Protocol B5411003

A multicenter, open-label, single-arm study to assess the efficacy and safety of PF-06462700 administered intravenously at 40 mg/kg/day for 4 days in Japanese Participants with moderate and above aplastic anemia

Statistical Analysis Plan (SAP)

Version: 2

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APPENDICES

1. VERSION HISTORY

Version/ Date	Associated Protocol Amendment	Rationale	Specific Changes
Original 14 Feb 2020	Original 24 Dec 2019	N/A	N/A
Version 2 7 May 2021	N/A	Additional detail added about the analysis plans. Some summary tables are included additionally. Update analysis plan based on the BDR meeting (a process of DMB09).	Sections 2.1.1 and 2.1.2: For clarification, additional detail added about the evaluation of the dependence upon exogenously administered growth factors or transfusion for the consideration of hematologic response. Section 3.2.3 Clarify the definition of transfusion independence. Section 3.4 and Section 6.4.1: Update the description on the baseline variables. Sections 3.5.1 and 6.5.1: Move the description on 3-tier approach from section 3.5.1 to section 6.5.1 with additional explanation on special interest AEs and some modification. Section 5.2: Modify the description considering some summary tables are added in the analysis plan. Section 6.1.1.1: Clarify that transfusion or growth factor dependence for hematological response will be evaluated for each hematological test value. Section 6.2.1.1: Modify the description on the survival status appropriately. Section 6.4.2: Add the plan for summary tables of subject evaluation group and disposition event. Section 6.4.4: Add the plan for the summary table for prior and concomitant medications for primary diagnosis and transfusions, and the listing of the number and dose of transfusion. Section 6.4.6: Add the plan for the listing of radiography at the screening. Sections 6.5.1, 6.5.2 and 6.5.3: Add the plan for the standard summary tables on adverse events, laboratory data and vital signs. Overall: Minor edits for the description maintenance.

2. INTRODUCTION

This statistical analysis plan (SAP) provides the detailed methodology for statistical analyses of the data collected in Study B5411003. This document may modify the plans outlined in the protocol; however, any major modifications of the primary endpoint definition or its analysis will also be reflected in a protocol amendment.

2.1. Study Objectives, Endpoints, and Estimands

The study objectives, endpoints and estimand are described in the below table.

Objectives	***Endpoints***	***Estimands***
Primary:	***Primary:***	***Primary:***
• *To investigate the efficacy of PF-06462700 administered intravenously at 40 mg/kg/day for 4 days in Japanese participants with moderate and above aplastic anemia*	• *Hematologic response at Week 12*	*Population: Japanese participants with moderate and above aplastic anemia who are at least 2 years of age* *Variable: Hematologic response at Week 12* *Intercurrent events: The efficacy will be evaluated individually for each patient. Therefore, any missing data will not be imputed. All observations after prohibited concomitant medication will be included. Improvement in counts that are dependent upon exogenously administered growth factors or transfusion will not be considered as fulfilling hematologic response criteria in the evaluation.* *Population-level summary: Due to the limited sample size, summary statistics will not be shown.*
Secondary:	***Secondary:***	***Secondary:***
• *To investigate the efficacy of PF-06462700 administered intravenously at 40 mg/kg/day for 4 days in Japanese participants with moderate and above aplastic anemia*	• *Hematologic response at Week 24* • *Hematological test values at Day 4 and at Weeks 1, 2, 4, 6, 8, 10, 12 and 24 (Absolute neutrophil count, Platelet count, Reticulocyte count). In case of early termination (ET), at ET and follow-up ET*, these values will be evaluated. • *Survival status* • *Transfusion independence at Weeks 12 and 24*	*Population: Japanese participants with moderate and above aplastic anemia who are at least 2 years of age* *Variable: Each secondary endpoint* *Intercurrent events: The efficacy will be evaluated individually for each patient. Therefore, any missing data will not be imputed. All observations after prohibited concomitant medication will be included.* As for the hematologic response at Week 24, improvement in counts that are dependent upon exogenously administered growth factors or transfusion will not be considered as fulfilling hematologic response criteria in the evaluation. *Population-level summary: Due to the limited sample size, summary statistics will not be shown.*

