Statistical analysis plan

A prospective, observational long-term follow-up trial of kidney transplant patients treated with imlifidase or plasma exchange after an active/chronic active Antibody-Mediated Rejection episode

Sponsor:	**Hansa Biopharma AB**
Contract research organisation:	[redacted]
Sponsor protocol number:	**20-HMedIdeS-18**
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Signature page

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List of abbreviations and definition of terms

ADA	Antidrug Antibody
ADaM	Analysis data model
AE	Adverse event
AMR	Antibody Mediated Rejection
AUC	Area under the concentration versus time curve
BMI	Body mass index
BP	Blood pressure
CI	Confidence interval
C_{max}	Maximum observed concentration
C_t	Observed plasma concentration for a given time point.
CRF	Case report form
CV	Coefficient of variation
DSA	Donor Specific Antibody
ECG	Electrocardiogram
eGFR	estimated Glomerular Filtration Rate
FAS	Full analysis set
HLA	Human Leukocyte Antigen
HR	Heart rate
IgG	Immunoglobulin G
IMP	Investigational medicinal product
ITT	Intention-to-treat
MedDRA	Medical Dictionary for Regulatory Activities
PK	Pharmacokinetics
PP	Per-protocol
PT	Preferred term
Randomised	Patient randomised to study treatment
SAE	Serious adverse event
SAP	Statistical analysis plan
Screened	Patient who enters the screening phase of the study
SD	Standard deviation
SOC	System organ class
Sqrt	Square root
STDM	Study data tabulation model
TLF	Tables, listings and figures
T_{max}	Time of observed C_{max}
$T_{½\ el}$	Elimination half-life

1 Introduction

This document describes the planned statistical analyses for 20-HMedIdeS-18 which will include data processing, data analyses and data presentation. This Statistical Analysis Plan (SAP) is based on protocol version 2.1_AUT_22Nov2021 (for Austria), protocol version 1.1 (for France) and protocol version 3.0_DEU (for Germany) (Doc. No.: 2020-059).

1.1 Study objectives and endpoints

1.1.1 Objectives

As started in the protocol the primary objective of this trial is:

- To evaluate kidney graft survival in patients that have been treated with imlifidase or plasma exchange in association with Antibody Mediated Rejection (AMR) in the trial 16-HMedIdeS-12 (referred to as the feeder trial).

As stated in the protocol the secondary objectives of this trial are to:

- Evaluate overall graft survival at Year 1 and 2
- Evaluate patient survival at Year 3
- Evaluate kidney function at Year 1, 2 and 3
- Evaluate Donor Specific Antibody (DSA) levels at Year 1, 2 and 3
- Evaluate anti-imlifidase Immunoglobulin G (IgG) levels at Year 1, 2 and 3
- Investigate the occurrence of AMR episodes after completion of the feeder trial (classified according to Banff 2017 or later version
- Investigate the occurrence of other graft rejection episodes (classified according to Banff 2017 or later version)

1.1.2 Endpoints

The primary endpoint of this trial is:

- To evaluate the overall graft survival at Year 3.

Secondary endpoints of this trial are:

- Overall graft survival evaluated at Year 1 and 2.
- Patient survival, evaluated at 3 years after start of treatment in feeder trial.
- Kidney function, as evaluated by estimated Glomerular Filtration Rate (eGFR), S/P-creatinine and albumin/creatinine ratio in urine at 1, 2, and 3 years after start of treatment in feeder trial.
- Proportions of patients with presumed or biopsy proven AMR episodes (classified according to Banff 2017 or later version) within 3 years after start of treatment in feeder trial.
- Proportion of patients with presumed or biopsy-proven rejection episodes, (other than AMR episodes) (classified according to Banff 2017 or later version) up to 3 years after start of treatment in feeder trial.

- DSA levels at 1, 2, and 3 years after start of treatment in feeder trial.
- Anti-imlifidase IgG at 1, 2, and 3 years after start of treatment in feeder trial.

