STATISTICAL ANALYSIS PLAN

Project Title: A randomized, open-label, adaptive, proof-of-concept clinical trial of Donated Antibodies Working agaiNst COVID-19: DAWN-PLASMA

NCT04429854

Versions

- Final version 1.0, 27 Oct 2020
- Amendment 1.0, 5 Feb 2021
- Amendment 2.0, 12 Feb 2021

DAWN-PLASMA Study

PLASMA Specific Statistical Analysis Plan

Final Version 1.0

Project Title: A randomized, open-label, adaptive, proof-of-concept clinical trial of Donated Antibodies Working agaiNst COVID-19: DAWN-PLASMA

Date: 27 October 2020

Version: Final Version 1.0

Signature Page

This statistical analysis plan is approved by:

Coordinating Investigator:

27-10-2020
Date

Prof. Dr. Geert Meyfroidt

Local PI, UZ Leuven

28-OCT-2020
Date

Prof. Dr. Timothy Devos

Statistician

27 October
Date

Ann Belmans, M.Sc.

DAWN Coordinator

Date

Prof. Dr. Peter Verhamme

Signature Page

This statistical analysis plan is approved by:

Coordinating Investigator:

Date	Prof. Dr. Geert Meyfroidt

Local PI, UZ Leuven

Date	Prof. Dr. Timothy Devos

Statistician

27 October 2020	Ann Belmans, M.Sc.
Date	

DAWN Coordinator

28/6/2020	Prof. Dr. Peter Verhamme
Date	

2 Contents

3 Purpose

In addition to the Global SAP, this document provides further details for the statistical evaluation of the primary, secondary and exploratory endpoints of the PLASMA compound within the DAWN study

4 Description of the Study

A total of 483 patients were to be randomised in a 2:1 ratio to either Standard of Care (SOC, 161 patients) or convalescent plasma (PLASMA, 322 patients). Randomisation was stratified by study site.

5 Master Statistical Analysis Plan

Due to the fast-changing addition of novel treatments and hypotheses of interest, a Master SAP describes the general statistical methods to be used for statistical evaluation of the primary, secondary, exploratory and safety endpoints of all compounds within the DAWN study.

This PLASMA-Specific SAP provides additional and specific details about the statistical methods for the evaluation of the outcomes for DAWN-Plasma at the time of (early) termination.

6 Study Objectives and Endpoints

6.1 Study Objective

The objective of the DAWN study is to evaluate the efficacy of various compounds of interest in the treatment of hospital-admitted COVID-19 patients.

6.2 Study Endpoints

6.2.1 Primary Outcome

The primary outcome for the evaluation of PLASMA is:

Number of patients alive without mechanical ventilation at Day 15.

This endpoint, among other, will be derived from the Daily assessment of Clinical Status up to Day 15 on a 11-point ordinal scale:

0. Uninfected. Non-viral RNA detected
1. Ambulatory, Asymptomatic, viral RNA detected
2. Ambulatory, Symptomatic, Independent
3. Ambulatory, Symptomatic, Assistance needed
4. Hospitalized, mild disease, No oxygen therapy needed
5. Hospitalized, mild disease, Oxygen by mask of nasal prongs
6. Hospitalized, severe disease, Oxygen by NIV or High flow
7. Hospitalized, severe disease, Intubation and mechanical ventilation (pO2/FiO2>=150 OR SpO2/FIO2>=200)
8. Hospitalized, severe disease, Mechanical ventilation (pO2/FiO2<150 OR SpO2/FIO2<200) OR vasopressors (norepinephrine >0.3 microg/kg/min)
9. Hospitalized, severe disease, Mechanical ventilation pO2/FiO2<150 AND vasopressors (norepinephrine >0.3 microg/kg/min), OR Dialysis OR ECMO
10. Death, Dead

6.2.2 Secondary Outcomes

The following secondary outcomes will be of interest:

