Common Terminology Criteria for Adverse Events
DL	Detection Limit
DLT	Dose Limiting Toxicity
DR	Duration of Response
ECOG	Eastern Cooperative Oncology Group
[redacted]	[redacted]
EOC	Epithelial ovarian cancer
[redacted]	[redacted]
IEC	International Ethics Committee
[redacted]	[redacted]
LOAd	Lokon oncolytic adenovirus
MedDRA	Medical Dictionary for Drug Regulatory Activities
Min	minimum
Max	maximum
[redacted]	[redacted]
MTD	Maximum Tolerated Dose
NE	Non-evaluable
ORR	Overall Response Rate (defined as Partial response or better)
OS	Overall Survival
ORR	Overall Response Rate
PC	Pancreatic cancer
PD	Progressive Disease
[redacted]	[redacted]
PFS	Progression Free Survival
PI	Principal Investigator
PK	Pharmacokinetics
[redacted]	[redacted]
PR	Partial Response
PT	Preferred Term

[redacted]	[redacted]
RECIST	Response Evaluation Criteria In Solid Tumors
SAP	Statistical Analysis Plan
SAE	Serious Adverse Event
SD	Standard Deviation
SD	Stable Disease
SoC	Standards of Care
SOP	Standard Operating Procedures
[redacted]	[redacted]
TTP	Time to Tumor Progression
UCR	Uppsala Clinical Research Center
VP	Virus Particle
WHO	World Health Organization

2.1. RECIST (Response Criteria)

Tumor regression or progression will be evaluated accordingly with RECIST 1.1 criteria.

Complete Response (CR)	Complete macroscopic disappearance of all tumors.
Partial Response (PR)	A reduction of at least 30% in the sum of all tumor diameters from baseline.
[redacted]	[redacted]
Stable Disease (SD)	Neither partial response nor progressive disease.
Progressive Disease (PD)	At least a 20% increase in the sum of all tumor diameters from the smallest tumor size and/or the appearance of new tumor lesion/s.
Clinical Benefit Rate (CBR)	CBR is defined as MR or better.
Overall Response Rate (ORR)	ORR is defined as PR or better.

Note:
Due to the potential immunostimulatory capacity of the investigational product LOAd703, it is possible that the induced immune stimulation may induce an inflammatory swelling of the tumor that initially may be mistaken for progression. Therefore, PD during the study participation leading to discontinuation of repeated treatment needs to be confirmed by radiological imaging at a later time point ([redacted]) and/or by a biopsy confirming tumor progression by histochemical analysis.

2.2. Diagnosis (Patient populations)

This study will enroll subjects with following cancer diagnosis:

- Colorectal cancer
- Pancreatic cancer
- Biliary cancer
- Ovarian cancer

[redacted]

3. Study Objectives and Endpoints

3.1. Objectives

3.1.1. Primary Objective(s)

The primary objective is to determine the tolerability of increasing doses of LOAd703 intratumoral injections during standard of care or added conditioning gemcitabine chemotherapy.

3.1.2. Secondary Objective(s)

The secondary objective is to determine the effects of LOAd703 intratumoral injections during standard of care or added conditioning gemcitabine chemotherapy.

3.2. Endpoints

3.2.1. Primary Endpoints

Phase I

1. Tolerability is determined by physical examination, hematological and clinical chemistry analysis using common terminology criteria for adverse events (CTCAE) v 4.03

[redacted]

Phase II

1. Tolerability is determined by physical examination, hematological and clinical chemistry analysis using CTCAE v 4.03

[redacted]

3.2.2. Secondary Endpoints

Phase I

[redacted]

3. Local and distant anti-tumoral effects assessed by appropriate imaging dependent on tumor localization and by monitoring tumor marker/s when available. Responses are judged accordingly to response evaluation criteria in solid tumors (RECIST) 1.1.
4. Effects are evaluated as time to tumor progression (TTP), progression free survival (PFS) and overall survival (OS).

Phase II

1. Local and distant anti-tumoral effects on tumor assessed by appropriate imaging dependent on tumor localization and by monitoring tumor marker/s when available. Objective responses are measured using RECIST 1.1.
2. Effect evaluated as TTP, PFS and OS.