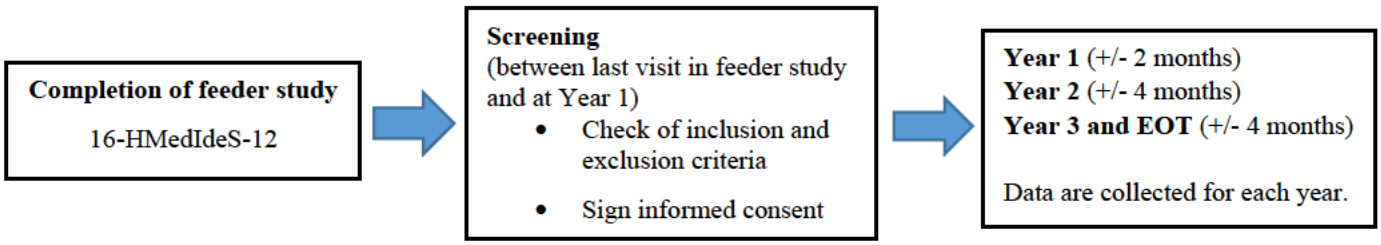
2 Study design

The study is an observational 3-year follow up trial after the 16-HMedIdeS-12 study. The purpose of this trial is to investigate the long-term outcomes of imlifidase or plasma exchange treatment on active or chronic active AMR in kidney transplant recipients. The long-term outcomes are evaluated by considering the overall graft survival, patient survival, kidney function, DSA levels, AMR episodes, and anti-imlifidase IgG.

A maximum of 30 patients can be included depending on patients' willingness to participate in the trial after the 16-HMedIdeS-12 study.

The flow of the study is displayed in Figure 1 below.

Figure 1.



2.1 Overview of study procedures

Screening
Screening may be conducted at the earliest at the last visit in the feeder trial and at the latest at Year 1 (one year after start of treatment in the feeder trial +/- the visit window of 2 months).

These assessments and activities are performed:

- Check inclusion and exclusion criteria.
- Sign informed consent.
- Assessment of graft survival. For patients with graft loss after last visit in the feeder trial, date, reason for graft loss, medical history, and root cause will be recorded.
- Record patient survival (if applicable, date and cause of death).
- Pregnancy test (only for Austrian patients due to an exclusion criterion in the protocol version 2.1_AUT_22Nov202 regarding “Lactating or pregnant females”).

At Year 1, Year 2, and Year 3 (End-of-Trial)

The following visit windows applies:
Year 1: (+/- 2 months)
Year 2 and Year 3 (End-of-Trial): (+/- 4 months)

The following activities and assessments will be performed:

- Assessment of graft survival. For patients with graft loss since last visit in the feeder trial or screening (if screening is done at a separate visit), date, reason for graft loss, medical history, and root cause will be recorded.
- Record patient survival (if applicable, date and cause of death).
- Collect information about graft rejection episodes, (classified by Banff 2017 or later).

  For-cause biopsies together with contemporaneous local DSA analyses, kidney function parameters (P-creatinine, U-albumin/creatinine ratio) and treatments (e.g. plasma exchange and IVIg) will be collected to assess the rejection episodes.
- Record information from standard of care biopsies, if available.
- Sample for S/P-creatinine will be drawn (eGFR will be calculated by the Sponsor)
- Collect urine sample for measurement of albumin/creatinine ratio.
- Sample for HLA antibodies, including DSA, and ADA will be drawn.
- Record comorbidity such as infections, malignancy, diabetes mellitus, cardiovascular events and comorbidity leading to worsening of kidney function, that occurred after end of trial in feeder trial.
- Record current immunosuppressive medications.
- Record treatment with dialysis.
- AEs related to trial procedure or IMP in feeder trial will be recorded.

The study flow chart is displayed in Table 1.

Table1. Flow chart.

