Supplementary Online Content


Supplement 2. Statistical analysis plan
A randomised, controlled clinical trial assessing the efficacy of Lanreotide to halt disease progression in ADPKD

The DIPAK 1 Study

STATISTICAL ANALYSIS PLAN
Part 1 out of 2: regarding renal endpoints

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1. **STUDY OBJECTIVES**

We refer to the study protocol version 4.9 (April 2017) for a description of the study objectives. According to this protocol, the objectives of the study are: 1) to evaluate whether Lanreotide attenuates progression of the renal phenotype in Autosomal dominant polycystic kidney disease (ADPKD) patients as measured by change in rate of renal function decline and change in renal volume growth, and 2) to evaluate whether Lanreotide modifies progression of the liver phenotype in the subset of ADPKD patients with moderate to severe polycystic liver disease.

The analyses for the two objectives will be performed separately, using different data-sets, statistical methods and outcome tables and figures, which will result in two different outcome papers. Therefore, it was decided to write two separate Statistical Analyses Plans: part 1 regarding renal endpoints and part 2 regarding liver endpoints.

1.1 **Endpoints**

1.1.1 General considerations

Treatment used in this study (Lanreotide) is known to induce a small, acute and reversible hemodynamic effect on renal function, that is not indicative for structural benefit that may be achieved by drug treatment. Therefore the primary endpoint of change in renal function will not include this acute hemodynamic effect and will be calculated from 3 months after start of treatment until the end of the treatment phase (EQT) (i.e., "on treatment only").

1.1.2 Primary efficacy endpoint

The primary efficacy outcome variable will be the change in renal function, as assessed by the per-individual slope through serial estimated glomerular filtration rate (eGFR) measurements over time during the treatment phase of the study. The eGFR measurements starting at month 3 until the end of the treatment phase (EQT) will be used for analysis of the primary efficacy endpoint.

eGFR will be calculated using the CKD-EPI formula for serum creatinine (unit: ml/min×1.73m²). Serum creatinine values for this analyses will be measured in a central laboratory (UMCG) from samples that have been stored frozen in the central laboratory at -80 degrees Celsius. All serum creatinine measurements per subject will be performed in the central laboratory in one run in order to avoid inter-assay variation. Vena puncture will performed at the study site, and the blood collected will be directly shipped at room temperature to the central laboratory site at the UMCG. Blood samples will then be stored frozen at -80 degrees Celsius until performing the measurement. Of note: having blood samples stored at room temperature for up to 4 days does not influence creatinine concentration, nor does frozen storage at -80 degrees Celsius during prolonged periods of time.
1.1.3 Secondary efficacy endpoints

Renal:
- Change in renal function (expressed as change in eGFR in ml/min*1.73m²) will be calculated as the absolute difference and percentage difference between pre-treatment eGFR and eGFR 12 weeks after cessation of the treatment (obtained at follow-up (FU) visit). Pre-treatment eGFR will be defined as the average eGFR measurements from the screening visit (SV) and baseline visit (BV).

   In accordance to the primary outcome measure eGFR will be assessed using the CKD-EPI equation; serum-Creatinine values will be used measured in 1 run per patient in the central laboratory facility.

- Incidence of worsening renal function, as defined as a sustained (2 consecutive measurements) 30% decrease in eGFR and/or incidence of need for renal replacement therapy computed from pre-treatment eGFR.

   In accordance to the primary outcome measure eGFR will be assessed using the CKD-EPI equation; serum-Creatinine values will be used measured in 1 run per patient in the central laboratory facility.

   The need for renal replacement therapy is set at the date of first dialysis or date of renal transplantation. Time to first event (need for renal replacement therapy or 30% decrease in eGFR) will be analyzed.

- Change in height adjusted total kidney volume (hTKV) will be calculated as percentage difference between height adjusted hTKV at the baseline visit (BV) and hTKV obtained 12 weeks after cessation of the treatment (FU visit).

   The method for measurement of total kidney volume (TKV) is described in the methods section of the study protocol.

