I.R.I.S.

INSTITUT DE RECHERCHES INTERNATIONALES SERVIER

Document title	**STATISTICAL ANALYSIS PLAN**
Study title	A multicentre, Phase II Randomized study, Open-label, with 2-arm Parallel Group, comparing the pharmacokinetics of the Liquid and the Lyophilized Formulations of pegaspargase (S95014) in Treatment of Paediatric Patients with Newly-Diagnosed Acute Lymphoblastic Leukemia (ALL)
Test drug code	S95014 / pegaspargase / Oncaspar®
Indication	Acute Lymphoblastic Leukemia
Development phase	Phase II
Protocol code	CL2-95014-002
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List of abbreviations

ADA	:	Anti-Drug Antibodies
AE	:	Adverse Event
AEOSI	:	Adverse Event Of Special Interest
ALL	:	Acute Lymphoblastic Leukemia
ALT	:	Alanine aminotransferase
ANOVA	:	Analysis of Variance
AUC	:	Area Under the Curve
AST	:	Aspartate aminotransferase
BMI	:	Body Mass Index
BSA	:	Body Surface Area
CBI	:	Central Business Intelligence
CI	:	Confidence Interval
Cmax	:	maximum Concentration
CoV	:	Coefficient of Variation
CTC	:	Common Terminology Criteria
CTCAE	:	Common Terminology Criteria for Adverse Events
DAP	:	Data Analysis Plan
ECG	:	ElectroCardioGram
FAS	:	Full Analysis Set
GMR	:	Geometric Mean Ratio
IAS	:	Immunogenicity Analysis Set
IMP	:	Investigational Medicinal Product: a pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial (test drug / placebo / reference product)
I.R.I.S.	:	Institut de Recherches Internationales Servier
IRS	:	Interactive Response System
MedDRA	:	Medical Dictionary for Regulatory Activities
NCI	:	National Cancer Institute
PAA	:	Plasma Asparaginase Activity
PEG	:	PolyEthylene Glycol
PK	:	PharmacoKinetics
PKAS	:	Pharmacokinetic Analysis Set
RDI	:	Relative Dose Intensity
SAE	:	Serious Adverse Event
SAP	:	Statistical Analysis Plan
SAS	:	Safety Analysis Set
SD	:	Standard Deviation
TEAE	:	Treatment Emergent Adverse Event
TLG	:	Tables, Listings and Graphs
%CV	:	Coefficient of Variation in percent

1. INTRODUCTION

This Statistical Analysis Plan (SAP) details the planned analyses to be performed, in accordance with the main characteristics of the study protocol v2.0, dated on December 2nd, 2020, and the non substantial Protocol Amendment, dated on Apr 7th, 2021. The templates for Tables, Listings and Graphs (TLG) are described in a separate document.

1.1. Study objectives

1.1.1. Primary objective

To compare the pharmacokinetics (PK) of both lyophilized and liquid S95014 formulations during the induction phase after a single IV dose in newly diagnosed paediatric patients with ALL

1.1.2. Secondary objectives

- To describe the PK of S95014 after administration of either lyophilized or liquid formulation
- To evaluate the occurrence of treatment emergent adverse events (TEAEs) including serious adverse events (SAEs), regardless of causality and severity, based on National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) 5.0
- To evaluate the achievement of plasma asparaginase activity (PAA) of ≥0.1 U/mL after the administration of either lyophilized or liquid S95014
- To assess the immunogenicity of both lyophilized and liquid S95014 formulations

1.2. Study design

This is a multicentre, national, randomized, open-label, phase II clinical study comparing the pharmacokinetics of lyophilized and liquid Pegaspargase (Oncaspar®, S95014) formulations in the treatment of newly diagnosed, untreated pediatric patients with ALL.

1.2.1. Study plan

Each patient will be randomly assigned (1:1) to either lyophilized (arm 1) or liquid (arm 2) S95014 intravenously at the dose of 2500 U/m² at Day 3, in combination with other backbone chemotherapy agents as per local practice.