Safety objective:	***Safety endpoints:***	***Estimands:***
• *To investigate the safety of PF-06462700 administered intravenously at 40 mg/kg/day for 4 days in Japanese participants with moderate and above aplastic anemia*	• *Treatment-emergent adverse events (TEAEs)* • *Serious AEs (SAEs) and AEs leading to discontinuation* • *Vital signs* • *Clinical laboratory values*	*N/A*

Abbreviation: AE = adverse event

2.1.1. Primary Estimand

The hematologic response at Week 12 in the target population:

- *Population: Japanese participants with moderate and above aplastic anemia who are at least 2 years of age.*
- *Variable: Hematologic response at Week 12.*
- *Intercurrent events:*

  The efficacy will be evaluated individually for each patient. Therefore, any missing data will not be imputed. All observations after prohibited concomitant medication will be included. Improvement in counts that are dependent upon exogenously administered growth factors or transfusion will not be considered as fulfilling hematologic response criteria in the evaluation.*

  * Dependence upon exogenously administered growth factors or transfusion will be evaluated for each hematological test value (absolute neutrophil count, platelet count and reticulocyte count). When improvement in hematological test value is judged to be dependent upon growth factors or transfusion, it will not be considered as fulfilling the criteria described in section 3.1, regardless of the test values.

- *Population-level summary: Due to the limited sample size, summary statistics will not be shown.*

2.1.2. Secondary Estimand(s)

The secondary estimand in this study is as follows:

- *Population: Japanese participants with moderate and above aplastic anemia who are at least 2 years of age*
- *Variable: Each secondary endpoint*
- *Intercurrent events: The efficacy will be evaluated individually for each patient. Therefore, any missing data will not be imputed. All observations after prohibited concomitant medication will be included.*

As for the hematologic response at Week 24, improvement in counts that are dependent upon exogenously administered growth factors or transfusion will not be considered as fulfilling hematologic response criteria in the evaluation*.

* Dependence upon exogenously administered growth factors or transfusion will be evaluated for each hematological test value (absolute neutrophil count, platelet count and reticulocyte count). When improvement in hematological test value is judged to be dependent upon growth factors or transfusion, it will not be considered as fulfilling the criteria described in section 3.1, regardless of the test values.

- *Population-level summary: Due to the limited sample size, summary statistics will not be shown.*

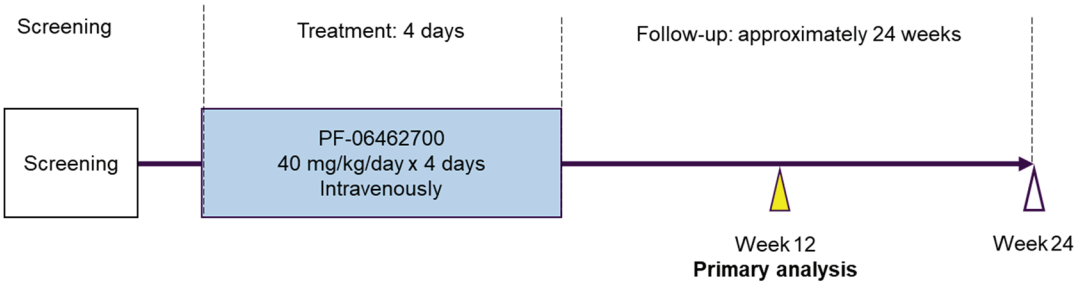
2.1.3. Additional Estimand(s)

N/A.

2.2. Study Design

Study B5411003 will assess the efficacy and safety of PF-06462700 in participants with moderate and above aplastic anemia. This is a multicenter, open-label, single-arm study. The study will have a maximum duration of approximately 28 weeks. This includes an up to 4-week screening period, a 4-day treatment period and a 24-week follow-up period. This study will enroll the minimum of 3 participants. The study will be initially conducted at 3 sites. It is acceptable to assign to investigational product more than 3 subjects. The target sample size was determined based on the study feasibility perspective.

Figure 1. Study Design



3. ENDPOINTS AND BASELINE VARIABLES: DEFINITIONS AND CONVENTIONS

3.1. Primary Endpoint

Hematologic response at Week 12.