Visit (post treatment in feeder trial)	**Screening**	**Year 1**	**Year 2**	**Year 3 (EOT)**
Visit window	**EOT feeder trial to year 1**	**+/- 2 months**	**+/- 4 months**	**+/- 4 months**
Assessment				
Informed consent	x[1]			
Inclusion and exclusion criteria	x[1]			
Enrolment and allocation of patient no	x[1]			
Pregnancy test	x[6]			
Graft survival status	x	x	x	x
Patient survival status	x	x	x	x
Graft rejection episodes[2]		x	x	x
Kidney graft biopsy reports[3]		x	x	x
S/P-creatinine, eGFR calculation (performed by Sponsor)		x	x	x
U-Albumin/creatinine ratio		x	x	x
Sample for HLA antibodies, including DSA (donor-specific antibodies) and ADA (Anti-imlifidase IgG) *central lab*		x	x	x
Comorbidity[4]		x	x	x
Current immunosuppressive medication		x	x	x
Treatment with dialysis		x	x	x
Adverse events related to trial procedure or IMP in feeder trial[5]		x	x	x

Footnotes

1) Signing of Informed consent and check of inclusion and exclusion criteria can be done any time between end of trial in feeder trial and at year 1 or at year 1.
2) Record graft rejection episodes (classified by the Banff 2017 or later version), creatinine and DSAs taken in standard of care due to the episode and treatments (e.g. plasma exchange and IVIg).
3) If standard of care kidney biopsies are collected for any reason, at any time-point, e.g. suspected graft rejections, record information in the eCRF.
4) Record comorbidity that are medically relevant and registered in the patient`s medical record. Medically relevant comorbidity are infections, malignancy, diabetes mellitus, cardiovascular events and comorbidity leading to worsening of kidney function.
5) Only Adverse events caused by a procedure in the protocol (e.g. blood sampling) or related to IMP in feeder trial will be recorded.
6) Only for Austrian patients.

2.2 Determination of sample size

No calculation for the sample size is made. The 30 patients included in the feeder trial (16-HMedIdeS-12) are considered and asked if they want to participate in this trial. Therefore, the sample size will be 30 as maximum.

2.3 Blinding

The study is an open-label study. The study is fully unblinded.

2.4 Data pre-processing

The export data sets from data management will be transformed into Study Data Tabulation Model (SDTM) structure and format following the implementation guide version 3.2 (current version) and also following "Therapeutic Area Data Standards User Guide for Kidney Transplant Version 1.0 (Provisional)". Analysis Data Model (ADaM) data sets will be prepared based on the SDTM data sets following implementation guide version 1.1 (current version). Tables, figures, and listings will be prepared from the ADaM data sets.

3 Analysis sets

3.1 Full analysis set

The Full Analysis Set will be defined as all patients enrolled in 20-HMedIdeS-18. Because of the observational nature of this trial no other analysis set will be defined, and all data presentations and all outputs will be based on the FAS.

4 Statistical analyses and presentation of data

4.1 General considerations

All results are based on the FAS.

4.1.1 Data presentation

No formal hypothesis test will be applied in the study. The data will be presented descriptive where 95 % confidence intervals will be presented.

Numerical data will be presented in summary tables by number of patients, arithmetic mean, median, standard deviation (SD), minimum and maximum. Categorical data will be presented by number and percent of patients as well as number events (where applicable).

Summary tables will in general be presented by treatment and total unless this is not appropriate for specific tables.

All data will be listed by patient.

4.1.2 Baseline definition

Baseline value is defined as the baseline value in the feeder trial. Change from baseline will be calculated for patients with relevant endpoints, and for these the baseline value is defined as the baseline value in the feeder trial. Change will be calculated as the difference between post values for a given timepoint and baseline values (post value to a timepoint minus baseline value). If the post value or baseline value are missing, then the change from baseline will also be missing.

4.1.3 Multiplicity

The study is descriptive, so no correction for multiple testing will be done.

4.1.4 Data imputation

In general, missing data will remain as missing and the missing values will not be adjusted. There are some expectations described below.