The study duration will be approximately 1.5 month, including the screening (14 days) and the treatment period consisting of the induction phase (approximately 30 days). After completing the induction phase, patient will be discontinued from the study and, provided that the inclusion/non-inclusion criteria are fulfilled, will be proposed to enter into a roll-over study (CL2-95014-003) for the consolidation phase.

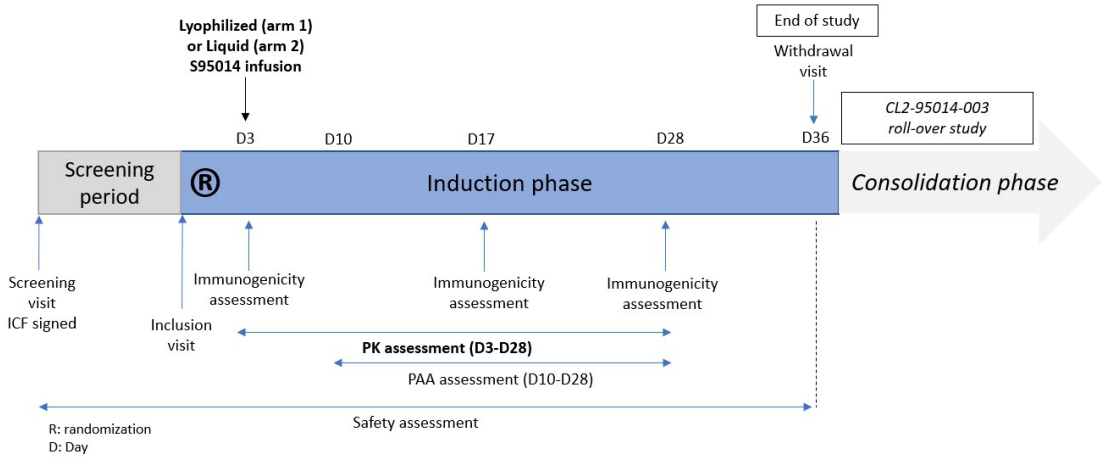
The study will be divided into the following periods for each participant:

- **Screening visit (up to 14 days prior to inclusion):** to obtain informed consent and check the screening/non-screening criteria.
- **Screening period (Day -14 to Day -1):** to check the eligibility of the patient to be included in the study within 14 days prior to the inclusion.
- **Inclusion visit (Day 0)**: to check inclusion/non-inclusion criteria and to confirm inclusion of the patient in the study

- **Randomization (Day 0):** the day of inclusion visit (i.e. Day 0), included subjects will be randomly assigned to one of the two treatment groups:
  - Lyophilized S95014 (arm 1)
  - Liquid S95014 (arm 2)
- **Infusion (Day 3):** the day of S95014 infusion, based on randomized treatment group.
- **Treatment period:** treatment period will start from the day of S95014 infusion, corresponding to Day 3 of the induction phase, until the withdrawal/end-of-study visit.
- **Withdrawal/End-of-study visit:** at least 30 days after S95014 infusion (i.e. Day 33) and before starting the consolidation phase. During the withdrawal/end-of-study visit, subjects will be proposed to participate in a roll-over study (CL2-95014-003) for the consolidation phase using lyophilized S95014 in combination with backbone regimen as per ALL-MB 2015 protocol.

The study plan is shown in Figure (1.2.1) 1.

Figure (1.2.1) 1 - Study plan



1.2.2. Type of randomisation

The treatment groups will be allocated via IRS using a central randomization (1:1) to either lyophilized S95014 (arm 1) or liquid S95014 (arm 2) of the induction phase.

1.3. Determination of sample size

This study is a two-arm parallel 1:1 randomized trial to evaluate the PK comparability between liquid and lyophilized S95014 formulations. For both AUC and C_{max}, the coefficient of variation (CoV) of 0.30 is assumed based on the estimation of CoV for liquid S95014 from a previous study (Angiolillo, 2014). It is also reasonable to assume a geometric mean ratio (GMR) of 100% for C_{max} and AUC, since no impact of an absorption phase is expected because both formulations are delivered intravenously. In addition, the CMC investigations have shown that once the lyophilised formulation is reconstituted, the characteristics of the solution are comparable to the ones of the liquid formulation. Assuming a CoV of 0.30 and a true GMR of 100%, a total of 78 evaluable subjects (39 subjects per arm) will provide approximately 90% power to establish the PK comparability. Assuming that 10% of the included subjects could not

be evaluable (e.g. missing PK timepoint etc.), 88 subjects are expected to be included to allow 78 evaluable subjects.