"Effective" when 2 or more of the following criteria are met*:*

- *Absolute neutrophil count ≥500/µL.*
- *Platelet count ≥20,000/µL.*
- *Reticulocyte count ≥ 60,000/µL.*

Improvement in counts that are dependent upon exogenously administered growth factors or transfusion will not be considered as fulfilling response criteria.

For subjects of Stage 2b or 3 (see Appendix 1*) at the baseline*, hematological response will be "effective" when* 2 or more of *the above-mentioned criteria are met, and Stage is improved at the same time. (*If the baseline assessment is omitted, severity at the screening will be referred.).*

3.2. Secondary Endpoint(s)

3.2.1. Hematological response at Week 24

Hematological response described in Section 3.1 will also be evaluated at Week 24.

3.2.2. Hematological test values

Hematological test values (absolute neutrophil count, platelet count and reticulocyte count) will be evaluated at Weeks 12 and 24. These values will also be evaluated at Day 4 and at Weeks 1, 2, 4, 6, 8 and 10. In case of early termination (ET), these values will be evaluated at ET and follow-up for ET.

As described in Schedule of Activities (SoA) section of the protocol, *Hematology test on Day 1* (baseline) *can be omitted, if the interval between Screening visit and Day 1 visit is within 7 days*. In such cases, the values on screening visit will be used as baseline values.

3.2.3. Transfusion independence

Transfusion independence will be evaluated at Weeks 12 and 24.

The transfusion independence will be evaluated every 12 weeks. Participants who do not have any transfusion records from the time of the first dose of the investigational product at Day 1* to the day of Week 12 visit (inclusive) will be regarded as transfusion independent at Week 12. Participants who do not have any transfusion records from the day after Week 12 visit to Week 24 visit (inclusive) will be regarded as transfusion independent at Week 24. As for transfusion independence at baseline, participants who do not have any transfusion records from 84 days before Day 1 visit (inclusive) to the time of the first dose of the investigational product at Day 1* will be regarded as transfusion independent at baseline.

* If the time of the first dose of the investigational product or time of transfusion at Day 1 visit are not available, transfusion at Day 1 will contribute to transfusion dependence at Week 12 but not at baseline.

3.2.4. Survival status

During the study period, the survival status of each participant will be recorded.

3.3. Other Endpoint(s)

N/A.

3.4. Baseline Variables

No covariates and stratification variables are defined in this trial. The baseline variables to be listed are follows:

- Demographic characteristics.
- Details of aplastic anemia diagnosis (eg. Severity criteria of aplastic anemia, transfusion independence, prior rabbit-anti-thymocyte globulin [rabbit-ATG] treatment status).

3.5. Safety Endpoints

3.5.1. Adverse Events

MedDRA will be used to classify all AEs with respect to system organ class (SOC) and preferred term (PT). *The time period for actively eliciting and collecting AEs and SAEs ("active collection period") for each participant begins from the time the participant provides informed consent, which is obtained before the participant's participation in the study (ie, before undergoing any study-related procedure and/or receiving investigational product), through the last study visit (Week 24 visit or Follow-up visit of ET case).*

An adverse event is considered treatment emergent relative to a given treatment if the event started on or after Day 1 (start of PF-06462700 administration) and through the last study visit (Week 24 visit or Follow-up visit of ET case).

3.5.2. Laboratory Data

The following safety laboratory tests will be performed at times defined in the SoA section of this protocol. Additional laboratory results may be reported on these samples as a result of the method of analysis or the type of analyzer used by the clinical laboratory; or as derived from calculated values. These additional tests would not require additional collection of blood. Unscheduled clinical laboratory measurements may be obtained at any time during the study to assess any perceived safety concerns.

As described in SoA section of the protocol, *Hematology, Blood chemistry, Urinalysis, Coagulation, and Pregnancy test on Day 1* (baseline) *can be omitted, if the interval between Screening visit and Day 1 visit is within 7 days*. In such cases, the values on screening visit will be used as baseline values.

