SUPPLEMENT

Investigator Statement

Title:	**A prospective outcome study on patients with profound combined immunodeficiency**
Coordinating Investigator	Prof. Dr. Stephan Ehl

Trial Centre:

Principal Investigator in the Centre:

The Principal Investigator at each collaborating clinical site should print, sign, and date at the indicated location below. A copy should be kept for your records and the original signature page sent to the P-CID Coordinating Center.

I confirm that I have read the Study Protocol and hereby commit myself to adhere to all actions and terms as specified in the relevant sections of the clinical, ethical and general paragraphs.

I confirm that I and my colleagues will abide by the local legislation (in Germany, the German Pharmaceutical Law with the appropriate amendments). I further confirm that the Study will be carried out in compliance with the Declaration of Helsinki and ICH-GCP guidelines.

I acknowledge that all confidential information in this document will not be used or circulated without the prior written consent of the Coordinating Investigator.

Under my supervision I put copies of this Study Protocol and possible updates as well as access to all information regarding the carrying out of this Study Protocol at the disposal of my colleagues. I will discuss this Study Protocol in detail with my colleagues and ensure that they are comprehensively informed about the trial compound/preparation and the execution of the study.

Furthermore I commit myself not to commence patient enrolment before the approval of the authorities and acceptance by the relevant/responsible Ethics Committee.

Date (DD/MM/YY) Signature of the Principal Investigator

List of Abbreviations

ADA	Adenosine Deaminase
CBC	Complete Blood Count
CCI	Centre of Chronic Immunodeficiency
CID	Combined Immunodeficiency
CMV	Cytomegalovirus
CRF	Case Report Form
DNA	Deoxyribonucleic acid
EBV	Epstein-Barr Virus
FISH	Fluorescence In Situ Hybridization
GvHD	Graft-versus-host disease
HSCT	Hematopoetic stem cell transplantation
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HPV	Human Papillomavirus
ICF	Immunodeficiency Centromeric Instability Facial Anomalies
Ig	Immunoglobulin
IVIG	Intravenous Immune Globulin
KREC	Kappa recombination excision circles
LOCID	Late-onset combined immunodeficiency
MNC	Mononuclear Cell
NK	Natural Killer (cell)
PBMC	Peripheral Blood Mononuclear Cell(s)
P-CID	Profound combined Immunodeficiency
PCR	Polymerase Chain Reaction
PEG	Polyethylene Glycol
PICF	Patient Informed Consent Form
PICU	Pediatric Intensive Care Unit
PID	Primary Immune Deficiency
PHA	Phytohemagglutinin
PNP	Purine Nucleoside Phosphorylase
QOL	Quality of Life
RFLP	*Restriction Fragment Length Polymorphism*
RDCRN PIDTC	Rare Disease Clinical Research Network Primary Immune Deficiency Treatment Consortium
SCETIDE	*Stem Cell Transplantation for Immunodeficiencies*
SCID	Severe Combined Immunodeficiency
STR	*Short Tandem Repeats*
TCR	*T Cell Receptor*
TRECs	T Cell Receptor Excision Circles

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Procedures

General Study Procedures

Information on the study, all procedures and all study documents (including PICF and questionnaires) can be downloaded at the website (www.pcid-study.org). Baseline and follow-up questionnaires (CRFs) can be printed out and then be mailed to the study coordination office together with copies of available histology/immunological test reports. Reports will be forwarded with blackened name and added patient identification number instead. Alternatively, they can be filled in electronically (pdf forms) and can be returned via email attachment. All CRFs and all further test reports will be saved in pseudonymized form (Section 7.1 in Supplementary table of contents).

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In case that the patient withdraws his consent, the referring investigator will inform the study coordinator immediately.

Biological Specimens

Blood Samples

Blood will be obtained by venous puncture of antecubital or other peripheral veins or via indwelling central lines. Aseptic technique will be used. Indwelling lines are not suitable for dried blood spots for TREC samples (due to interference of heparin with DNA quality); finger sticks are acceptable for dried blood spots, as is peripheral venous blood not exposed to heparin. (Section 7.2.1 in Supplementary table of contents).

Samples for Diagnostic Genetic Testing

Subjects lacking genetic diagnosis of their primary immune deficiency disease may be tested by evaluation of a blood or other tissue sample as appropriate. Alternative tissue samples may include hair follicles, a buccal swab or brushing and/or skin biopsy or other pathologic samples. (Section 7.2.2 in Supplementary table of contents).

Other Research Samples

In the event a subject for the purpose of their clinical care would be undergoing a needed procedure such as bone marrow aspiration or a procedure requiring a general anesthetic, the site investigators may wish to collect a small amount of bone marrow aspirate or skin or other tissue. Such samples may be used for storing stem cells and generating skin fibroblast lines and will be stored in a standardized fashion at the local centres for future genetic and non-genetic research studies to learn more about the immune system and PID. Separate consents will be obtained for such procedures at the individual participating sites. (Section 7.2.3 in Supplementary table of contents).

Banking of Samples

Samples will be collected at specified intervals as described in the Flow Charts. Samples will be stored decentrally at the specific site and information on storage will also be archived in the virtual

biomaterial bank. This biomaterial bank will be under the governance of the P-CID steering committee and will be conducted in compliance with international guidelines (OECD, BBMRI). Other investigators may apply for use of samples (Section 7.3 in Supplementary table of contents).