Quality of life:
Impact of lanreotide on quality of life will be scored as change on a ADPKD Impact scale. This PKO specific questionnaire is developed using 18 items (based on questionnaires used in other ADPKD trials and is included as addendum to the study protocol, concerning question 17-34 of the annual questionnaire). Change in quality of life will be determined as percentage change on the ADPKD Impact scale from Baseline visit to End of Treatment phase (EOD).
Subgroup analyses

The primary and secondary efficacy endpoint analyses will also be performed in the following a priori defined subgroups:
- age group at inclusion (≤ versus > 45 years)
- sex (males versus females)
- eGFR at inclusion (≤ > 45 ml/min*1.73m2)
- height adjusted Total Kidney Volume (≤ > 1000 ml/m)
- Mayo classification (low risk (class 1A, 18 or 2) versus high risk (class 1C-1E)) [ref 6].
- DNA classes (PKD1 truncating versus PKD1 non-truncating or PKD2)
- 24hr urinary volume at inclusion (≤ > 2500 ml)

1.1.4 Safety endpoints

(Severe) adverse events will be assessed as safety parameters. Vital signs (blood pressure, pulse), physical examinations, and clinical laboratory tests will be performed according to the study protocol. Clinically relevant findings at the time of physical examination or changes in vital signs and laboratory values out of range will be reported as Adverse Event.

The Serious adverse events and Adverse events will be categorized as Preferred Terms using the MedDra HGLT systematics (MedDRA= Medical Dictionary for Regulatory Activities). An overview will be given for Serious Adverse events and Adverse Events occurring in more than 2% of the subjects, addressed as possibly related or occurring significantly more often using the Fisher's exact test in the Lanreotide treated group compared to control group and vice versa.

The number of patients in which Lanreotide has been down-titrated due to a suspected dose related side effect will be assessed as additional safety parameter. An overview including reasons for down-titration will be given.

1.1.6 Interim analyses

The statistical analysis plan does not incorporate nor allow an interim analysis, unless demanded by the independent Data Safety Monitoring Board of the study.

1.1.6 Post-hoc endpoints

In addition to the above mentioned analyses, a number of post-hoc analyses will be performed including, but not limited to:
- Difference between pre-treatment eGFR and eGFR after 12 weeks of treatment
- Difference between end of treatment eGFR (EQT) and eGFR 12 weeks after cessation of the therapy (FU visit)
- Change in pre-treatment eGFR to eGFR after 12 weeks of treatment versus change in eGFR from end of treatment to follow-up (FU visit).
- Change in eGFR (pre-treatment towards eGFR at follow-up visit) versus change in hTKV (baseline towards hTKV obtained at follow-up visit)
- Difference in quality of life (assessed by APKD Impact scale) between baseline (BL) versus follow up (FU).
- Quality of life divided in three domains: physical (question 17-21; 31-32 of the questionnaire), fatigue (question 26; 33; 34) and emotional (question 22, 27-29). Changes in these domains form baseline to end of treatment (EQT) and follow-up (FU) visit will be analyzed.
- Blood pressure at the various time points in the study
- Weight at the various time points of the study
- 24hr Creatinine excretion at the various time points of the study
- 24hr Urinary volume at the various time points of the study
- eGFR cystatin C at the various time points of the study
- eGFR creatinine at the various time points of the study, using creatinine that has been measured routinely at the various sites
- 24hr Creatinine clearance at the various time points of the study

It is anticipated that other exploratory post-hoc analyses will be performed after the main results of the DIPAK 1 Study have been obtained. A description of these post-hoc analyses, however, is beyond the scope of the present statistical analysis plan.
2. STATISTICAL METHODS AND REPORTING

2.1 Sample size
For the sample size considerations we refer to the protocol text (section 4.3):

In a cohort of ADPKD patients participating in the MORD Study (baseline GFR between 55-25 mUmin), mean slope of GFR decline on treatment was 5.1 mUmin/yr with a standard deviation of 4.2. In the recent Everolimus ADPKD Study (baseline eGFR 90-30 mUmin/1.73m²), the mean change in eGFR was 4.2 mUmin/1.73m² with a similar standard deviation of 4.3. Annual slope of eGFR in this study is expected to be similar to the MORD Study and higher than in the Everolimus ADPKD Study, since in the present study only subjects with already impaired renal function will be included (baseline eGFR between 60-30 mUmin). Assuming an average change in eGFR of 5.1 mUmin/1.73 m²/yr in untreated patients, an average 30% reduction in rate of renal function loss deemed clinically significant, with 80% power to detect this difference and a two-sided alpha of 0.05, then approximately 123 subjects per study group are needed. Taking into account the possibility of 20% protocol violators and/or dropouts, inclusion of 150 subjects per group is aimed. About 300 subjects will therefore need to be enrolled in the trial.