2. ANALYSIS SETS / TREATMENT GROUPS

2.1. Analysis sets

Full Analysis Set
The full analysis set (FAS) will consist of all subjects who have been randomized in the study. Subjects will be classified according to their assigned treatment arm.

Safety Analysis Set
The safety analysis set (SAS) is defined as the set of all subjects who have received at least one dose of S95014 in the study. Subjects will be classified according to treatment received. The safety endpoints will be analysed in the safety analysis set, unless otherwise specified.

Pharmacokinetic Analysis Set
The pharmacokinetic analysis set (PKAS) will include all subjects who have received at least one dose of IMP and are evaluable for PK analysis. The PK dataset will include only the subjects who had enough samples collected to provide interpretable PK results with no deviations that might have affected the PK interpretation (e.g. infusion interrupted for any reason, deviation in the theoretical administered dose > 10%, at least one missing PK sample during the 48 first hours, ≥ 2 missing PK samples after the 48-hour time point). The ADA-positive subjects will be a priori included in the PKAS since no impact was identified based on the population PK model. Nevertheless, if any evidence of such an impact can be identified in the study, a second PKAS will be defined that will include the subjects for whom none of the ADA assessment time points is positive.

Immunogenicity Analysis Set
The immunogenicity analysis set (IAS) will include all subjects who have received at least one dose of IMP and have at least one post-dose sample evaluable for immunogenicity testing.

2.2. Treatment groups

S95014 liquid (reference drug) and S95014 lyophilizate (test drug) are both IMPs.

- Arm 1: lyophilized S95014 reconstituted will provide 5 mL of extractable volume with the concentration of 750 U/mL. The vial of lyophilized powder (3.750 U/vial) is reconstituted with 5.2 mL of SWFI to obtain a 750 U/mL solution for single use.
- Arm 2: liquid S95014 is provided as 3.750 U per 5 mL solution in a single use vial to obtain a 750 U/mL solution for single use.

S95014 (either liquid or lyophilized) will be intravenously administered over 1 hour at the dose of 2500 U/m^2 at Day 3 of the induction phase. Subjects will receive other backbone chemotherapy agents as per ALL-MB 2015 protocol.

3. STATISTICAL METHODS

3.1. General considerations

All data processing, summarization, and analyses will utilize SAS® software package, Version 9.3, unless otherwise specified.

3.1.1. Descriptive statistics

For **qualitative data**, number of observed values, number and percentage of subjects per class will be presented. Unless otherwise specified in the TLG, no class "Missing" is considered.

For **quantitative data**, number of observed values, mean, standard deviation, median, first and third quartiles, minimum and maximum will be presented. For lognormal-distributed PK parameters, coefficient of variation (CoV), geometric mean and geometric CoV will also be presented.

3.1.2. General definitions

Unless specified otherwise in section 3.3 to 3.5, the following definitions will be considered:

- Analysable value will be defined as any non-missing value.
- Baseline value will be defined as the last analysable value prior to the first IMP intake (i.e. before or the same date as the first IMP intake date).
- Post-baseline value will be defined as any value recorded at a given timepoint after baseline.
- Change from baseline will be defined as the arithmetic difference between a post-baseline value and the baseline value for a given variable at a given time point.

3.2. Handling of Missing, Unused, and Spurious Data

No specific imputation method will be used for missing data, unless otherwise specified.

Adverse events (AEs)

- Handling of unknown causality assessment:
  - If a subject experience an AE with a missing causality assessment, the relationship of the AE will be counted as “related”.
- Handling of unknown severity grades:
  - If a subject experiences more than one AE categorized under the same preferred term, one of them is categorized as “severe” and one of them is categorized as “unknown”, the severity of this AE should be counted as “severe”.
  - If a subject experiences more than one AE categorized under the same preferred term, one of them is categorized as “mild” or “moderate” and one of them is categorized as “unknown”, the severity of this AE should be counted as “unknown”. A column “UNK” should be inserted for those AEs at the end of the table (before the “Total” column if applicable).