1.1 Study Visits for all patients

Time Intervals for Visits:

Time intervals for study visits will be one year for the time period of five years. Patients undergoing stem cell transplantation will have an additional study visit at 6 months after HSCT. The regular study visits will then be rescheduled to occur yearly after HSCT instead of yearly after study inclusion (Section 7.4.1 in Supplementary table of contents).

Study Informed Consent

1. Signed P-CID Study Informed Consent Form (required prior to Eligibility Assessment)
2. Consent forms for registration in the European registry for primary immunodeficiency (ESID registry) are also offered to all patients. (Section 7.4.2 in Supplementary table of contents).

Baseline Visit (Visit 1) (Section 7.4.3 in Supplementary table of contents).

Study Forms

1. Eligibility Form (see 6.3)
2. P-CID Visit 1 Form
    a. Registration form
    b. Baseline assessment
    - Medical history (documentation of severe events in past medical history)
    - Documentation of past and current therapy
    - Physical exam (including Neuro-developmental testing, if indicated)

    c. HSCT decision form
3. Peds QL Generic Core Scales (Child Self Report and/or Parent proxy Report, depending on recipient's age)

Laboratory

4. Documentation of CMV and EBV status, including PCR, if appropriate*
5. CBC with differential *
6. Quantitative Immunoglobulins *
7. Vaccine titers and isohemagglutinins (if not performed previously)
8. T, B, NK cell counts *
9. Fraction of naïve CD4+ T cells *
10. Fraction of CD3+ T cells expressing γ/δ TCR *
11. In vitro lymphocyte proliferation (PHA) *
12. T cell diversity by Vβ usage (optional) *
13. TRECs and KRECs (central analysis of dried blood spots from Guthrie cards; see below)
14. sCD25
15. Genetic investigations (if appropriate)

Biobanking

16. Frozen PBMC archive
17. Fibroblast/EBV/HVS cell line (if not established previously)
18. Serum archive
19. Registration of prior tissue biopsies (e.g. skin, gut, liver, lymph node, bone marrow)

* only required, if these values have last been obtained >6 months prior to study inclusion. If the baseline visit occurs within 6 months after the diagnosis of P-CID has been

established, the values submitted for eligibility assessment can be used.

Yearly Follow-Up Assessments Year 1-5 +/- 2 months (Section 7.4.4 in Supplementary table of contents).

Study Forms

1. P-CID Visit 2+ Form
    a. Assessment of Death or Loss to Follow-Up
    b. Follow-up Assessment
    - Medical history (documentation of severe events since last visit)
    - Documentation of current therapy
    - Physical exam

    c. HSCT decision form
    d. (SCETIDE Follow-up: leave blank prior to HSCT)
2. Peds QL Generic Core Scales (Child Self Report and/or Parent proxy Report, depending on recipient's age)

Laboratory

3. CBC with differential
4. Quantitative Immunoglobulins (IgG only if without substitution)*
5. T, B, NK cell counts
6. Fraction of naïve CD4+ T cells
7. Fraction of CD3+ T cells expressing γ/δ TCR
8. In vitro lymphocyte proliferation (PHA)
9. TRECs and KRECs (central analysis of dried blood spots from Guthrie cards; see below
10. sCD25
11. Genetic investigations (if appropriate)

Biobanking

12. Frozen PBMC archive
13. Fibroblast/EBV/HVS cell line (if not established previously)
14. Serum archive
15. Registration of any tissue biopsies performed since last visit (e.g. skin, gut, liver, lymph node, bone marrow)

Study Visits for patients undergoing HSCT

Informed consent

Additional signed SCETIDE consent form are required for all patients undergoing HSCT. They are obtained prior to documentation of the SCT1 visit. (Section 7.5.1 in Supplementary table of contents).

Time Intervals for Visits

Time intervals post-transplantation are as follows. HSCT will be reported to the study coordinator immediately (HSCT report form). Further details on HSCT will be assessed at Visit SCT1 at 6 months (+/- 1 month) post transplant. All further follow-up visits will be rescheduled yearly after HSCT instead of yearly after study inclusion (Section 7.5.2 in Supplementary table of contents).

HSCT Report form (Immediately at HSCT)

Date of HSCT will be reported immediately to study coordination (Section 7.5.3 in Supplementary table of contents).

Visit SCT1 (6 months after HSCT) (Section 7.5.4 in Supplementary table of contents).