Table 1. Protocol-Required Safety Laboratory Assessments

Hematology	*Chemistry*	*Urinalysis*	*Other*
Hemoglobin *Hematocrit* *RBC count* *Reticulocyte count* *MCV* *MCH* *MCHC* *Platelet count* *WBC count* *Total neutrophils (Abs)* *Eosinophils (Abs)* *Monocytes (Abs)* *Basophils (Abs)* *Lymphocytes (Abs)*	*BUN* *Creatinine* *Glucose (fasting)* *Calcium* *Sodium* *Potassium* *Chloride* *AST, ALT* *Total bilirubin* *Alkaline phosphatase* *Uric acid* *Albumin* *Total protein*	*Glucose (qual)* *Protein (qual)* *Blood (qual)*	*At screening only:* • *FSH[a]* • *Pregnancy test (β-hCG)[b]* • *Hepatitis B core antibody, surface antibody and surface antigen (HBV DNA if necessary)* • *Hepatitis C antibody (HCV RNA if antibody is positive)* • *HIV* • *HTLV-1* • *EBV antibody (EBV DNA if antibody is positive and conduct at Week 2 and after)* • *CMV antibody (CMV DNA if antibody is positive and conduct at Week 2 and after)*

Abbreviations: Abs = absolute; ALT = alanine aminotransferase; AST = aspartate aminotransferase; β-hCG = beta-human chorionic gonadotropin; BUN = blood urea nitrogen; CMV = cytomegalovirus; DNA = deoxyribonucleic acid; EBV = Epstein-Barr virus; FSH = follicle-stimulating hormone; HBV = hepatitis B virus, HCV = hepatitis C virus; HIV = human immunodeficiency virus; HTLV-1 = human T-cell leukemia virus type 1; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; qual = qualitative; RBC = red blood cell; RNA = ribonucleic acid; WBC = white blood cell.

a. For confirmation of postmenopausal status only.

b. Local urine testing will be standard for the protocol unless serum testing is required by local regulation or institutional review board/ethics committee (IRB/EC). Serum or urine β-hCG for female participants of childbearing potential.

3.5.3. Other Safety Endpoints

- SAEs and AEs leading to discontinuation;
- Vital signs.

4. ANALYSIS SETS (POPULATIONS FOR ANALYSIS)

Data for all participants will be assessed to determine if participants meet the criteria for inclusion in each analysis population prior to releasing the database and classifications will be documented per standard operating procedures.

For purposes of analysis, the following analysis sets are defined:

Analysis Set	***Description***
Enrolled	*"Enrolled" means a participant's, or their legally authorized representative's, agreement to participate in a clinical study following completion of the informed consent process. Potential participants who are screened for the purpose of determining eligibility for the study, but do not participate in the study, are not considered enrolled.*
Full analysis set (FAS)	*All participants assigned to investigational product and who take at least 1 dose of investigational product. For participants who discontinue study and/or receive blood transfusion or prohibited concomitant medication, any missing data will not be imputed and all observations after blood transfusion or prohibited concomitant medication will be included in the analysis.*
Safety	*All participants assigned to investigational product and who take at least 1 dose of investigational product.*

5. GENERAL METHODOLOGY AND CONVENTIONS

The primary analyses will be performed after study participant data set release after last participant last visit.

5.1. Hypotheses and Decision Rules

Any formal hypothetical testings will not be conducted and there is no decision rule in this study.

5.2. General Methods

Because of the limited sample size, the result of each endpoint will be individually listed, and summary statistics will not be presented unless otherwise specified.

5.2.1. Analyses for Binary Endpoints

The result of each endpoint will be individually listed.

5.2.2. Analyses for Continuous Endpoints

The result of each endpoint will be individually listed. As for hematological test values, the values of each timepoint will be plotted for each participant individually.

5.2.3. Analyses for Categorical Endpoints

The result of each endpoint will be individually listed.

5.2.4. Analyses for Time-to-Event Endpoints

N/A.

5.3. Methods to Manage Missing Data

For efficacy endpoints, observed values will be used at each designated visit without imputation for missing values.