Immunogenicity

Missing baseline anti-drug antibody results will be imputed as negative.

Missing dates

If no specific management of dates is defined, missing information is substituted as shown below:

Table (3.1.2) 1 - Substitution rules of dates if no specific management is defined

Date to substitute		Substituted date
Date	../mmm/yyyy	01/mmm/yyyy
	../…/yyyy	01/JAN/yyyy
	../.../….	No substitution

Note:
../mmm/yyyy = missing day
../.../ yyyy = missing day and month
../.../.... = missing date

3.3. Disposition and baseline characteristics

Disposition of subjects and baseline characteristics will be described by treatment group, to assess their comparability, and overall.

3.3.1. Disposition of subjects

Subject disposition will be tabulated for all subjects by summarizing the number and percentage of subjects who are included in each analysis set, randomized, completed study treatment, discontinued from the study and by the primary reason for discontinuation.

A listing will present dates of study discontinuation and the primary reason, if applicable for each subject.

3.3.2. Protocol deviations

Important protocol deviations will be summarized for the full analysis set by treatment group, using the number and percentage of subjects who had each type of deviation for the SAS. COVID-19 related protocol deviation will be also summarized. Protocol deviations will be reviewed by the sponsor prior to the database lock. In addition, these protocol deviations will be listed:

- Inclusion/Exclusion criteria not respected
- Study Withdrawal criteria not respected
- Study Treatment: incorrect dose or wrong treatment
- Primary Endpoint interpretation may be compromised
- Safety/Rights affected

3.3.3. Demographic data and other baseline characteristics

Demographic and baseline measurements will be summarized for the safety analysis set and the pharmacokinetic analysis set, using standard descriptive summaries or categorical summaries, as appropriate. In addition to the summary tables, a listing will be provided for all demographic and baseline characteristics data. In addition, listing of medical history will be provided.

The following baseline characteristics will be summarized based on descriptive statistics:

- Age at screening (years; if not available, then it will be calculated as the biggest integer <= [(ICF date – date of birth + 1) / 365.25])

  - As continuous variable
  - By categories (age <10, 10 - <16, and >= 16 years)

- Sex
- Race
- Ethnicity
- Height (cm)
- Weight (kg)
- Body Mass Index (BMI (kg/m^2) = weight (kg) / height $(m)^2$)
- Body Surface Area (BSA(m^2) = sqrt (height (cm) x weight (kg) / 3600))

Baseline disease characteristics will be separately tabulated in a similar fashion for the following variables:

- ALL subtype and therapeutic group (B-Cell type Acute Leukemia (therapeutic groups: A/B/C/D1/D2/F/1221-SR/1221-IR)/E / T-Cell type Acute Leukemia (therapeutic groups: F/T-SRG/T-ImRG/T-HRG)

3.4. Treatments of subjects

3.4.1. Extent of exposure

Each patient will receive either lyophilized (arm 1) or liquid (arm 2) S95014, per the randomization at D000, intravenously over 1-hour at the dose of 2500 U/m² at Day 003 during the induction phase. Exposure to test drug for each subject will be listed. The following metrics will be summarized:

- Total exposure (U)
- Relative dose intensity (RDI): total exposure (U) / (2500 U/m^2 x BSA)

The safety analysis set will be used for exposure analysis. Listings of all dose change/interruption and reasons will be produced.

3.4.2. Concomitant treatments

Concomitant medication for each subject in the safety analysis set will be listed.

3.5. Pharmacokinetics analysis

The pharmacokinetic analysis will be performed on the Pharmacokinetic Analysis Set (PKAS). The PKAS is defined as all included participants having completed the treatment period without deviation affecting pharmacokinetic interpretation.

Final analysis will be performed after the database lock and the electronic transfer of PK data (i.e. PAA). The dataset needed for final analysis will be prepared by extraction under the supervision of Clinical Data Management Department from the I.R.I.S database.