[redacted]

4. Study design

Single arm, open-label, multi-center trial.

Site 01: [redacted]
Site 02:

Phase I: 3 patients received up to 8 LOAd703 injections at three dose levels ($5x10^{10}$ virus particle (VP), $1x10^{11}$ VP, $5x10^{11}$ VP) during standard of care (SoC) or gemcitabine conditioning, 9 dose-limiting toxicities (DLT) evaluable patients.

DLT is defined as any grade 3 or higher toxicity according to the NCI CTCAE version 4.03 that is attributed (definitely, possibly or probably) to LOAd703. A DLT attributed to LOAd703 precludes any further LOAd703 injections. [redacted]

Maximum tolerated dose (MTD) is defined as the highest safe dose tested in Phase I, in which DLT does not occur in >1 of 3, or >2 of 6 treated patients.

[redacted]

[redacted]

LOAd703 will be given as add-on, biweekly, to standard of care chemotherapy, or with gemcitabine conditioning if there are no standard options available. Each patient will receive up to a total of 8 LOAd703 injections. Missed LOAd703 doses will not be made up in order to maintain adherence to the protocol schedule.

[redacted]

The LOAd703 treatment schedule and study visits are described in Figure 1 (flow chart) and Table 1 (Schedule of events).

Figure 1

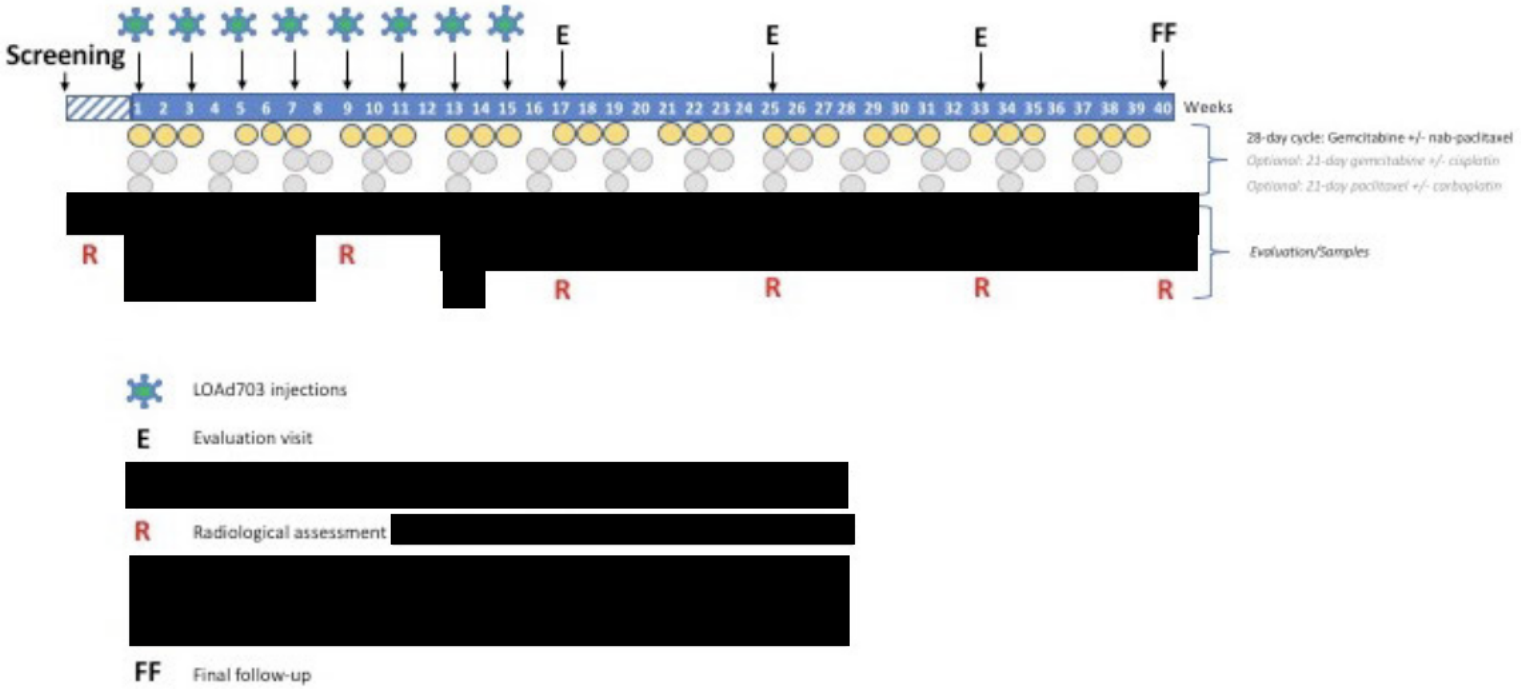
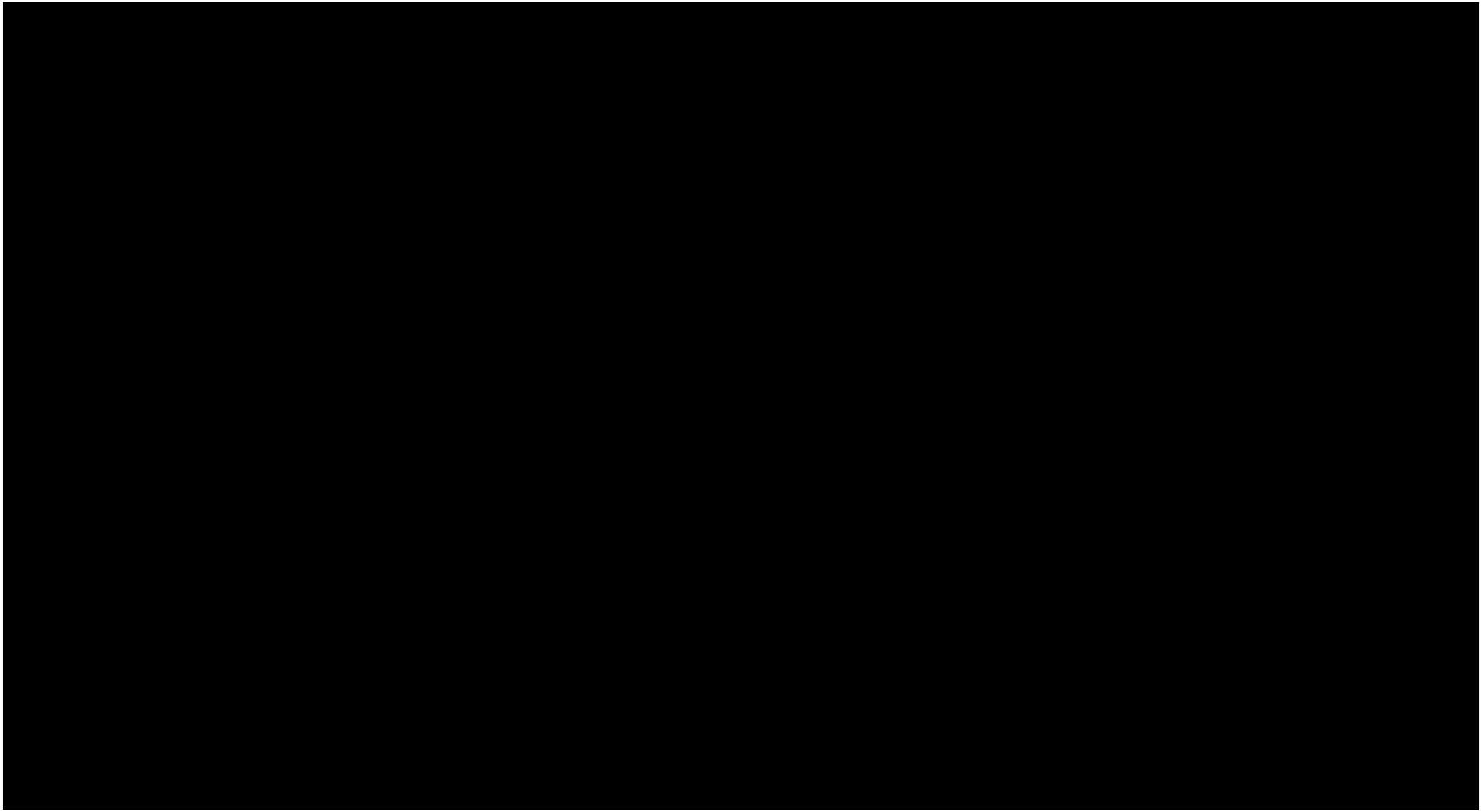