1. Daily clinical status on Days 15 and 30;
2. Time to sustained clinical improvement or life discharge, whichever comes first: time from Day 0 to sustained clinical improvement or life discharge, whichever comes first, whereby a sustained clinical improvement is defined as an improvement of $\geq$ 2 points vs the highest value of Day 0 and 1 and sustained for at least 3 days.
3. Proportion of patients on mechanical ventilation or death within 30 days and 90 days after randomisation;
4. Duration of hospital stay;
5. All-cause mortality on Days 15 and 30;
6. Duration of supplemental oxygen;
7. Duration of mechanical ventilation;
8. Incidence and duration of ICU stay;
9. Incidence and duration of ECMO;
10. Time to Death;
11. Incidence of venous thromboembolism: deep vein thrombosis and pulmonary embolism;
12. Incidence of transfusion related side effect;
13. Correlation between clinical outcome and titer of anti-SARS-Cov-2 neutralizing antibodies in transfused plasma units;
14. Safety of convalescent plasma;
15. Quality of life 30 days after randomisation;
16. Adverse events graded as severe or SAEs;
17. Laboratory data assessed on days 1, 3, 5, 8, 11, 15 and 30;
18. Vital signs:
    a. Daily highest temperature measured during hospitalization with a maximum of 14 days after hospitalization;
    b. Highest amount of oxygen given daily during hospitalization with a maximum of 14 days after hospitalization.

6.2.3 Long-Term Exploratory Outcomes

1. Qualitative and quantitative PCR for SARS-CoV-2 in swab on Day 1 and 6.

7 Study Methods

7.1 Overall Study Design and Plan

The DAWN study evaluates several potential candidates in a randomized design vs Standard of Care (SOC) as a control group.

In the PLASMA sub-protocol, patients are randomized in a 2:1 ratio to either convalescent plasma or Standard of Care (SOC).

7.2 Selection of Study Population

Adult patients who tested positive for SARS-CoV-2 and are admitted to the hospital are screened for eligibility. Once eligibility is confirmed, informed consent is sought from the patient to participate in the study. Patients who consented are randomized to either Plasma or SOC.

7.3 Method of Treatment Assignment and Randomisation

Patients are randomized in a 2:1 ratio, stratified by study site.

7.4 Blinding of Study Treatment

This was an open-label study, meaning both patients and study personnel were aware of the assigned treatment.

8 Sequence of Planned Analyses

8.1 Interim Analyses

No formal interim analyses to check for early efficacy or futility have been planned.

A regular review of the safety data was performed by a Data Safety Monitoring Board (DSMB).

8.2 Early Termination

Early termination of the study can only be considered for the following reasons:

1. Recommendation by the DSMB to stop due to safety concerns.
2. Clear evidence (positive or negative) from other, external trials.

In case the study is terminated early, the following additional analyses will be performed for the primary endpoint:

1. Bayesian logistic regression :

   A Bayesian Proportional Odds (PO) logistic regression model will be fit on the primary endpoint. For the base model the ordinal response will be regressed on treatment (binary), study period (categorical) and study site (categorical). A test will be performed to verify whether the interaction term of treatment with study period is important. The interaction term is considered important when the 95% equal tail credible interval of the interaction term excludes zero. The PO assumption will be verified by fitting a Partial-Proportional Odds (PPO) model to the data. Deviation from the PO assumption will be concluded when the difference between the DIC of the PO model and the PPO model is greater than 10.

   The prior distribution of the regression parameters will be vague. On the other hand, when there is external information available at the time of the analysis, then that information will be used in the above analysis.

   - In case of no extra information, vague independent priors suggested by Gelman et al (2008) for a binary logistic regression will be extended to the PO (or PPO) model. More specifically, a Cauchy prior with location 0 and scale 2.5 will be taken for the

slopes and a Cauchy prior with location 0 and scale 10 for the intercept. To ensure practical independence of the scale of the covariates, the authors suggested to standardize the continuous covariates such that their mean is 0 and their SD equals 0.5. Binary covariates should also be rescaled such that they have mean 0 and range 1. More specifically, when the observed proportion for outcome 0 is *a*, and thus for outcome 1 equal to *1-a*, then the binary outcome should be rescaled as *(1-a)* for outcome 0 and to *-a* for outcome 1. In this way the mean of the binary covariate is 0 and the range = 1. Interactions are based on the standardized covariates.

The purpose of the Bayesian approach is to estimate the posterior probability $Pr(\beta_{Treat} < 0|data)$, whereby a negative regression coefficient implies a beneficial effect of the plasma therapy. Note that this probability statement assumes the original binary treatment covariate (0=control, 1=PLASMA).

In case there is external information at the time of the analysis, the statistical analysis will depend on how that information is presented. The following describes what will be done if there is just 1 extra source of information.