A non-compartmental pharmacokinetic analysis will be performed on the individual PAA-time profiles, using the actual administration and sampling times after IMP administration. Calculated PK parameters will be described according to a separate Data Analysis Plan (DAP) such as: area under the PAA-time curve from time 0 to the time of the last observed non-zero PAA ($AUC_{0\text{-}Tlast}$); area under the PAA-time curve from time 0 extrapolated to infinity (AUC_{inf}); percent of AUC_{inf} extrapolated ($AUC_{\%ext}$) ; maximum observed PAA (C_{max}); time to maximum PAA (T_{max}); observed PAA 14 days post-dose($C_{day\ 14}$); last observed PAA (C_{last}); time to last observed PAA (T_{last}); terminal half-life ($T_{1/2}$). The above PK parameters will be calculated for each patient (details of the PK parameter derivation will be specified in a separate document) and the corresponding descriptive statistics of PK parameters will be tabulated by treatment group (e.g. number of subjects, arithmetic mean, SD, geometric mean, geometric CoV, median, min, max).

Descriptive statistics of PAA (e.g. number of subjects, arithmetic mean, geometric mean, % coefficient of variation [%CV], SD, minimum, median, and maximum) will be also calculated and tabulated per nominal sampling time by treatment group. Plasma asparaginase activity over time will be plotted in semilogarithmic and linear formats as mean ± SD and as median (Q1, Q3), using the nominal timepoints.

Bioequivalence tests will be used to compare the primary endpoints (AUC and C_{max}) in lyophilized formulation versus liquid formulation using the PKAS. Individual values of $C_{max,}$ $AUC_{0\text{-}Tlast}$ and AUC_{inf} will be logarithmically transformed prior to statistical analysis. An additional comparison will also be performed on $C_{day\ 14}$ for information purpose. To be specific, an analysis of variance (ANOVA) will be performed for the natural logarithms of PK parameters ($AUC_{0\text{-}Tlast}$, AUC_{inf}, C_{max} and C_{day14}). The ANOVA model will include fixed effect for formulation. The two one-sided tests procedure will be performed on the geometric mean ratio (GMR) between test (lyophilized pegaspargase) and reference (liquid pegaspargase) treatments. This will be done via a 90% confidence interval for the ratio obtained in the framework of the ANOVA for the logarithms. The confidence interval will be obtained by exponentiation of the upper and lower 90% confidence limits for the difference of logarithm means. PK comparability between the test treatment and the reference treatment will be concluded if the 90% confidence interval for AUC and C_{max} GMR is within the [80.00%; 125.00%] range.

Exploratory analysis may be performed to evaluate potential covariates impacting the PK parameters.

3.6. Safety analysis

All subjects included in the safety analyses will be evaluated by the actual treatment arms unless otherwise specified.

3.6.1. Adverse Events

A treatment-emergent adverse event (TEAE) is defined as any untoward medical occurrence in a subject who received IMP, which occurs within the period from the S95014 Infusion (i.e. Day 3), until the withdrawal/end-of-study visit, which is at least 30 days after S95014 infusion (i.e. Day

33) and before starting the consolidation phase. TEAE will be assessed according to the National Cancer Institute (NCI) Common Terminology Criteria Adverse Events (CTCAE) v5.0. Laboratory abnormalities will be classified into Common Terminology Criteria (CTC) grade according to CTCAE v5.0 and will be reported as adverse events.

Adverse events will be summarized in hierarchical tables, presenting the number and percentage of subjects having at least one AE, and having at least one AE in each primary system organ class and for each preferred term using MedDRA coding. AEs will be sorted by descending frequency, and alphabetically where frequency is tied. Such summaries will be produced for all AEs, serious adverse events (SAEs), grades 3 or 4 AEs, AEs leading to study discontinuation, and AEs leading to dose-adjustment or interruption of any of the drugs of the treatment. All of these summaries will be by system organ class, preferred term, treatment group and, in some case, maximum grade. Most of the summaries will be produced twice, once for all events regardless of study treatment relationship, and once for events suspected to be study treatment related. All deaths will be listed, and separate summaries will be produced by treatment.