Figure 1. *Treatment schedule for LOAd703 treatments, sampling and follow-up visits.* [redacted] *The LOAd703 study will be added to standard care (SoC) chemotherapy treatment which will commonly be given in a 28-day cycle (yellow circles indicate infusion days). If no SoC is available, the PI can use gemcitabine conditioning which is also given at a 28-day cycle. Some patients may need SoC using a 21-week cycle and infusion days are shown in as grey circles. Chemotherapy can be omitted at any time as per hospital guidelines. Independently of chemotherapy regimen, the study visits are always at the same weeks.* [redacted]



[redacted]

5. Definition of Analysis Populations

5.1 Definitions

Screened patient	Patient who has signed the Informed Consent Form
Enrolled patient	Patient who has fulfilled all inclusion criteria but no exclusion criteria
Safety evaluable patient	Patients that have [redacted]
Efficacy evaluable patient	Patients that have [redacted]
Screening failure	Patient who has been screened but is not eligible
Non-qualified	Patients that have been screened but have not received any investigational product, (i.e. screen failures and patients who have been eligible for the trial but never received any LOAd703 treatment)
Responder	Patient who has shown at least partial response at any time during the study
Permanently discontinued from LOAd703 treatment	Patients prematurely discontinued from the LOAd703 treatment
Interruption of LOAd703 treatment	Temporary interruption of LOAd703 treatment
Withdrawal from study	Patients prematurely withdrawn from the **study**
Completed patient	Patient completed the study to Final follow-up ([redacted])
Dose Limited Toxicity (DLT)	DLT is defined as a CTC adverse event (CTCAE) grade 3 or higher that can be attributed (definitely, possibly or probably) to LOAd703 treatment. DLT precludes further LOAd703 injections or leads to dose reduction.

5.2 Analysis populations

The analysis of data will be based on different subsets according to the purpose of the analysis.

Safety	All patients who have [redacted] The patients analysed for the safety endpoints will be grouped by the LOAd703 doses they have been assigned to at enrolment. Only observed data will be used.
Efficacy	All patients that have [redacted] The patients analysed for the efficacy endpoints will be grouped by the LOAd703 doses they have been assigned to at enrolment.

6. Description of statistical analysis

In this study, no formal hypothesis testing will be performed. The outcome variables will be evaluated by descriptive methods. All variables will be presented in summary tables and per-patients data listings. The outcome variables will be summarized by dose ($5x10^{10}$ VP, $1x10^{11}$VP, $5x10^{11}$ VP), LOAd703 treatment number and cancer diagnosis as applicable. Descriptive statistics are defined as frequency tables for qualitative variables and as number of observations, means, standard deviations, medians, minimum and maximum values for quantitative variables.

Tables and graphs that presented all patients together will also be constructed.

[redacted]

6.1. Study conduct and Subject/Patient disposition

The number of screened patients, number of screening failures and the number of enrolled patients will be presented. Also, the number of patients that received LOAd703 and the number of patients that did not receive LOAd703 will be presented.
The number of patients with their actual number of received LOAd703 injections will be presented. The number of patients that discontinued from LOAd703 or were withdrawn from the study, in total and for each pre-defined withdrawal reason, and the allocation of patients to each analysis population group (safety, efficacy) will be presented in the table. Number of patients will be presented by LOAd703 dose, cancer diagnosis and in total.

A patient for whom the dose of LOAd703 treatment was modified during the treatment phase will be included in the dose group to which the patient was assign to at enrollment.