Adverse events

Missing values will be treated as missing except for causality, toxicity, intensity, seriousness and outcome of an AE, in which case a “worst case” approach will be taken. If causality is missing, it will be set to “related”. If intensity is missing, it will be set to “severe”. If seriousness is missing, it will be considered as a Serious Adverse Event (SAE). If outcome is missing and no date is given for the outcome it will set to “not yet recovered”.

Dates for adverse event and concomitant medical

Missing or partial start dates for adverse events and concomitant medication data will be handled in the following way:

- If the start date is totally missing, the date will still be missing.

- If the start day is partial where only the year are given, then the two cases are considered:
    - If the available partial date (year) is equal to or after the year of treatment start date, then the start date will be imputed as start of the year (1st January YYYY) (if the year is not same as that of the treatment start date) or treatment start date (if the year is same as the treatment start date).
    - For the partial end dates will similar procedure be used as for the start dates. The only difference is the imputation for missing days. Missing days is imputed to the end of the month instead of the start of the month. It will be checked that the imputed end dates fall after the corresponding start dates.

4.2 Patient disposition

All patients screened will be accounted for. The number of patients in the analysis set and the number of patients who completed the trial will be summarized by treatment group. Furthermore, the number of patients who withdraw from the trial will be summarised overall and by treatment group and reason for withdrawal. Patients screened but not found eligible will be listed with reason(s) for screening failure.

4.3 Demographics and other baseline characteristics

4.3.1 Patient Characteristics

Data from the clinical assessments will be summarised or listed by treatment group and each time point using descriptive techniques. Summary statistics (n, arithmetic mean, standard deviation, median, minimum and maximum values) will be presented for continuous variables (absolute values at each time point and, if relevant, changes from baseline) and counts and, if relevant, percentages will be presented for categorical variables. Where appropriate, the presentation of results will include confidence intervals of estimated treatment differences and plots.

4.3.2 Demographics and Other Baseline Characteristics

The following demographic variables will be summarised by treatment group: Age, sex, race, height, weight and Body Mass Index (BMI). The demographics variables will additionally be listed by patient.

Previous kidney transplant history will be presented in a summary table by treatment group and listed by patient. The donor type (living or deceasing) will be listed by patient.

4.3.3 Recent and Concomitant Medication

Recent and concomitant immunosuppression medication will be summarised by anatomical therapeutic chemical (ATC) code and generic drug name for each treatment group.

4.4 Efficacy

Data is for the patients included in 20-HMedIdeS-18 and will be presented by treatment group.

4.4.1 Primary efficacy analysis

Overall graft survival at Year 3. Graft survival is defined as time from start of treatment (imlifidase or plasma exchange) of active or chronic active AMR in feeder trial to graft loss. Graft loss is defined as permanent return to dialysis for at least 6 weeks, re-transplantation, or nephrectomy. If dialysis is used to define graft loss, the date of graft loss will be the first day of the last ongoing dialysis period reported.
The primary endpoint, overall graft survival at Year 3, will be analysed by the Kaplan-Meier survival method. The overall graft survival will be tabulated and presented graphically by treatment.
The following events will be censored at the time of occurrence: withdrawal from the trial without graft loss, evaluation time point and end of trial without graft loss.
The reason for graft loss will be tabulated by time.

4.4.2 Secondary efficacy analyses

4.4.2.1 Graft survival at Year 1 and 2

Graft survival at Year 1 and Year 2 will be listed by patient and summarized by treatment and time point and analysed using the same methods as for the primary efficacy analysis .

4.4.2.2 Patient survival

Patient survival, defined as time from start of treatment in feeder trial to death, evaluated at Year 3 will be listed by patient, summarized by treatment, and analysed by the Kaplan-Meier survival method. The patient survival will be tabulated and presented graphically by treatment.

4.4.2.3 Kidney function

Kidney function is evaluated by estimated Glomerular Filtration Rate (eGFR), S/P-creatinine and albumin/creatinine ratio in urine at 1, 2, and 3 years after start of treatment in feeder trial.