- When the maximum likelihood estimate (MLE) and standard error are available of each regression coefficient, independent Gaussian priors will be taken with the same mean as the MLE and with an inflated standard deviation of equal to twice the reported standard error. This is roughly equivalent to decreasing the importance of the external data by 4. This will be done once for all slopes (not intercept) and once for all slopes except for the treatment slope. Note: this procedure is only advisable when the regression models upon which the estimates are based are similar to the one used in this study. If these estimates turn out to be too different, two options can be taken: (1) the priors can be adapted adequately, by experts; (2) in case it is not possible to adapt the prior in a justified manner, the prior information will be neglected. The aim is to estimate $Pr(\beta_{Treat,current} < 0|data)$, with the assumption that the estimate for the regression coefficient of the current study is based on the current and external data.
- If individual level external data are available, a Gaussian hierarchical model will be set up whose corresponding regression coefficients in both studies will have a common Gaussian prior distribution and its parameters are estimated as hyperparameters. However, we intend to use this model only with a limited number of interaction terms, a method called the meta-analytic prior approach. The aim is to estimate $Pr(\beta_{Treat,current} < 0|data)$, and to base the estimate for the regression coefficient of the current study on the current as well as external data.

In case of *K* sources of prior information, the prior mean of the regression coefficient will be taken equal to the weighted average of the means of the *K* studies, where the weights are the individual precision estimates. The prior standard error of the regression coefficient will be taken equal to ¼ of the inverse of the sum of the *K* precisions. Second, the Gaussian hierarchical will be based on the *K* external sources and the data of the current study.

The calculations will be done using JAGS 4.3.0 in combination with R or SAS.

2. Conditional power:

The conditional power for the originally planned sample size (n=483) will be calculated based on data collected up to time of termination and making the following assumptions for the non-recruited patients:

a. Event rate in SOC: as observed in recruited patients.
b. Treatment effect: varying assumptions to assess sensitivity of results:
    i. No treatment effect
    ii. Treatment effect observed in recruited patients
    iii. Treatment effect postulated in sample size calculations
    iv. Treatment effect observed in external study (if available).

8.3 Final Analysis and Reporting

Upon final database lock, statistical analyses of the data will be performed according to the methods described in this document and the Master SAP.

All deviations will be documented. The analysis populations and analysis plan will be finalised at a Blinded Review Meeting prior to database lock where all attendees will be kept blind from randomised study treatment. All decisions taken at this meeting will be fully documented in a Blind Review Document that will be dated and signed prior to final database lock.

The agenda of the Blind Review Meeting will include (but not necessarily be limited to) the following:

- Definition of Per Protocol Set (see Section **Error! Reference source not found.**);
- Definition of Study Periods (see Section **Error! Reference source not found.**);
- Pooling of Study Sites (see Section **Error! Reference source not found.**).

9 Sample Size Determination

Approximately 20% of patients hospitalized for a SARS-CoV-2 infection are admitted to the ICU with respiratory failure. When admitted to the ICU, 80% of these patients need mechanical ventilation. With the administration of convalescent plasma as early as possible, we hope to decrease the proportion of patients who have a clinical decline and need ICU support. We assume by providing passive immunity with convalescent plasma, we are able to reduce the proportion of patients admitted to the ICU from 20% to 15%. Furthermore, we assume that when admitted to ICU, the need for mechanical ventilation is reduced from 80% to 50%. With a power of 0.8, a delta of 8.5% (16% in the control group and 7.5% in the invention group), a randomization ratio of 2:1 and a two-sided alpha of 0.05, sample size estimates to detect a difference between both groups, is 483 patients with 322 patients and 161 patients in the intervention and SOC group, respectively (using a Pearson Chi-square test for a difference in proportions).

A pragmatic initial sample size of 483 patients (161 control patients, 322 in the plasma intervention group) was used.

10 Analysis Populations

The following analysis sets will be of interest:

10.1 Full Analysis Set (FAS)

The FAS will include all randomised patients according to their randomised treatment. However, the following patients will be excluded from the FAS:

a) Covid-negative patients: violation of inclusion criterion 5
b) Patients randomised to receive plasma, but plasma could not be administered because none was available at the site.

The FAS will be used for the evaluation of all efficacy and safety endpoints.

10.2 Safety Set (SS)

No separate Safety Set will be defined.

The FAS will be used for the evaluation of all safety parameters.

10.3 Per Protocol Set (PPS)

If warranted, patients from the FAS with major protocol deviations will be excluded from the per protocol set (PPS).

The Per Protocol Set will be reviewed and finalized prior to database lock at the Blind Review Meeting (see Section 8.3).

The PPS will be used for the evaluation of all efficacy endpoints.

11 General Issues for Statistical Analysis

11.1 Analysis Software

All analyses will be performed using SAS software version 9.41 or higher for Windows 10 or higher. In case of early termination of the trial (see Section 8.2), the extra Bayesian calculations will be done using JAGS 4.3.0 in combination with R or SAS.