2.2 Level of significance
For all analyses used for assessment of primary and secondary endpoints, a p-value of less than 0.05 (2-tailed) will indicate statistical significance. For primary and secondary outcome measures a p-value below 0.05 will be considered as confirmatory to the hypothesis.

2.3 Study analysis set

2.3.1 Efficacy analysis set

Full analysis set
The full analysis set includes all randomized subjects according to the Intention-To-Treat principle (ITT), for whom post-baseline primary efficacy data is present.

Per-protocol set
One per-protocol set will be analyzed. The Per-protocol set includes all subjects from the full analysis set, excluding:

- Subjects violating inclusion/exclusion criteria
- Subjects deviating from the protocol guidelines during the trial, in a sufficiently serious manner to warrant data (not subject) exclusion.
- Subjects who withdraw from the trial resulting in missing data, in a sufficiently serious manner to warrant subject exclusion. When on-treatment data is available for a period less than 1 year, subjects will be excluded.
- Subjects who were withdrawn by the investigators due to a change in study protocol (Amendment 2), in which 'a history of liver cyst infection' was added as exclusion criterion (and thus violate the finally established inclusion/exclusion criteria).
- Only on-treatment data, this is when treatment is in accordance to randomization outcome, is used.
**Patient classification document**

Subject eligibility for study analysis sets will be documented in the Patient Classification Document. The reasons for exclusion from analyses, as documented in the Patient Classification Document, will be tabulated.

### 2.3.2 Safety analysis set

**Safety analysis set**

The safety analysis population includes all randomized subjects. After early discontinuation of treatment in a subject, efforts will be made to collect safety data during the rest of the planned study period. No safety data will be collected after a subject reaches end stage renal disease (a study end point) or when in a subject the study protocol is stopped in order to start other experimental medication for the treatment of ADPKD. The status of the subjects, and reasons for exclusion from the safety analysis will be documented in the Patient Classification Document.

### 2.4 Efficacy parameters

#### 2.4.1 General considerations

Longitudinal models will be used to handle missing measurements in analyses of the primary efficacy outcome. For secondary outcome analyses looking at change in eGFR and hTKV, baseline values and values obtained 12 weeks after cessation of the treatment (FU visit) will be needed to perform these analyses. For the secondary outcome time to event analysis, incidence of worsening renal function and time to reach Incidence of worsening renal function are needed to perform this analysis. Other values will be noted as not available, and the nature of missingness will be explored (missing (completely) at random, not at random).

#### 2.4.2 Statistical methods used for efficacy parameters

The statistical methods used are described above: linear mixed models and survival analyses will be used.

**Primary efficacy endpoint**

A mixed-model, repeated-measures analysis will be used to evaluate changes in eGFR during the treatment phase of the study between treatment groups. Unless otherwise specified, the repeated-measures mixed-model will have the following characteristics:

- The response variable will be the observed eGFR measurements (as opposed to any transformation, such as change from baseline). Only measurements during the treatment phase (from month 3 until end of the treatment phase (EQT)) will be considered response values. If End Stage Renal Disease (ESRD) or death occurs, only eGFR measurements obtained before these events will be used in analysis.
- Repeated measurements from each patient will be identified by patient identifier.
- Within-patient correlations will be modeled using an unstructured covariance structure. In the unlikely situation that this model does not converge (i.e., the study has too few observations for the number of parameters estimated), the model will use the Toeplitz structure, which makes the same assumption as the Toeplitz structure,
but is mathematically more restrictive. Finally, if the model using the AR(1) structure does not converge, the model will use a compound symmetry structure which assumes equal correlation for a patient's measurements, regardless of how far apart in time they were taken.

- The following categorical covariates will be used in the models as FIXED EFFECTS:
  - Treatment (lanreotide or placebo)
  - Time as defined by visit number
  - Treatment-by-time interaction

- An analysis will be performed of essential baseline characteristics (i.e. the stratification factors for randomization, (ht)TLV and selected other characteristics that are associated with disease outcome). When significant or clinically relevant differences are found between the two study groups, adjustment for these baseline characteristics will be incorporated in the mixed-effects models.

- Sensitivity analysis will be performed by including the last week a patient could have had an eGFR measurement to the longitudinal model to assess the impact of dropout on the outcome measurement.

For graphical representation of the primary efficacy endpoint, mean eGFRs with 95% confidence intervals per time point of the study will be plotted per lanreotide and placebo group from baseline until the end of follow-up.