Study Forms

1. P-CID visit 2+ Form
    a. Assessment of Death or Loss to Follow-Up
    b. Follow-up Assessment
    - Medical history (documentation of severe events since last visit)
    - Documentation of current therapy
    - Physical exam
    c. HSCT decision form
    d. SCETIDE Follow-Up

2. P-CID SCETIDE initial report Supplement
    a. Clinical status at time of HSCT
    b. Donor information, recipient and donor histocompatibility
    c. HSCT: conditioning, transplantation, prophylaxis of GVHD and infections
    d. HSCT results:
        i. Engraftment and chimerism at 6 months
        ii. Hematological reconstitution after myelosuppression
        iii. Immunological reconstitution at 6 months
        iv. Severe events at 6 months
3. Peds QL Generic Core Scales (Child Self Report and/or Parent proxy Report, depending on recipient's age) and the Peds QL Transplant Module

Laboratory

4. CBC with differential
5. Quantitative Immunoglobulins (IgG only if without substitution)*
6. T, B, NK cell counts
7. Fraction of naïve CD4+ T cells
8. Fraction of CD3+ T cells expressing γ/δ TCR
9. In vitro lymphocyte proliferation (PHA)
10. TRECs and KRECs (central analysis of dried blood spots from Guthrie cards; see below)
11. Lineage-specific chimerism

Biobanking

12. Frozen PBMC archive
13. Fibroblast/EBV/HVS cell line (if not established previously)
14. Serum archive
15. Registration of any tissue biopsies performed since last visit (e.g. skin, gut, liver, lymph node, bone marrow)

Yearly Follow-Up Assessments post HSCT Year 1-5 +/- 2 months (for scheduling see 7.5.2) (Section 7.5.5 in Supplementary table of contents).

Study Forms

1. P-CID Visit 2+ Form
    a. Assessment of Death or Loss to Follow-Up
    b. Follow-up Assessment
    - Medical history (documentation of severe events since last visit)
    - Documentation of current therapy
    - Physical exam
    c. (HSCT decision form, not filled in after patient has been transplanted)

SCETIDE Follow-Up (includes Clinical Status one year after HSCT, severe events after HSCT, Engraftment and chimerism, immunological reconstitution one year after HSCT)

2. Peds QL Generic Core Scales (Child Self Report and/or Parent proxy Report, depending on recipient's age) and the Peds QL Transplant Module

Laboratory

3. CBC with differential
4. Quantitative Immunoglobulins (IgG only if without substitution)*
5. T, B, NK cell counts
6. Fraction of naïve CD4+ T cells
7. Fraction of CD3+ T cells expressing γ/δ TCR
8. In vitro lymphocyte proliferation (PHA)
9. TRECs and KRECs (central analysis of dried blood spots from Guthrie cards; see below)
10. sCD25
11. Genetic investigations (if appropriate)
12. Lineage-specific chimerism

Biobanking

13. Frozen PBMC archive
14. Fibroblast/EBV/HVS cell line (if not established previously)
15. Serum archive
16. Registration of any tissue biopsies performed since last visit (e.g. skin, gut, liver, lymph node, bone marrow)

Genetic Testing

Diagnostic Genetic Testing

An attempt at identifying the genetic basis of P-CID in patients entered into this study will be made as part of standard medical care at the local site. The steering committee may add recommendations, when reviewing individual cases. A standardized case presentation form for case discussion with the steering committee is available at the P-CID website. It is assumed that some level of gene sequencing is indicated for medical and genetic management and will be reimbursable via the centre caring for the patient. Genotype reports should be submitted to the P-CID study coordination to ensure consistency of mutation reporting. Gene sequencing is not uniformly available for all patients, nor is a definitive mutation diagnosis always found. Therefore specific gene diagnosis is not required for study enrolment (Section 7.6.1 in Supplementary table of contents).

The following strategies for phenotypic and functional screening for genetically defined P-CID disorders are offered to assist referring local physicians in ordering functional diagnostic tests and molecular diagnostic tests as part of their patient's medical care.

1. Family history should be reviewed and mutation diagnosis conducted based on available data in any relatives affected with the same condition as the patient. A standardized extended family tree is mandatory.

2. An ADA enzyme assay should be considered in all lymphopenic P-CID patients. A PNP assay is needed if the biochemical or clinical picture is consistent with PNP deficiency.

3. Radiosensitivity testing should be considered, in particular (but not exclusively) in patients with microcephaly, small stature and/or cytopenia.

4. In patients with small stature, consider X-ray studies to investigate for CHH or Schimke disease.

5. Consider AFP determination to exclude ATM.

6. To define priorities for sequencing to establish the diagnosis of atypical SCID, consider the following tests:

a. T(-) B+ phenotype: CD45, CD3, CD132 and CD127 expression, STAT-5 phosphorylation assay, JAK3 expression
b. T(-) B(-) NK+ phenotype: radiosensitivity testing, in vitro (VDJ) recombination assay
c. syndromic features, heart defect: 22q11 FISH analysis
7. If these tests can not be performed at the patient's home institution, a list of centers offering appropriate functional and genetic tests is available in the appendix of this study protocol.

To facilitate an orientation in the immunological and genetic differential diagnosis of patients with CID, the following graphical illustrations of diagnostic algorithms for CID are also accessible via the website:

- Diagnostic algorithm for CID patients according to the pattern of lymphocytopenia
- Diagnostic algorithm for CID patients according to manifestations of immune dysregulation
- Diagnostic algorithm for CID patients according to syndromal manifestations

For all patients in whom no genetic diagnosis can be established, the following procedure should be followed:
1. DNA should be banked on all patients. If not saved at the patient's home institution, contact the study coordinator to arrange storage.
2. EBV transformed B cell lines and skin fibroblast lines should be banked. In addition, attempts should be made to generate and bank herpesvirus saimiri transformed T cell lines. If this is not possible at the patient's home institution, contact the study coordinator to arrange generation of cell lines and storage (Section 7.6.1 in Supplementary table of contents).