11.2 Summary Statistics

Continuous variables will be summarized by treatment group by the number of non-missing data points, mean, standard deviation, median and interquartile range.

Categorical and ordinal variables will be summarized by treatment group by observed frequencies and percentages relative to the total number of non-missing items.

All summary statistics will be presented by treatment group and, where possible, overall.

Data collected at several time points during the trial will be presented by planned visit, regardless of when the visit actually took place.

If applicable, changes from 'baseline' will be calculated whereby 'baseline' is defined as the last available measurement prior to randomization, unless specified otherwise.

Day 0 is defined as the day of randomization.

11.3 Statistical Comparisons between Groups

Unless specified otherwise, the following methods will be used to compare treatment groups:

- Normally distributed continuous data: 2-sample t-test.
- Continuous data showing serious deviations from normal distribution: Wilcoxon rank-sum test.
- Categorical data: chi-square or Fisher's exact test if cells with expected counts of <5 patients.
- Ordinal data: Wilcoxon rank-sum test or chi-square test for trend.

- Survival data: log-rank test.
- Competing risk data: Gray's test.

For each treatment comparison of interest, the treatment effect will be estimated by an appropriate measure (i.e., difference of the means, odds ratio, risk ratio, hazard ratio, …) and presented along with its associated 95% confidence interval.

11.4 Selective Randomization

Not applicable.

11.5 Period Effect

Due to the changing nature of the enrolled patient population and the Standard of Care, time will be accounted for in all statistical analyses by dividing the recruitment period into distinct periods and adjusting all analyses for said periods.

The choice of cutoff dates between the periods will be made prior to database lock during the Blind Review Meeting (see Section 8.3).

11.6 Centre Effect

All statistical analyses of the outcomes of interest will be adjusted for study site.

In case of small sites, the possibility and mechanism of pooling sites will be decided and documented prior to database lock during the Blind Review Meeting (see Section 8.3).

11.7 Choice of Controls

Not applicable.

11.8 Factorial Design

Not applicable.

12 Methods for Withdrawals, Missing Data and Outliers

Clinical Status will be recorded for all patients daily during hospital stay. Patients discharged prior to Day 15/30 will be contacted on Day 15/30 in order to obtain their clinical status.

Since several of the endpoints are determined based on the in-hospital clinical status, it is important that all scores are available. Missing clinical status during hospital stay will be imputed using the following steps:

I. Stage 1: **Single day missing, with preceding and following day known**: in these cases, the missing value will be imputed by the maximum of the two surrounding values.
II. Stage 2: The remaining missing data will be imputed using multiple imputation methodology. The fully conditional specification method will be used with a multinomial logistic regression and will be done in a consecutive manner as follows:
    1. Step 1: impute Day 1 based on clinical variables and Day 0;
    2. Step 2: impute Day 2 based on clinical variables and imputed Day 1 status
    3. Step 3: impute Day 3 based on clinical variables and imputed status at Days 1-2;
    4. And so on, but for each step, the value of **at most 5** previous days will be used.

This method will be used up to hospital discharge or Day 30 (which occurs first), whereby (1323+Day-Number) will be used as seed number in each step (e.g. for day 1, seed 1324; day 2, seed 1325, …).

The clinical variables that will be included in the imputation model will be the following:

- Randomized treatment group
- Age
- Weight at baseline
- Oxygen flow at baseline
- CRP recorded on the previous day

For the imputation of the clinical variables, the fully conditional specification method will be used with a normal or logistic regression.

Missing Clinical Status on Day 15 and on Day 30 will also be accounted for by multiple imputation using the clinical status at discharge (clinical status<4), clinical status at Day 15 (only applicable for imputation of Day 30) and the number of days since discharge as variables in the imputation model in addition to randomized treatment, age and BMI.

13 Data Transformations

When necessary, a log-transformation can be applied to the data in order to satisfy the normality assumption when analyzing data using a general linear model.

14 Multicenter Study

All analyses will be adjusted for study site (see Section 0).

15 Stratification Factors

Randomization was stratified for study site. Therefore, all models that are used for the estimation of treatment effects will be adjusted for study site (see Section 0).

16 Multiple Comparisons

Since only 1 primary endpoint is defined, no adjustment of the significance level is required.

For secondary efficacy endpoints, due to the exploratory nature of the efficacy analyses, no adjustment for multiple comparisons will be made.

17 Planned Subgroups, Interactions and Covariates

Subgroup analyses will be performed for the primary outcome:

- Number of patients alive and without mechanical ventilation at Day 15.