6.2. Baseline Characteristics

Demographic variables including age, sex, race, smoking status will be summarized by dose group, cancer diagnosis and all patients combined. Baseline characteristics including, height, weight, body mass index (BMI), blood pressure, body temperature and heart rate will be summarized by dose group and over all subjects combined. Continuous variables will be summarized by number of observations (n), mean, standard deviation (SD), median, minimum (min) and maximum (max) by dose, visit and overall. Categorical variables will be summarized in frequency tables (presenting frequencies and proportions) by dose, number of treatments and overall.

Medical and surgical history, oncological surgical history, baseline oncological disease details and baseline symptoms will be listed as a per-patient data listing and presented by Preferred term (PT) and System organ class (SOC) using Medical Dictionary for Regulatory Activities (MedDRA) terminology (version 21.0).

Medical and surgical history, oncological surgical history, baseline oncological disease details will be coded in [redacted] whereas baseline symptoms will be coded outside [redacted].

Baseline oncologic disease details include information about extent of disease, time between the date of diagnosis and date of signed informed consent and tumor mutation status.

6.3. Pregnancy test

Pregnancy test results (done for women of childbearing potential) will be presented in a listing.

6.4. Treatment Administration/Compliance

Information regarding all doses administrated, time points, and other information captured in the eCRF will be displayed in a per-patient listing and summarized in a table using descriptive statistics. The listing will indicate whether the medication is continuing or not.

6.5. Concomitant medication and anti-cancer therapy

Concomitant medication, concomitant chemotherapy and previous chemotherapy will be coded according to the WHO Drug dictionary. Coding will be done within [redacted] and the B3 format of WHO Drug dictionary will be used.

Concomitant medication and previous chemotherapy will be coded within [redacted] whereas concomitant chemotherapy will be coded outside [redacted].

Concomitant medication and anti-cancer therapy (concomitant chemotherapy and previous chemotherapy) will be presented in per-patient data listings.

6.6. Efficacy analyses

The efficacy evaluable population will be used for these analyses.

6.6.1. Primary efficacy analyses

[redacted]

Overall response rate (ORR) and clinical benefit rate (CBR) with 90% and 95% exact binomial confidence interval will be presented for all patients as well as summarized by dose and cancer diagnosis.

Sum of RECIST target lesions will also be explored with graphical methods. Figures will be constructed showing results at each corresponding visit by dose, cancer diagnosis and in total. Overall survival (OS), duration of response (DR), time to progression (TTP), and progression free survival (PFS) will be presented in a per-patient data listing and as Kaplan-Meier curves together with median survival time and time to progression, respectively. Figures with OS, DR, TTP and PFS data will present results by dose and cancer diagnosis.

[redacted]

[redacted]

6.7. Safety analyses

6.7.1. Adverse events

Adverse Events (AEs) will be coded using MedDRA terminology (version 21.0) in [redacted]. Adverse events will be presented in per-patient data listings and summarized in frequency tables by number of LOAd703 treatments. AEs (including serious adverse events [SAEs], AEs and DLTs) will be summarized as number of events and number of patients, respectively, by body system/system organ class, preferred term, intensity/CTCAE, seriousness and relationship. Separate tables will be produced by dose, cancer diagnosis and in total.

6.7.2. Vital signs

The vital signs parameters [redacted] will be presented in per-patient data listings and summarized as observed value and as change from baseline by dose group, cancer type, visit and scheduled time points [redacted]) using descriptive statistics (n, mean, SD, min, median, max). [redacted]

6.7.3. Laboratory parameters

The laboratory parameters (blood chemistry and haematology) will be presented in per-patient data listings, including reference and CTCAE ranges where applicable, and summarized as observed value and as change from baseline by scheduled time point, cancer diagnosis and dose group using descriptive statistics (n, mean, SD, min, median, max). [redacted]

Laboratory parameter values below a detection limit (DL) will be set to DL/2 and values above a DL will be set to DL in the summary tables, but in the per-patient data listings the original values will be displayed with a comment showing if the value is below/above the DL.

6.7.4. Physical examination and ECOG

Physical examination and ECOG will be presented in per-patient data listings and summarized in frequency tables. Physical examination will be presented as (frequency and proportion) and shift table by scheduled time point. ECOG will be presented in tables by scheduled time point. Tables will be constructed by dose, cancer diagnosis and in total.