**Secondary efficacy endpoints**

**Renal**
- Change in renal function (expressed as change in eGFR in ml/min*1.73m²) between pre-treatment and 12 weeks after cessation of the therapy (FU visit) will be assessed with a repeated measures mixed model. Pre-treatment eGFR is defined as the average eGFR measurements from the screening and baseline visits. Treatment (lanreotide or placebo), time, and a treatment-by-time interaction will be included in the model, and adjustment for any significant or clinically relevant baseline differences between groups.

- Incidence of worsening renal function, as defined as confirmed 30% decrease in eGFR from pretreatment and/or incidence of need for renal replacement therapy, whatever comes first. Pre-treatment eGFR is defined as the average eGFR measurements from the screening and baseline visits. Time to event analysis will be performed using Cox proportional hazards models, using the time to incidence of worsening renal function as dependent variables and treatment allocation as independent variable. Subjects will be censored when they reach the follow-up visit. Kaplan Meier graphs will be made to visualize the probability of worsening renal function between treatment groups.

- Change in height adjusted total kidney volume between baseline and 12 weeks after cessation of the therapy (FU visit) will be assessed with a repeated measures mixed model. Treatment (lanreotide or placebo), time, and a treatment-by-time interaction will be included in the model, and adjustment for any significant or clinically relevant baseline differences between groups.

**Quality of life**
- Change in quality of life will be determined between pretreatment and end of treatment phase. It will be assessed with a repeated measures mixed model. Treatment (lanreotide or placebo), time, and a treatment-by-time interaction will be included in the model, and adjustment for any significant or clinically relevant baseline differences between groups.

**Subgroup analyses**
- Subgroup analyses will be performed using mixed-model, repeated-measures analysis or ANCOVA or any other appropriate test, with the respective primary or secondary outcome as the dependent variable, treatment with Lanreotide (yes/no) and subgroup as independent variables and an interaction term of treatment group AND subgroup variable. If this interaction term appears to be significant than the subgroup variable can be considered as a moderator for treatment effect.

### 2.5 Safety parameters

#### 2.6.1 General considerations
Safety parameters are recorded as Adverse and Serious Adverse Events. All adverse events (including common and less frequent adverse events observed with Lanreotide administration) occurring during the study will be recorded in the patient's medical records. These will be coded using the most recent version of the MedDRA codelist. Clinical significant changes from baseline in safety laboratory parameters (see paragraph 7.1.3 of the study protocol) and in physical examinations are reported as Adverse Event.

#### 2.6.2 Primary safety endpoint specification
The incidence of adverse events will be summarized by treatment group and severity.

#### 2.6.3 Secondary safety endpoint specifications
Not applicable

#### 2.6.4 Statistical methods used for safety parameters
Fischer's Exact test will be used to compare the incidence of the (Serious) Adverse Events between the groups
3. VALIDATION OF SOFTWARE

The statistical analysis and reporting will be done using SAS software (version 9.3 or higher, SAS Institute, Cary, NC, USA), IBM SPSS Statistics version 22® for WindowsTM, or the current available version of R (currently 3.2.2).
4. CHANGES FROM PROTOCOL AND REMARKS

No major changes from the study protocol are introduced in this Statistical Analyses Plan. However:

1. We decided to pull apart the endpoints regarding the renal and the liver phenotype. Therefore the analysis plan is split into two parts, the first regarding renal endpoints and the second regarding liver endpoints.

2. Statistical analyses and assessment of parameters are described in more detail.

3. The CKO-EPI equation to estimate GFR has become available since the design of the trial and is now standard in clinical practice. Therefore, the equation to estimate GFR has been changed from the MORD equation to the CKD-EPI equation.

4. Since the design of the trial, height adjusted total kidney volume and height adjusted total liver volume have become the standard to express kidney and liver volume, respectively. Therefore TKV and TLV have been replaced by hTKV and hTLV.

5. Subgroup analyses were described in the original protocol (in section 9.2 statistical analyses), but not mentioned in the section 7.1.2 secondary outcome parameters. We now added the subgroup-analyses to the list of secondary outcome parameters. Moreover, we better specified the subgroups and added subgroups based on Mayo hTKV classification and genotype.

Data collection is performed by study personnel. Data monitoring and data management is performed by an independent agency (Trial Coordination Center; TCC, Groningen, The Netherlands). Data concerning randomization and end-points will only be given to study personnel after finalization of the study and database lock. Analyses will be performed by an independent statistician.
6. REFERENCES