The following subgroups will be of interest:

1. Duration of symptoms prior to enrolment (according to observed median);
2. Age groups (according to observed median);
3. Period (see Section 11.5);
4. Blood group (A, B, AB, O);

5. Size of study site (according to median);
6. Admission to ICU at hospital admission (yes vs no).

Appropriate summary statistics per treatment and estimated treatment differences, will be presented for each subgroup. In addition, the interaction between the above subgroups and randomized treatment will be tested to assess whether the treatment effect differs according to subgroup. The treatment difference per subgroup will be estimated from an appropriate statistical model (e.g., ANOVA, ANCOVA, logistic regression, Cox regression, …) that includes a factor for treatment, subgroup and their interaction.

Subgroup analyses will only be performed for the FAS.

18 Study Subjects

18.1 Disposition of Subjects and Withdrawals

A summary by treatment group will be provided for the following:

- Number of randomized subjects,
- Number treated according to randomization
- Number in Full Analysis Set (FAS);
- Number in Per Protocol Set (PPS)
- Exclusions from FAS
- Exclusions from PPS
- Number of subjects who died in hospital up to Day 15
- Number of subjects who were discharged up to Day 15
- Number of subjects who died out-of-hospital up to Day 15
- Number of subjects who died in hospital up to Day 30
- Number of subjects who were discharged up to Day9
- Number of subjects who died out-of-hospital up to Day 30

18.2 Protocol Violations and Deviations

Not applicable.

19 Demographics and Other Baseline Characteristics

All data recorded at baseline will be summarized by treatment group and compared using the methods described in Section 11.3.

Summaries will be presented for FAS and PPS separately.

The following baseline information will be presented:

- Demographic characteristics
- Medical history
- Maintenance medications
- Thromboembolic Event at Baseline
- Vital Signs at Baseline
- Chronic Respiratory Support at Home
- Medication at Baseline
- Imaging at Baseline
- ECG at Baseline

- Disease Triage at Admission and Time Differences
- Blood Type and Plasma Availability

20 Primary and Secondary Endpoints

20.1 Primary Efficacy Endpoint

Summary and statistical analysis of the primary endpoint will be done using the Full Analysis Set and, if defined, the Per Protocol Set.

20.1.1 Number of Patients Alive and Without Mechanical Ventilation

The treatment effect will be estimated as an odds ratio and will be obtained from a logistic regression that includes treatment, study site and period as factors in the model.

20.2 Secondary Efficacy Endpoints

Summaries and statistical analyses of the secondary endpoints will be done using the Full Analysis Set and, if defined, the Per Protocol Set.

20.2.1 Clinical Status on Day 15 and 30

Clinical Status (imputed) on Day 15 and 30 will be analyzed by means of a proportional odds logistic regression model, performed on each day. The treatment effect will be estimated by the common odds ratio.

The proportional odds logistic regression model will include a factor for randomized treatment, period and study site.

20.2.2 Death or Mechanical Ventilation Within 30 Days and 90 Days

Event rates (i.e. death or mechanical ventilation) will be calculated using Kaplan-Meier methodology. 95% pointwise confidence intervals will be calculated using the log-log transformation. Comparison of the curves will be done using a log-rank test.

Median times (or other more suitable quantiles) will be presented by treatment group.

The treatment effect will be estimated as a hazard ratio, obtained using a Cox proportional hazards regression model that includes factors for treatment group, period and study site.

For the analysis on Day 30, all data beyond Day 30 will be censored at Day 30. Likewise, for the analysis of the Day 90 outcome, all data beyond Day 90 will be censored.

20.2.3 Time to Sustained Clinical Improvement or Discharge

Time to Sustained Clinical Improvement or Discharge will be derived from the imputed Clinical Status data.

Time to sustained clinical improvement or discharge will be analysed using methods for the analysis of competing risk data: the event of interest is clinical improvement or discharge, death without improvement will be considered to be the competing risk, patients for whom follow-up ended without clinical improvement will be censored. Comparisons of the CIF curves will be done using Gray's test.

Event rates over time will be estimated as cumulative incidence functions (CIF) and presented along with their 95% point-wise confidence intervals. A seed number of 1610 will be used for the simulation of the Gaussian distribution for the calculation of the 95% confidence intervals of the CIFs.

Median times (or other more suitable quantiles) will be presented by treatment group.

The treatment effect will be estimated by the subdistribution hazard ratio obtained from a Fine&Gray model.

The Fine&Gray model will include a factor for randomized treatment, period and study site.

20.2.4 Duration of Hospital Stay

Duration of hospital stay with be analysed by means of competing risk methodology as described in Section 20.2.3 with time to discharge as the outcome and in-hospital death the competing risk.