Investigational Genetic Testing

Genetic testing is not a prerequisite for entry into the study. In many patients, genetic testing will be initiated or continued after enrolment. For those patients in whom no genetic diagnosis can be established, a sample of pre-transplant genomic DNA will be stored locally according to a standard protocol. It is expected that next generation sequencing methods will allow complete Exome/Genome Sequencing at prices around $1000 within the course of the study. This will therefore eventually be the preferred approach to exclude/confirm mutations in the then known genes associated with combined immunodeficiency. Whether bioinformatics development will follow a similar pace and whether this approach will therefore be useful in the identification of new genes is still open (Section 7.6.2 in Supplementary table of contents).

Immune Status Testing (Standard of Care)

Rationale for Immune Status Testing

It is anticipated that the assessments requested in this study will be completed at the participating centers as standard of care for a patient with P-CID. Tests will be performed according to local practice. The immunologic methods used for these tests will involve *in vitro* studies of blood serum and mononuclear cells (MNC) from P-CID patients, using phenotypic and functional assays of cellular and humoral immunity and molecular studies to evaluate the molecular types of P-CID. A major goal of this prospective study will be measuring **key biomarkers** of T, B and NK cell numbers and function to determine if they predict the clinical course, i.e. the occurrence of severe events and the eventual need for HSCT (Section 7.7.1 in Supplementary table of contents).

Immunologic Tests

Immunologic testing is important for assessment of the function of the immune system for all patients with P-CID. A meeting of the coordinators of the main diagnostic centers within the first year of the study will be initiated with the goal to standardize methodology of T cell analysis as much as useful

and possible. This will include a comparison of standard procedures for storing of biomaterial (Section 7.7.2 in Supplementary table of contents).

Quantitative immunoglobulins: Serum IgG, IgA, IgM and IgE are measured at the local center labs. The reporting center should note whether the patient is currently on IVIG and, if so, the dose and date of last administration.

Isohemagglutinin titers: Naturally arising antibodies to the ABO carbohydrate antigens, induced by gut flora. Measurement of these isohemagglutinin titers will be performed at the local centers. The reporting center should include the patient's blood type.

Immunization response: The capacity to respond to vaccination will also contribute to the B cell evaluation. Pre- and post-immunization antibody responses to both protein (tetanus and polio, varicella and measles) and polysaccharide (pneumococcal serotypes) antigens will be evaluated.

PBMC phenotyping: PBMC phenotyping will be performed at the local centers on whole blood lysed samples using a locally evaluated fluorochrome design for each monoclonal antibody combination. The following combinations will be used: 1) B cells, NK cells and T cells: CD3/CD16/CD56/CD19; 2) T cells and subsets, activated T cells: CD3/CD4/CD8/HLA-DR ; 3) Naive T cells: CD3/CD4/CD45RA/CD31 or CD3/CD4/CD45RA/CD62L; 4) B cell subsets: CD19 (or CD20)/IgM/IgD/CD27.

Lymphocyte proliferation: Functional studies will include lymphocyte proliferative responses to: PHA (mandatory) and anti-CD3/28 (optional). One recall antigen, such as candida and/or tetanus or other specified antigen (optional) should be analyzed when cell numbers permit. The methodology will vary among labs. However, the results will be made uniform by expressing them as a percentage of the lowest normal control value for that particular laboratory.

TCR Vβ utilization: It is suggested to use a panel of antibodies covering the 7 most frequently used V beta chains (1, 2, 3, 8, 13.6, 17, 21.3) to stain both CD4 and CD8 T cells.

TREC and KREC analysis: During the *de novo* production of T cells in the thymus, the DNA encoding the T cell antigen receptor undergoes a recombinatorial process that results in an intervening segment of DNA being excised as a circular element. These T cell receptor excision circles (TRECs) are present in T cells as they emerge from the thymus, but are lost overtime. Quantification of TRECs shall be used as an index of thymopoiesis and – in transplanted patients - as an early indicator of T cell reconstitution. κ-deletion excision circles (KRECs) are generated during B-cell receptor gene rearrangement, after Vα-Jα rearrangement. The KREC is analogous to the TREC in its stability and thus dilutional nature. It can be used as a measure of mature B-cell homeostasis. TREC and KREC analysis will be done by collecting blood spots similar to the method used for the newborn screening (one circle of the filter card has to be filled with a drop of fresh venous or capillary blood). The cards (Whatman paper 907) will be mailed in a platic bag to the CCI and stored there in the dark at 4°C until analysis. Analysis will be performed in larger batches by Dr. Stephan Borte at the University of Leipzig. The test results will be batched and not reported back to the centers in real time.

A consensus conference for the standardization of immunological assays relevant for the investigation of patients with CID is part of this study and is scheduled for January 2012 (Section 7.7.2 in Supplementary table of contents).

Immunological tests performed after HSCT

Immunization response: When subjects develop initial signs of B cell reconstitution post-transplant [when any two of: 1. serum IgA>15 mg/dL, 2. IgM isohemagglutinin titers >1:4, or 3. appearance of switched memory B cells (CD19+/CD27+)], administration of IVIG will be stopped for 3 months. Then, routine clinical vaccinations (tetanus, diphtheria, and pneumococcus) will be administered. The center will provide the pre- and post-immunization antibody titers. The center will also include information regarding the time frame following immunization that the titers were obtained as well as information regarding the timing of the pre and postimmunization antibody titers relative to IVIG administration (Section 7.7.3 in Supplementary table of contents).