6. ANALYSES AND SUMMARIES

6.1. Primary Endpoint

6.1.1. Hematological response at Week 12

6.1.1.1. Main Analysis

- Estimand strategy: Primary estimand (Section 2.1.1);
- Analysis set: FAS (Section 4).
- Analysis methodology:

  The result of hematological response at Week 12 will be listed individually for each patient. Because of the limited sample size in this study, no summary statistics will be provided. Any missing data will not be imputed.

  Severity of aplastic anemia (Appendix 1), hematological test values, and transfusion or growth factor dependence for each hematological test value at Week 12 will also be listed. In addition, prior rabbit-anti-thymocyte globulin (rabbit-ATG) treatment status at baseline visit will be listed.

6.1.1.2. Sensitivity/Supplementary Analyses

N/A.

6.2. Secondary Endpoints

6.2.1. Endpoints

Secondary endpoints are described as follows:

- *Hematologic response at Week 24.*
- *Hematological test values at Day 4 and at Weeks 1, 2, 4, 6, 8, 10, 12 and 24(Absolute neutrophil count, Platelet count, Reticulocyte count). In case of ET, at ET and follow-up ET*, these values will be evaluated.
- *Survival status.*
- *Transfusion independence at Weeks 12 and 24.*

6.2.1.1. Main Analysis

- Estimand strategy: Secondary estimand (Section 2.1.2).
- Analysis set: FAS (Section 4).
- Analysis methodology:

  The result will be listed individually for each participant. Because of the limited sample size in this study, no summary statistics will be provided. Any missing data will not be imputed.

  As for the hematological response at Week 24, the listing will be prepared in the same manner as described in Section 6.1.1.1.

  As for the hematological test values (absolute neutrophil count, platelet count and reticulocyte count), individual plots of the test values for each participant will be shown up to Week 24. As for survival status, the final survival status in the study and date last known to be alive will be listed. If a participant dies during the study, the date of death and the cause of death will be shown.

6.2.1.2. Sensitivity/Supplementary Analysis

N/A.

6.3. Subset Analyses

N/A.

6.4. Baseline and Other Summaries and Analyses

6.4.1. Baseline Summaries

Details of aplastic anemia diagnosis

Primary diagnosis of aplastic anemia will be listed. In addition, the severity criteria for aplastic anemia, transfusion independence and prior rabbit-ATG treatment status will be listed.

Hematological test values (absolute neutrophil count, platelet count and reticulocyte count)

Hematological test values (absolute neutrophil count, platelet count and reticulocyte count) will be listed, separately from other hematologic laboratory data. The collection date of the blood sample will also be shown.

Other baseline variables

Other baseline variables described in section 3.4 will be listed for each participant according to CDISC and Pfizer Standards (CaPS).

6.4.2. Study Conduct and Participant Disposition

The data related to study conduct and participant disposition will be listed for each participant according to CaPS. Such data includes followings:

- Assignment to each analysis set (Enrolled, FAS and Safety);
- Participant disposition (eg, discontinuation from study, reason for discontinuation, completed study, etc);
- Medication errors;
- Inclusion and exclusion criteria.

Summary table for subject evaluation group and disposition event will be provided according to CaPS.

6.4.3. Study Treatment Exposure

Study treatment exposure will be listed for each participant according to CaPS.

6.4.4. Prior and Concomitant Medications and Nondrug Treatments

Prior and concomitant medications and nondrug treatments will be listed for each participant according to CaPS.

Prior and concomitant medications for primary diagnosis and transfusions will be summarized and listed separately. The number and dose of transfusion will be listed for each participant every 4 weeks from Week -4 to Week 24. As for the summary table for transfusion and the listing of the number and dose of transfusion, transfusions will be shown by blood cell type (red blood cells, platelets and others).

6.4.5. Medical History

Medical history will be listed for each participant according to CaPS.

6.4.6. Radiography

The result of radiography (chest x-ray) at the screening visit will be listed for each participant.

6.5. Safety Summaries and Analyses

All safety analyses will be performed on the safety population.

6.5.1. Adverse Events

SAEs and AEs will be listed for each participant according to CaPS. In addition, AEs leading to discontinuation will be listed for each participant. The following safety tables will be provided according to CaPS.