20.2.5 Time to death and All-Cause Mortality on Day 15 and Day 30

Mortality rates over time will be estimated by Kaplan-Meier methodology. 95% point-wise confidence intervals will be calculated using the log-log transformation. Comparisons of the curves will be done using a log-rank test.

Median times (or other more suitable quantiles) will be presented by treatment group.

The treatment effect will be estimated as a hazard ratio, obtained using a Cox proportional hazards regression model.

The Cox regression will include a factor for randomized treatment, period and study site.

For the analysis of mortality on Day 15, all data beyond Day 15 will be censored at Day 15. Likewise, for the analysis of all-cause mortality on Day 30, all data beyond Day 30 will be censored.

20.2.6 Incidence and Duration of Supplemental Oxygen

In addition to duration, the incidence of supplemental oxygen will also be assessed.

Incidence and duration of supplemental oxygen will be calculated from the imputed Clinical Status data.

Incidence of supplemental oxygen will be analysed using competing risk methodology as described in Section 20.2.3 above, whereby death without the administration of supplement oxygen is considered a competing risk.

Competing risk methodology will also be used to evaluate the duration of the supplemental oxygen. The event of interest will be the end of supplemental oxygen when alive, competing risk is the end of supplemental oxygen because of death.

Two separate analyses will be done for the duration: first, patients who did not have any supplemental oxygen will be excluded from the analysis. Second, patients who did not have any supplemental oxygen will be included with a duration of zero days.

20.2.7 Incidence and Duration of Mechanical Ventilation

In addition to duration, the incidence of mechanical ventilation will also be assessed

Incidence and duration of mechanical ventilation will be calculated from the imputed Clinical Status data.

The statistical methodology for the evaluation of this endpoint will be the same as for incidence and duration of supplemental oxygen (see Section 20.2.6).

20.2.8 Incidence and Duration of ICU Stay

Incidence of ICU stay will be analyzed using the competing risk methodology described in Section 20.2.6, considering death without ICU admission as competing risk.

Duration of ICU stay will be analyzed by means of competing risk methodology as described in Section 20.2.6 with in-ICU death the competing risk. For patients who have multiple ICU stays, the durations will be added up.

20.2.9 Incidence and Duration of ECMO

Incidence of ECMO stay will be analyzed using the competing risk methodology described in Section 20.2.6, considering death without ECMO as competing risk.

Duration of ECMO will be analyzed by means of competing risk methodology as described in Section 20.2.6 with death during ECMO the competing risk. For patients who have multiple ECMO administrations, the durations will be added up.

20.2.10 Incidence of Venous Thromboembolism

A venous thromboembolism will be defined as the incidence of a deep vein thrombosis or pulmonary embolism.

The number and proportion of patients experiencing a venous thromboembolism will be summarized by treatment group.

20.2.11 Incidence of Transfusion Related Side Effects

The incidence of transfusion related side effects will be summarised in the same as described in Section 20.2.10.

20.2.12 Correlation between Clinical Outcome and Titers of Anti-SARS-Cov-2 Neutralizing Antibodies in Transfused Plasma Units

The correlation between plasma titers and the primary outcome will be assessed using a logistic regression in the plasma patients where the titer is included as a continuous and linear variable. This analysis is equivalent to the Cochran-Armitage test for a linear trend. It should be kept in mind, it has increased power for detecting a linear trend at the expense of statistical power to detect other trends.

The logistic regression model will include factors for study site and period.

To visualize the correlation, a bar chart of the titers by outcome will be presented.

20.2.13 Safety of Convalescent Plasma

The safety of convalescent plasma will be evaluated in the Full Analysis Set by the following:

- Incidence of thromboembolism (see Section 20.2.10);
- Incidence of adverse events (see Section 22).

20.2.14 Quality of Life 30 Days after Randomization

Quality of Life (QoL) will be analysed using a general linear model that includes a factor for treatment, period and study site.

The effect of treatment will be estimated as a difference of estimated means and will be calculated using the general linear model and presented along with its associated 95% confidence interval.

20.2.15 Vital Signs

Daily highest temperature during hospitalisation up to Day 14 and highest amount of oxygen given daily during hospitalisation up to Day 14 will be analyzed using the methodology described in Section 20.2.14.

21 Exploratory Outcomes

21.1 PCR for SARS-CoV-2 on Day 6

Viral load will be analyzed using a general linear model with treatment group, study site and period as factors and the baseline value as a covariate. The viral load data will be $\log_{10}$-transformed prior to analysis. The treatment effect will be estimated as a ratio of geometric means between treatments by back-transforming the estimated treatment difference from the above model.