Assessment of Chimerism: The extent of engraftment may be determined by measuring donor chimerism in peripheral blood, or for selected cell lineages. Chimerism analysis will be performed on positively selected circulating T cell, B cell, Myeloid, and NK cell populations by restriction fragment length polymorphism (RFLP), short tandem repeats (STR) or fluorescence in situ hybridization (FISH) at 6 and 12 months by the center performing the transplant (or the supporting lab used by the center) for reporting purposes in this project (Section 7.7.3 in Supplementary table of contents).

Advanced immune function studies using fresh and archived samples (investigational)

Further characterization of phenotypic and functional abnormalities in immune cells will help to establish a molecular diagnosis in P-CID patients. This may include Ca-flux measurements, studies of signalling events in response to stimulation via antigen, stimulatory/inhibitory or cytokine receptors, studies of cytokine production, T reg function, cytotoxicity or apoptosis and others. At the CCI, an advanced diagnostic T cell signalling platform has been initiated that focuses on developing miniaturized Western Blots for the detection of proteins and protein interactions in patients with PID. Analysis from this platform will be made available to all study participants upon request. A list of laboratories offering particular tests is available via the P-CID website to all participants and fresh samples can be sent for functional analysis. In addition, a decentral biomaterial archive will be established, that is available to the core labs of the P-CID study for research studies related to the molecular basis of these diseases. The archive will include: frozen PBMC, fibroblast/EBV/HVS cell lines, a serum archive, and samples for whole blood gene expression profiling. Access to biomaterial will be granted by the P-CID study steering committee upon presentation and review of a study proposal (Section 7.7.4 in Supplementary table of contents).

Premature Termination or Suspension of a Study

Safety Reporting

This is a non-interventional study without any study-specific procedures apart from collection of questionnaire data. All samples are to be taken additionally during routine procedures. Therefore, there is no safety reporting as defined for interventional studies and as defined through ICH Guideline E2A: Clinical Safety data Management (Section 8.1 in Supplementary table of contents).

Premature Termination or Suspension of a Study

If the study is prematurely terminated or suspended for any reason, the study coordinator will promptly inform the participating investigators. Where required by the applicable regulatory requirements, the competent authority(ies) and the ethics committee(s) will also be informed. The

investigator has the right to terminate the study at one of the centres (Section 9 in Supplementary table of contents).

Data Collection and Management

Details on data management (procedures, responsibilities, data corrections) will be described in a data management plan. During the study, the performance of data management and any deviations from the data management plan will be documented in a data management report. Information on patient identity will be kept separately (see 6.3). All further personal and medical information in the register will be collected and saved in pseudonymized form. The register database will contain clinical information of the questionnaires as well as immunological/laboratory test results. All information on biomaterial storage will be kept separately in a biobank log (patient identification number, place of storage, documentation on entry and shipments). SAS software will be used to review the data for completeness, consistency and plausibility (Section 10 in Supplementary table of contents).

Quality Assurance System

Study Management

P-CID Steering Committee Responsibilities

Coordinating Investigator, Coordination Center and Steering Committee representatives will meet yearly at the EBMT IEWP meetings and every two years at the ESID meeting to discuss study status, recruitment, compliance, deviations, review data issues or interim analyses, clinical centre participation, and any new concerns (Section 11.1.1 in Supplementary table of contents).

Investigator Responsibilities

Each investigator is required to keep accurate records to ensure the conduct of the study is fully documented. The investigator is required to ensure that all case report forms (CRFs) are completed for every participant entered in the trial. The period of record retention should be consistent with the record retention policies of the applicable regulatory agencies (Section 11.1.2 in Supplementary table of contents).

Quality Control (Monitoring)

No site-monitoring with regular visits has been planned. Quality control will be guaranteed through central monitoring and a defined query process which is described in detail in the data management plan. Nevertheless, single centers might be visited if the coordinator decides that visits are necessary and helpful for the conduct of the study. In such case, the investigator will grant the study personal access to the patients' personal medical records for the verification of the proper documentation of study data. The CRA is bound by medical secrecy when comparing the CRFs with the source documents. The investigator must allow sufficient time for these visits. Between visits, the project coordination of the study will maintain regular telephone or e-mail contact with the study centres (Section 11.2 in Supplementary table of contents).