- Treatment-emergent adverse events (all causalities/treatment-related)
- Treatment-emergent adverse events by system organ class and preferred term (all causalities/treatment-related)
- Incidence and severity of treatment-emergent adverse events by system organ class and preferred term (all causalities/treatment-related)

Special interest AEs are pre-specified events of clinical importance and are maintained in a list in the product's Safety Review Plan. A 3-tier approach will not be used in this study due to the limited sample size. The specific summary table for special interest AEs will not be provided and these AEs can be visually confirmed in the above summary tables and listings.

6.5.2. Laboratory Data

Results of each laboratory data will be listed for each participant according to CaPS. Incidence of laboratory test abnormalities will be summarized according to CaPS.

6.5.3. Vital Signs

Results of vital signs will be listed for each participant according to CaPS. Categorization of vital signs data will be provided according to CaPS.

6.5.4. Electrocardiograms

Results of electrocardiograms (ECG) will be listed for each participant according to CaPS.

7. INTERIM ANALYSES

No formal interim analysis will be conducted for this study. As this is an open-label study, the sponsor may conduct clinical reviews of the data during the course of the study for the purpose of safety assessment, and/or supporting clinical development.

8. REFERENCES

1. Study Group on Idiopathic Hematopoietic Disorders, Research Program on Rare and Intractable Diseases, Health, Labour and Welfare Sciences Research Grants (Principal Investigator: Syunya Arai). Reference Guide for Management of Aplastic Anemia, Revised in 2018. Revised on May 13, 2018. [in Japanese]

9. APPENDICES

Appendix 1. Severity criteria for aplastic anemia (Modified in 2017)[1]

Stage 1	*Mild*	*No blood transfusion is required other than the following:*
Stage 2	*Moderate*	
	a	*Two or more of the following criteria are met, and no red blood cell transfusion is required.*
	b	*Red blood cell transfusion is required, but the frequency is less than 2 units per month.* *Reticulocytes < 60,000/μL* *Neutrophils < 1,000/μL* *Platelets < 50,000/μL*
Stage 3	*Slightly severe*	*Two or more of the following criteria are met, and transfusion of 2 units or more of red blood cells is required every month.* *Reticulocytes < 60,000/μL* *Neutrophils < 1,000/μL* *Platelets < 50,000/μL*
Stage 4	*Severe*	*Two or more of the following criteria are met:* *Reticulocytes < 40,000/μL* *Neutrophils < 500/μL* *Platelets < 20,000/μL*
Stage 5	*Very severe*	*In addition to neutrophils < 200/μL, at least one of the following criteria is met:* *Reticulocytes < 20,000/μL* *Platelets < 20,000/μL*

Appendix 2. Data Derivation Details

Appendix 2.1. Definition and Use of Visit Windows in Reporting

Visit windows in reporting are not defined in this study and the visit day for each value will be shown as necessary in the listings. No summary statistics will be reported.

Appendix 2.2. Endpoint Derivations

None.

Appendix 2.3. Definition of Protocol Deviations That Relate to Statistical Analyses/Populations

None.

Appendix 3. Data Set Descriptions

None.

Appendix 4. Statistical Methodology Details

None.

Appendix 5. List of Abbreviations

Abbreviation	Term
Abs	Absolute
AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATG	anti-thymocyte globulin
BDR	Blinded data review
β-hCG	beta-human chorionic gonadotropin
BUN	blood urea nitrogen
CaPS	CDISC and Pfizer Standards
CDISC	Clinical Data Interchange Standards Consortium
CMV	cytomegalovirus
DNA	deoxyribonucleic acid
EBV	Epstein-Barr virus
ECG	electrocardiogram
ET	early termination
FAS	full analysis set
FSH	follicle-stimulating hormone
HBV	hepatitis B virus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HTLV-1	human T-cell leukemia virus type 1
IRB/EC	institutional review board/ethics committee
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
N/A	not applicable
PT	preferred term
Qual	qualitative
RBC	red blood cell
RNA	ribonucleic acid
SAE	serious adverse event
SAP	statistical analysis plan
SoA	Schedule of Activities
SOC	system organ class
TEAE	Treatment-emergent adverse events
WBC	white blood cell