The eGFR will be calculated as a part of ADaM programming, using the Modification of Diet in Renal Disease Study (MDRD) equation (2):

$$\text{eGFR (mL/min/1.73 m}^2) = 175 \times (\text{Cr})^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female}) \times (1.212 \text{ if African American}),$$

where Cr stands for creatinine value and Age are measured in years at the visit date. This formula assumes that Cr is given in the mg/dL unit.

Variables will be listed by patient and summarized by treatment at Year 1, 2, and 3 after start of treatment in feeder trial.

4.4.2.4 Proportion of patients with rejection episodes

Proportion of patients with presumed or biopsy-proven AMR episodes (classified according to Banff 2017 or later) and proportion of patients with biopsy-proven rejection episodes, (other than AMR episodes) within 3 years after start of treatment in feeder trial will be listed and summarised by treatment group.

4.4.2.5 DSA level at 1, 2, and 3 years after start of treatment in feeder trial

DSA levels (measured using SAB-HLA assay) at 1, 2 and 3 years after start of treatment in the feeder trial will be summarized, listed, and presented graphically. The individual plots, will include sum of DSA as a separate line. Mean plots will display the mean of "sum of DSA".

4.4.2.6 Immunogenicity

Anti-imlifidase antibody at 1, 2, and 3 years after start of treatment in the feeder trial, will be listed and summarised.

4.5 Safety

Safety parameters will be evaluated for the FAS (which in this study is the same as the safety analysis set).

4.5.1 Adverse events

Adverse events (AEs) will be classified according to the Medical Dictionary for Regulatory Activities (MedDRA version 23.1).

4.5.1.1 Adverse Event

An AE is the development of an undesirable medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product or trial procedure. No IMP will be administered in this trial and only AEs that the investigator judges as related to IMP administered in the feeder trial, and adverse events caused by a procedure in the protocol (such as blood sampling) will be captured and reported in this trial.

An AE overview summary table will be prepared including the number of patients reporting an AE, the percentage of patients (%) with an AE, and the number of events (E) reported for the following categories:

- All AEs
- AEs – by System Organ Class (SOC) and Preferred Term (PT)
- AEs – by causality

- AEs – by severity
- Serious AEs (SAEs)
- Serious AEs (SAEs) – by causality
- Serious AEs (SAEs) – leading to death
- Serious AEs (SAEs) – leading to withdrawal

Data listings will be provided for:

- All AEs sorted by patient number
- All AEs sorted by MedDRA SOC and PT
- SAEs

5 Interim analyses

No interim analyses were performed.

6 Deviations from protocol analysis

The study was terminated prematurely in January 2023 due to an internal decision that will allow Hansa to focus on prioritised projects and allocate resources in the way the company deems most effective according to its strategic priorities.

7 Quality control

The quality control of the data will be performed as follows:

- By Data Management for all CRF data following the data validation plan
- By Statistics and Programming for all data received

The QC depends on the process below, but it will follow [redacted] SOP 703 Single Use Programs:

SDTM data sets will be generated by programs in the Statistical analysis system (SAS) by reading in lab data and export data to SDTM formats following the SDTM specifications. The QC will include:

- Independent Code review
- CRF annotation review vs SDTM content
- Run of Pinnacle verification
- QC of CRF input vs output in SDTM on a sample of data
- Format and fonts in annotated CRF

ADaM data sets will be based on the SDTM data sets. The programming of the ADaM datasets will be performed in SAS and QC of the programs will include

- Independent code review
- Run of Pinnacle verification
- QC of SDTM input data vs output in ADaM or listings

Tables, listings and figures (TLF) will be created by SAS programs and based on the ADaM data sets. The programming of TLFs will be reviewed by an independent person.

Since there are no formal statistical analyses, double programming will not be applied in this study.

The programming and execution of programs for producing data sets and tables, figures and listings follow the [redacted] SOPs 701, 702 and 703 on standard programs, programming environment and single use programs respectively.

SAS Viya version 03.05 will be used for data handling and presentation.