Biostatistical Planning and Analysis

Study Design

This study will be performed as a prospective observational cohort study of patients with P-CID. The study cohort will be heterogenous with respect to the presence and the nature of the molecular diagnosis, which implies that the extent of T cell deficiency will be variable. To address this issue, the numeric and/or functional T cell deficiency will be quantified in each patient by a set of 4 simple parameters that can be determined in each centre. These parameters will be obtained at diagnosis

(retrospectively), and prospectively at study entry and yearly thereafter. The different molecular diagnoses will also imply that in some patients, elements of the immune system in addition to T cells are affected, which will increase the risk for severe events. This can include B cells, NK cells and myeloid cells. Baseline tests for these additional parameters will also be performed to control for these confounders. Another important issue is that patients will have a different age at the diagnosis of P-CID and a different age at study entry. Therefore, the time of exposure will differ and will have an impact on the frequency of severe events prior to study entry. Events prior to study inclusion are therefore summarized in a morbidity score that is obtained at study inclusion and that is weighted for the time of exposure (i.e. chronological age and time of first severe event). Delayed entry will be acknowledged to avoid the length bias and HSCT will be treated as a time-dependent exposure (treatment) to avoid the time-dependent bias *(28,29)*

The decision for HSCT remains under the control of the treating investigator. This decision will be based on the number and severity of severe events and the immune status of the patient, but factors such as the family decision, history of affected relatives, donor availability and center policy will also have an important impact. The latter variables will be independent of the disease status and will introduce variability into transplant decisions that can be used for comparative analysis. Thus, patients with a similar disease status will be transplanted or not transplanted depending on these variables and can be used as matched pairs for outcome analysis.

Patients undergoing HSCT will also be analyzed with a second set of endpoints (12.2.4) to evaluate of the outcome of HSCT. Power analysis and statistical analysis as described in 12.4. and 12.5. only consider the primary set of endpoints. The analysis of the second set of endpoints will be part of the detailed statistical analysis plan (Section 12.1 in Supplementary table of contents).

Target Variable/Endpoints

Primary Target Variable

The primary endpoint is overall survival determined after year 5. The event analysed is death from any cause. The time to this event is the time from the first major infection or major manifestation of immune dysregulation (documented retrospectively at the time of diagnosis) to death.

The major aim is to compare three groups: patients with primary HSCT (expected 40%), secondary HSCT (expected 30%) and no HSCT (expected 30%). HSCT is a time-dependent exposure (i.e. group membership is time-dependent) and may occur after study entry. The following variables will be considered as potential baseline confounders:

- severity of T cell abnormalities (biomarkers, especially all T-cell markers)
- severity of other immumological abnormalities (mainly NK cell and B cell markers)
- severity and frequency of severe events (severe infections, severe manifestations of immune dysregulation, malignancy) before entry into the study
- age at first severe event
- age at diagnosis

The frequency and severity of severe events after entry into the study will be considered as a time-dependent confounder. (Section 12.2.1 in Supplementary table of contents).

Secondary Target Variables

The secondary endpoint is the time point of HSCT. The time to this event is the time from the first major infection or major manifestation of immune dysregulation to HSCT. Death without HSCT is a competing event. The variables listed in 12.2.1 will be considered as risk and prognostic factors (Section 12.2.2 in Supplementary table of contents).

Frequency of severe events

The tertiary endpoint is the frequency of severe events during the observation period. The time to the first severe event is the time from study entry. Furthermore, the frequency of severe events during the observation period (including events before, during and after HSCT) will be recorded. Death is a competing event (Section 12.2.3 in Supplementary table of contents).

Endpoints for Patients undergoing HSCT

Primary endpoint is the time point when immune reconstitution is achieved.
Secondary endpoint is engraftment assessed at 6 and 12 months after HSCT.
Tertiary endpoint is the clinical outcome based on follow-up data obtained at 6 and 12 months.
(Section 12.2.4 in Supplementary table of contents).

Sample Size Calculation

We assume that we include 200 patients in total in the study, 40% (80 patients) will receive primary HSCT, 30% (60 patients) secondary HSCT and 30% (60 patients) no HSCT. (Patients receiving alternative therapies (PEG-ADA and gene therapy) are not included in the power calculations/sample size calculation nor in the statistical analysis). We further assume a 5-year mortality of 20% (40 patients).

For the primary endpoint (impact of HSCT on death), we performed a power analysis (PROC POWER from the statistical software SAS 9.12) for 5-year mortality and assumed a Pearson Chi-square test for two proportions. Assuming a reference mortality of 20%, 60 patients per group are necessary to detect a relative risk of 2.2 with power of 80% (Section 12.3 in Supplementary table of contents). See below for more details:

Fixed Scenario Elements	
Distribution	Asymptotic normal
Method	Normal approximation
Reference (Group 1) Proportion	0.2
Sample Size Per Group	60
Number of Sides	2
Null Relative Risk	1
Alpha	0.05

Computed Power		
Index	**Relative Risk**	**Power**
1	2.0	0.670
2	2.1	0.747
3	2.2	0.813
4	2.3	0.867
5	2.4	0.909
6	2.5	0.941

For the secondary endpoint (risk and prognostic factors for the need of HSCT), we performed a power analysis (PROC POWER from the statistical software SAS 9.12) for the occurrence of HSCT within a small interval (e.g. within 6 months) and assumed a Pearson Chi-square test for two proportions. Assuming a reference incidence of 30% within a fixed time frame (e.g. 6 months), 200 patients in total (group weights 1:4) are necessary to detect for instance a relative risk of 1.9 with power of 88%. See below for more details:

Fixed Scenario Elements	
Distribution	Asymptotic normal
Method	Normal approximation
Reference (Group 1) Proportion	0.3
Nominal Total Sample Size	200
Number of Sides	2
Null Relative Risk	1
Alpha	0.05