In addition, the number (%) of patients that were positive for SARS-CoV-2 will be analyzed using logistic regression analyses, including baseline result (positive/negative), treatment group, period and study site as factors in the model.

21.2 NT50 Values

NT50 values were recorded at baseline and on Day 6.

Appropriate summary statistics by treatment group will be provided for values observed at each visit and for changes from baseline at Day 6. Boxplots of the data will be provided by visit. Change plots will be provided by treatment group.

The difference between treatment groups at Day 6 will be assessed using a general linear model that includes treatment, study site and period as factors in the model and baseline value as a covariate.

The treatment effect will be estimated for the above model as a treatment difference and presented along with its 95% confidence interval.

In addition, differences at baseline and Day 6 (unadjusted for baseline) will be assessed using a general linear model, adjusted for treatment, study and period.

The association of NT50 at baseline, D6 and changes in NT50 with the primary endpoint will be assessed separately using logistic regression analyses, including the NT50 values/changes as a continuous covariate. To allow for non-linear relationships, restricted cubic splines will be used to model the NT50 values and changes. If the non-linear terms of the spline are found to be non-significant ($p>0.05$), they will be dropped from the model and a linear association will be assessed.

All analyses of NT50 data will be done only for the FAS.

22 Adverse Events

The number of events and the number of patients experiencing adverse events will be summarized by treatment group.

Separate summaries will be presented for:

- Serious adverse events
- Serious or severe adverse events
- Treatment-related adverse events
- Treatment-related serious adverse events.

Summaries of adverse events will be presented only for the FAS.

23 Other Data

All other data will be summarized by treatment group and, if applicable, by day.

24 PK/PD Analyses

Not applicable.

25 References

1. SAS software, version 9.4 of the SAS System for Windows. Copyright © 2002 SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA
2. Liang KY, Zeger S Longitudinal data analysis of continuous and discrete responses for pre–post designs. Sankhya: The Indian Journal of Statistics (Series B) 2000; 62:134–148.
3. Andrew Gelman, Aleks Jakulin, Maria Grazia Pittau and Yu-Sung Su (2008), A Weakly Informative Default Prior Distribution for Logistic and Other Regression Models. The Annals of Applied Statistics, 1360- 1383

26 List of Tables and Figures

DAWN-PLASMA Study

Amendment to Plasma-Specific Statistical Analysis Plan

Amendment 1.0

Project Title: A randomized, open-label, adaptive, proof-of-concept clinical trial of Donated Antibodies Working agaiNst COVID-19: DAWN-PLASMA

Date: 05 February 2021

Version: Amendment 1.0

Signature Page

This statistical analysis plan is approved by:

Coordinating Investigator:

8 FEB 2021
Date

Prof. Dr. Geert Meyfroidt

Local PI, UZ Leuven

05/FEB/2021
Date

Prof. Dr. Timothy Devos

Statistician

5 Feb 2021
Date

Ann Belmans, M.Sc.

2 Contents

3 Purpose

This document serves as an amendment to the Plasma-Specific Statistical Analysis Plan (Final Version 1.0, dated 27 October 2020).

4 Rationale for the Amendment

Clinical monitoring of the data revealed some patients had withdrawn their consent to undergo treatment after randomization, but before treatment was administered. Some of said patients also withdrew their consent to record any further information for the purpose of the trial.

A meeting was held on 14Jan2021 to discuss whether and how such patients were to be included in the statistical analysis of the study. The following attendees were present at the meeting:

1. Principal Investigator – Prof. Dr. G. Meyfroidt
2. Local PI, UZ Leuven – Prof. Dr T. Devos
3. Study Statistician – A. Belmans.

In accordance with ICH-E9 (Statistical Principles for Clinical Trials; September 1998; CPMP/ICH/363/96; Section 5.2.1), it was agreed that, in order to stay as close as possible to the ITT principle, patients who withdrew consent prior to treatment, but agreed to have further data recorded would be included in the Full Analysis Set. Patients who also withdrew their consent to record any further data will be excluded from the FAS.

It should be noted that these withdrawals of consent for treatment/data collection were mostly done by patients randomised to receive plasma who felt too well to have their hospital stay prolonged in order to undergo the additional procedures for treatment administration. It is therefore unlikely that the exclusion of such patients will create bias in favour of plasma treatment.