6.7.5. Deaths

Deaths will be presented in a listings

6.7.6. Concomitant chemotherapy (Standard of care)

Concomitant chemotherapy (standard of care) at [redacted] will be presented in a table showing the number and percentage of chemotherapy regimen in total and by cancer diagnosis. Data for will also be presented in per-patient data listings.

6.8. Handling of Missing Data

Missing data will result in a reduced sample size for that parameter. Since the statistical analyses will be predominantly presentations in tables and individual listings, no action will be taken to handle missing data.

A patient who withdraws prior to the last planned observation will be included in the analysis up to the time of discontinuation.

In case of incomplete start dates and end dates of adverse events, the "worst case" date will be used. For example, if "December 2015" is recorded as the time of start or end of an adverse event, December 1, 2015 and December 31, 2015, will be used as start- and end-date, respectively. All data from the analysis populations defined in Section 5.2 will be included in the relevant listings and summary tables.

6.9. Other planned analyses

Not applicable

7. Determination of sample size

The number of patients to be included in this study is not based on statistical considerations but based on experiences from similar studies.

Phase I

[redacted]

Note: at least 1 of 3 or 2 of 6 patients per dose cohort should be represented by a patient within one of the orphan indications pancreatic, biliary or ovarian cancer.

[redacted]

Phase II

[redacted]

8. Interim analysis plan

Not applicable

9. Changes in the planned analyses

The subgroup analysis of ovarian cancer patients (with modified eligibility criteria) will not be performed due to only two subjects in the study fulfill these criteria.

10. Description of Derived Variables

BMI	The body mass index (BMI) is calculated as BMI (kg/m^2) = Weight (kg) / Height $(m)^2$
Smoking	Will be defined as (smokers/former smokers/non-smokers using data given in medical history
Duration of AE:	Stop date minus onset date expressed in days
Change from baseline:	For laboratory variables and vital signs, change from baseline will be calculated as the observed value subtracted by the baseline value. [redacted]
CTC:	CTC grades will be assigned to laboratory values using CTC grade criteria (National Cancer Institute, Common Terminology Criteria for Adverse Events (CTCAE) v4.03 http://evs.nci.nih.gov/ftp1/CTCAE/About.html), for parameters with available CTC criteria.
Laboratory parameters	Laboratory parameter values below a detection limit (DL) will be set to DL/2 and values above a DL will be set to DL before
Overall survival (OS)	Defined as time from enrolment until death from any cause. Status will be defined as 1=dead, 0=alive. Subjects without death are right censored at the date of last contact.
Time to progression (TTP)	Time from enrolment to objective tumour progression according to RECIST v1.1 (does not include deaths) 1=progression, 0=no progression. Subjects without progression are censored at the date of the last evaluable tumor assessment.

Progression free survival (PFS)	Time from enrolment to objective tumour progression according to RECIST v1.1 or death (whichever occurs first) 1=progression/death 0=no progression/alive. Subjects without progression/death are right censored at the date of the last evaluable tumor assessment.
Duration of response (DR)	Time from the first assessment of CR (complete response) or PR (partial response) until the date of the first occurrence of PD (progressive disease) according to RECIST 1.1, or until the date of death (if occurred within predefined time period)
Best tumor response	The smallest post-baseline value (PD/SD/PR/CR) according to RECIST 1.1
Time from diagnosis to informed consent	Date of signed informed consent minus date of diagnosis

11. Description of Output

See Appendix 1: Output shells LOKON002.

12. Statistical software

SAS [redacted]. R ([redacted]) may be used.

13. References

1. H. Young, R. Baum, U. Cremerius, K. Herholz, O. Hoekstra, A.A. Lammertsma, J. Pruim and P. Price. Measurement of Clinical and Subclinical Tumour Response Using [18F]-¯uorodeoxyglucose and Positron Emission Tomography: Review and 1999 EORTC Recommendations *European Journal of Cancer* Vol.35. No.13, 1999

2. Cunanan KM, Lasonos A, Shen R, Begg CB, Gönen M. An efficient basket trial design. Stat Med, 2017. 36:1568-1579.

3. Kramar A, Potvin D, Hill C. Multistage designs for phase II clinical trials: statistical issues in cancer research.