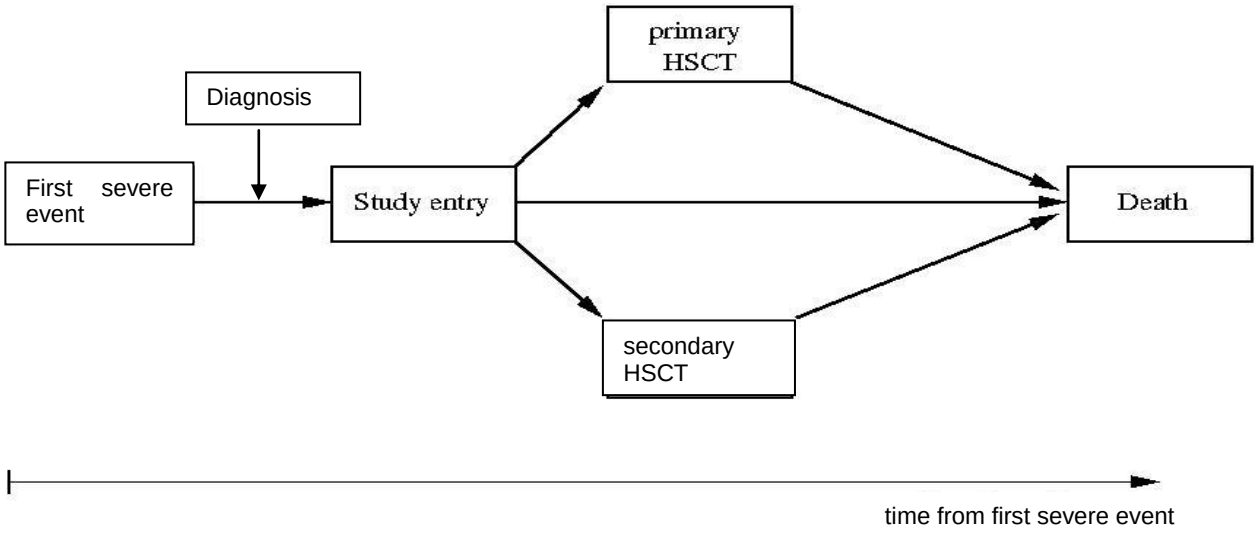
Computed Power

Index	Relative Risk	Weight1	Weight2	Actual N Total	Power
1	1.8	1	1	200	0.936
2	1.8	1	2	198	0.904
3	1.8	1	3	200	0.852
4	1.8	1	4	200	0.791
5	1.8	1	5	198	0.725
6	1.9	1	1	200	0.975
7	1.9	1	2	198	0.958
8	1.9	1	3	200	0.925
9	1.9	1	4	200	0.880
10	1.9	1	5	198	0.827

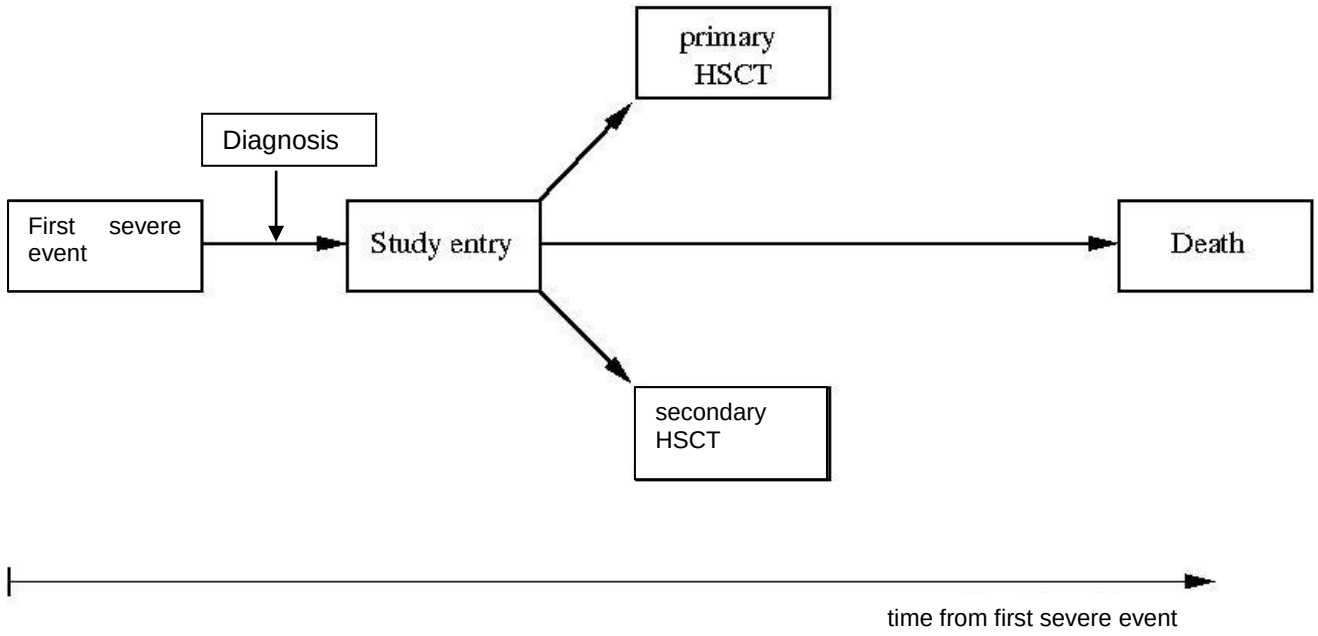
Methods of Analysis

Impact of HSCT on death (survival analysis using multistate models):

The following multistate will be considered and studied. Each arrow represents a hazard from one state to the other. Left-truncation (delayed entry) will be addressed. Primary and secondary HSCT will be treated as time-dependent exposures. Cumulative death hazards will be plotted for patients with primary HSCT, secondary HSCT and no HSCT and pairwise compared. To calculate death hazard ratios and account for confounding, a Cox regression model with time-dependent covariates will be applied (Putter et al 2008) (Section 12.4 in Supplementary table of contents).