8 Layout of output

The output will follow [redacted] standard output templates. Tables and listings will first be prepared as individual RTF (Rich text format, which is a standard text format) files. This file type may be opened and saved in the currently available Microsoft Word versions. Graphical output will be prepared in PNG (Portable network graphics) format, which is the standard graphical format in current Microsoft software packages and therefore easily importable into Microsoft Word documents.

Tables and figures will per default be created in portrait format while listings will be in landscape format. Margins and font will be chosen to respect the requirements for filing with EMA (European medicines agency) and FDA (the US Food and drug administration).

The output items (tables, listings, figures) will be collected in Word documents. Separate Word documents will be created for tables, figures, and listings, respectively.

9 Tables, listings and figures

The following sections contains lists of the tables, figures and listings (TLF) which will be produced.

9.1 Tables

Table 14.1.1 Patient disposition, including reason for discontinuation (All screened patients).
Table 14.1.2 Protocol Deviations (FAS).
Table 14.1.3 Demography and body measurements (FAS).
Table 14.1.4 Medical and surgical history (FAS).
Table 14.1.5 Concomitant medication (FAS).
Table 14.1.6 Previous transplant kidney history (FAS).
Table 14.2.1 MFI levels for DSA – by time point (FAS).
Table 14.2.2 Kidney function (based on S/P-creatinine analysis) (FAS).
Table 14.2.3 Proportion of patients with absence of graft loss at 1 year, 2 years and 3 years (FAS)
Table 14.2.4 Proportions of patients with presumed or biopsy proven AMR episodes (FAS).
Table 14.2.5 Proportion of patients with presumed or biopsy-proven rejection episodes, (other than AMR episodes) (FAS).
Table 14.2.6 Total IgG – by timepoint (FAS).
Table 14.2.7 Proportion of patients having reduction in total IgG (FAS).
Table 14.2.8. Patient survival (FAS)
Table 14.3.1 Overview of AEs (FAS).
Table 14.3.2 Summary of AEs – by SOC and PT (FAS).
Table 14.3.3 Summary of AEs – by causality (FAS).
Table 14.3.4 Summary of AEs – by severity (FAS).
Table 14.3.6 Summary of SAEs (FAS).
Table 14.3.7 Summary of SAEs – by causality (FAS).
Table 14.3.10 Summary of SAEs leading to death (FAS).
Table 14.3.11 Summary of SAEs leading to withdrawal (FAS).
Table 14.4.1 Summary of clinical chemistry variables (U-Albumin, Creatinine, U-Albumin/creatinine ratio) – by time point (FAS).
Table 14.4.2 Anti imlifidase IgG (ADA) (mg/mL) – by time point (FAS)

9.2 Listings

Listing 16.1.1 Patient disposition, including reason for discontinuation (All screened patients).
Listing 16.1.2 Protocol Deviations (FAS).
Listing 16.1.3 Demography and body measurements (FAS).
Listing 16.1.4 Medical and surgical history (FAS).
Listing 16.1.5 Concomitant medication (FAS).
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Listing 16.2.2 Kidney function (based on S/P-creatinine analysis) (FAS).

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Listing 16.3.1 AEs (FAS).
Listing 16.3.2 AEs – by SOC and PT (FAS).
Listing 16.3.3 Serious adverse event SAEs (FAS).
Listing 16.4.1 Clinical chemistry variables (U-Albumin, Creatinine, U-Albumin/creatinine ratio) – by time point (FAS).
Listing 16.4.2 Anti imlifidase IgG (ADA) (mg/mL) – by time point (FAS)

9.3 Figures

Figure 14.2.1 Individual plots DSA (including sum of DSA) vs time (FAS).
Figure 14.2.2 Mean plots of sum of DSA vs time (FAS).
Figure 14.2.3 Individual Anti-imlifidase IgG (ADA) vs time (FAS).
Figure 14.2.4 Mean Anti-imlifidase IgG (ADA) vs time (FAS).
Figure 14.2.5 Mean Anti-imlifidase IgG (ADA) box plot

10 Change log

Version	Effective date	Reason for revision
1.0	12-May-2023	New document