All adverse events will be listed, and any other information collected (e.g., start/end dates and duration of adverse event, severity or relatedness to study medication or backbone therapies) will be listed as appropriate.

Adverse events of special interest (AEOSI) will be summarized separately. AEOSI include:

- Grade ≥ 3 ALT/AST increase or grade ≥ 3 hyperbilirubinemia
- Grade ≥ 3 haemorrhage or grade ≥ 3 thromboembolic events
- Grade ≥ 3 pancreatitis
- Grade ≥ 3 hypersensitivity

All such events will be identified prior to database lock.

3.6.2. Other Safety Evaluations

Descriptive statistics of laboratory parameters and continuous variables of vital signs will be calculated by treatment group for each time point. Shift tables for qualitative urinalysis results at each time point after the start of administration will be created. Height, weight, body surface area (BSA) and body mass index (BMI) will be summarized over visit by treatment group.

Shift tables will be provided for laboratory parameters to compare a subject's baseline laboratory evaluation relative to the worst value during the treatment period using Common Terminology Criteria for Adverse Events (CTCAE) v5.0 grades. In addition, the normal laboratory ranges will be used to evaluate whether a particular laboratory test value was normal, low, or high for each visit value relative to whether or not the baseline value was normal, low, or high. Subjects with abnormal laboratory values will be listed and values outside the normal ranges will be flagged.

3.7. Analysis of Plasma Asparaginase Activity

Observed individual Plasma Asparaginase Activity (PAA) level 7 days (Day 10), 14 days (Day 17), 18 days (Day 21) and 25 days (Day 28) after administration of either liquid or lyophilized S95014 will be tabulated by treatment group together with descriptive statistics (Arithmetic mean, SD, geometric mean, geometric CV, median, min, max).

The number and proportion of subjects achieving a PAA of ≥ 0.1 U/mL after the administration of either liquid or lyophilized S95014 among subjects with evaluable Day 10, Day 17, Day 21 and Day 28 PAA data will be summarized by treatment group along with a 2-sided 95% Clopper-Pearson confidence interval (CI).

3.8. Immunogenicity Analysis

The immunogenicity analysis will be performed on the Immunogenicity Analysis Set (IAS). The assessment of anti-drug antibodies includes binding and neutralizing antibodies to S95014 and binding anti-PEG antibodies.

The number and proportion of subjects in the immunogenicity analysis set having anti S95014 ± anti-PEG antibodies at pre-dose, and 14 and 25 days (i.e. Day 17 and Day 28) after the administration of either liquid or lyophilized S95014 during the induction phase will be summarized by treatment group.

Results will be reported as follows:

- Pre-existing anti-S95014 or anti-PEG antibodies: number and proportion of samples confirmed positive at baseline.
- Seroconversion upon treatment: number and proportion of samples converted from negative at baseline to positive at Day 17 and Day 28. If a confirmed positive sample at baseline has titer increase at Day 17 or Day 28 by at least 4-fold (boosted ADA responses) then it will be included within the seroconverters. If a patient was negative at baseline, and ended up with a positive titer of "2" for example, post-treatment, this case was considered as a seroconversion despite the fact that the titer was not a 4 fold rise.

On a case-by-case approach, the impact of positive anti S95014 and anti-PEG antibodies will be investigated:

- On the pharmacokinetics through the temporal association of antibodies with the loss of asparaginase activity (neutralizing antibodies)
- On the safety through the temporal relationship with treatment-emergent hypersensitivity and anaphylactic reactions.

A patient with missing baseline will be considered as negative baseline to be conservative.

4. INTERIM ANALYSIS

No interim analysis is planned for this study.

5. APPENDICES

6. CHANGES FROM THE PLANNED STATISTICAL ANALYSIS

Not applicable.

7. REFERENCES

Angiolillo AL, Schore RJ, Devidas M, Borowitz MJ, Carroll AJ, G astier-Foster JM, et al. Pharmacokinetic and pharmacodynamic properties of calaspargase pegol Escherichia coli L-asparaginase in the treatment of patients with acute lymphoblastic leukemia: Results from Children´s Oncology Group Study AALL07P4. J Clin Oncol 2014; 32: 3874-82 [PE0150550]