Risk and prognostic factors for HSCT (survival analysis using multistate models):
The following multistate will be considered and studied. Left-truncation (delayed entry) will be addressed. Two competing risks models (for cause-specfic and subdistribution hazards) will be fitted to the data. Death without HSCT will be treated as a competing event. Appropriate regression models will be applied to study the risk and prognostic factors for HSCT.



Impact of HSCT on (frequency of) severe events (analysis of survival data with recurrent events):
This question will be addressed in two steps. First, we model the time to the first severe event and treat death as competing event. In the second step we also look at the frequency of severe events and will perform three models for analysing recurrent events: the intensity model (30), the proportional means model (31) and the conditional model (32). Left-truncation (delayed entry) will be addressed. Death without HSCT will be treated as a competing event (Section 12.4 in Supplementary table of contents).

Interim Analysis

An interim analysis is planned for October 2013, a time point when approximately half of the patients (100 patients) will have been recruited. The interim analysis and interim report will allow to re-evaluate the assumptions made in the sample size calculation and to re-evaluate the power of the study. An interim analysis of the data will be made as outlined in 12.4 in order to identify early trends (Section 12.5 in Supplementary table of contents).

Ethical and Legal Principles

Subject Informed Consent

Before enrolment in the study, the subject will be informed that participation is voluntary and that he/she may withdraw at any time without having to give reasons and without penalty or loss of benefits to which the subject is otherwise entitled.

The subject will be given ample time and opportunity to obtain answers to any open questions. All questions should be answered to the satisfaction of the subject and/or his/her legal representatives. In addition, the subject will be given a "Subject Information Sheet“, which contains all the important information in writing.

The subject's written consent must be obtained before any register-specific tests. For this purpose, the written consent form will be personally dated and signed by the trial subject/both legal representatives and the physician conducting the informed consent discussion.

By signing the consent form, the subject agrees to voluntarily participate in the register and declares that he/she agrees to be contacted in approximately yearly intervals. By signing the form, the subject also declares that he/she agrees to the recording of personal data, particularly medical data, for the register after pseudonymisation.

After signing, the subject will be given one copy of the signed and dated written consent form and any other written information to be provided to the subjects (Section 13.1 in Supplementary table of contents).

Ethical and Regulatory Requirements

The applicable national regulatory requirements (ethics committee, competent authority) will be complied with, according to the applicable legal requirements of the country concerned.

– If necessary, the investigator will be responsible for obtaining an extension of the ethics committee's approval in the course of the study.

In Germany, a favourable opinion from the competent ethics committee must be obtained before a study is started (Section 13.2 in Supplementary table of contents).

<u>Ethics Committee/ Competent Authority</u>

Before this study is started, the sponsor will apply for the following, according to the applicable legal requirements. In Germany all investigators (including sub-investigators) joining the team subsequently have to provide their relevant investigator qualification documents to the local ethics committee (Section 13.2.1 in Supplementary table of contents).

Data Protection and Confidentiality

The pertinent provisions on data protection must be fully complied with.

The trial subjects will be informed of the purpose and extent of the collection and use of personal data, particularly medical data.

Findings obtained in the course of the cohort study will be stored on electronic media and treated in strict confidence. For the protection of these data, organisational measures have been taken to prevent disclosure to unauthorised third parties. For example, the subject data will be captured in

pseudonymized form throughout the documentation and evaluation phase (Section 13.3 in Supplementary table of contents).

Archiving

After completion of the register, the Subject identification log and all essential study documents will be retained at the study centre for 10 years. The investigator will be responsible for the storage (Section 13.4 in Supplementary table of contents).

Reports and Publications

Study Reports

After completion of the analysis by the responsible biostatistician, the coordinating investigator will prepare and sign the final integrated medical and statistical report jointly with the biostatistician.

Except when required by law, no one will disclose a result of the study to third parties unless all parties involved have first agreed on the results of the analysis and their interpretation.

The final study report will be written and signed in co-operation between the coordinating investigator and the P-CID steering committee (Section 14.1 in Supplementary table of contents).

Publication

The right of publication rests primarily with the coordinating investigator, the P-CID steering committee and the other investigators involved. All data collected in connection with the study will be treated in confidence by coordinating investigator and all others involved in the study, until publication. Interim data and final results may only be published (orally or in writing) with the agreement of the coordinating investigator, the P-CID steering committee and the other investigators. This is indispensable for a full exchange of information between the above-named parties, which will ensure that the opinions of all parties involved have been heard before publication. The agreement, which does not include any veto right or right of censorship for any of the parties involved, may not be refused without good reason (Section 14.2 in Supplementary table of contents).