5 Description of the Amendment

The amendment to the Plasma-Specific SAP refers to the following section:

10.1 Full Analysis Set (FAS)

The FAS will include all randomized patients according to their randomized treatment. However, the following patients will be excluded from the FAS:

a) Covid-negative patients: violation of inclusion criterion 5
b) Patients randomized to receive plasma, but plasma could not be administered because none was available at the site.

The above section will be changed to the following:

10.1 Full Analysis Set (FAS)

The FAS will include all randomized patients according to their randomized treatment. However, the following patients will be excluded from the FAS:

a) Covid-negative patients: violation of inclusion criterion 5
b) Patients randomized to receive plasma, but plasma could not be administered because none was available at the site.

c) Patients randomized to plasma who did not receive treatment and for whom no data were recorded post-randomization OR patients randomized to SOC for whom no data were recorded post-randomization
d) Patients who are pregnant: exclusion criterion 2.
e) Patients with documented grade 3 allergic reaction to plasma: exclusion criterion 4
f) Patients with therapy restriction code excluding mechanical ventilation and/or intubation: exclusion criterion 5

DAWN-PLASMA Study

Amendment to Plasma-Specific Statistical Analysis Plan

Amendment 2.0

Project Title: A randomized, open-label, adaptive, proof-of-concept clinical trial of Donated Antibodies Working agaiNst COVID-19: DAWN-PLASMA

Date: 12 February 2021

Version: Amendment 2.0

Signature Page

This statistical analysis plan is approved by:

Coordinating Investigator:

12.02.2021
Date

Prof. Dr. Geert Meyfroidt

Local PI, UZ Leuven

12-FEB-2021
Date

Prof. Dr. Timothy Devos

Statistician

12 Feb 21
Date

Ann Belmans, M.Sc.

2 Contents

3 Purpose

This document serves as an amendment to the Plasma-Specific Statistical Analysis Plan (Final Version 1.0, dated 27 October 2020).

4 Rationale for the Amendment

On 11 February 2021, a Blind Review Meeting was held to discuss the following issues relevant to the statistical analysis of the study:

1. Analysis Sets: Full Analysis Set & Per Protocol Set
2. Selection of Cutoffs for Study Periods
3. Pooling of Study Sites for Statistical Analysis.

The amendments and additions described in this document accurately reflect the decisions made during this meeting.

5 Description of the Amendments/Additions

5.1 Full Analysis Set: Addition to Section 10.1

The following patients will be excluded from the Full Analysis Set:

Reason for Exclusion from FAS	**Patient Numbers**
No confirmed diagnosis of SARS-CoV-2	439-14
No post-randomisation data available	517-13; 529-2; 535-3; 523-29; 457-4

5.2 Per Protocol Set: Addition to Section 10.3

Patients with the following Protocol Violations will be excluded from the Per Protocol Set:

1. Patients randomised to PLASMA who received less than 4 units of convalescent plasma;
2. Patients randomised to SOC who received convalescent plasma within 30 days after randomization.

5.3 Cutoffs for Study Periods: Addition to Section 11.5

The study period will be divided into 2 study periods, using 09Aug2020, the end of the first wave[1], as the cutoff date.

5.4 Pooling of Study Sites: Addition to Section 11.6

The following study sites will be pooled for statistical analysis:

- Ambroise Paré (1 patient) with CHR Jolimont (7 patients)
- Bordet (6 patients) with St-Luc (7 patients).

5.5 Additional Subgroup Analysis: Amendment to Section 17

The following paragraph will be amended:

> The following subgroups will be of interest:
>
> 1. Duration of symptoms prior to enrolment (according to observed median);
> 2. Age groups (according to observed median);
> 3. Period (see Section 11.5);
> 4. Blood group (A, B, AB, O);
> 5. Size of study site (according to median);
> 6. Admission to ICU at hospital admission (yes vs no).

The above paragraph will be amended as follows:

> The following subgroups will be of interest:
>
> 1. Duration of symptoms prior to enrolment (according to observed median);
> 2. Age groups (according to observed median);
> 3. Period (see Section 11.5);
> 4. Blood group (A, B, AB, O);
> 5. Size of study site (according to median);
> 6. Admission to ICU at hospital admission (yes vs no);
> 7. Study Site Province: Antwerpen, Limburg, Vlaams-Brabant, West-Vlaanderen, Oost-Vlaanderen, Liege, Hainaut, Brussel.
> 8. Institution from which plasma was obtained: Croix Rouge, Rode Kruis.

5.6 References

1. F.S. Taccone et al., The role of organizational characteristics on the outcome of COVID-19 patients admitted to the ICU in Belgium, The Lancet Regional Health - Europe (2020), https://doi.org/10.1016/j.lanepe.2020.100019