Study protocol

Phase II/III Study of Allogeneic Hematopoietic Stem Cell Transplantation From Unrelated and Haploidentical Donors After TCR Alfa Beta Negative Selection in Pediatric Patients With Primary Immunodeficiency Diseases

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1. Background

For several decades now, hematopoietic stem cell trans- plantation (HSCT) has been the main curative treatment modality for patients with various primary immunodeficiency (PID) syndromes (1). Because only a small proportion of patients with inherited diseases have an HLA-identical related donor, transplantation of stem cells from a matched unrelated donor (MUD) or a haploidentical mismatched related donor (MMRD) is a widely used variant of HSCT. The most serious complications of HSCT from an alternative donor are acute and chronic graft-versus-host disease (GVHD) and graft failure for haploidentical donor HSCT (especially for non-SCID PID), which often leads to prolonged morbidity and late immune reconstitution with secondary immunodeficiency. Despite the fact that the frequency of acute GVHD after alternative donor HSCT does not exceed 20%, the incidence rate of life-threatening acute GVHD (higher than grade II) is more than 10% (2), (3). The use of immunomagnetic technology for T cell depletion or positive CD34 selection is a routine method of GVHD prevention after haploidentical HSCT. However, this method leads to prolonged immunoincompetence in patients, resulting in a high incidence of infections and associated complications, which affect the outcome of HSCT (4).

TCR alpha/beta depletion coupled with CD19 graft depletion is a promising technology, potentially capable of reducing the incidence of acute and chronic GVHD and enhancing immune reconstitution (5). T cells with alpha/beta -chain TCRs are the major inducers of GVHD, and their elimination from the graft can decrease the frequency of this complication (6). At the same time, T cells with gamma/delta-chain TCRs combine conventional adaptive features with rapid innate-like responses that place them in the initiation phase of immunoreactions (7). These cells are able to recognize target cells through MHC-independent mechanisms involving activating receptors (eg, gamma/delta TCR, NKG2D, TLRs, and DNAM-1), resulting in the rapid production of cytokines (IFN-g and tumor necrosis factor-a) and a cytotoxic response. Because their immunologic response does not depend on MHC, gamma/delta T cells, in contrast to alpha/beta T cells, play a limited role in the development of GVHD, the pathogenesis of which is based on HLA alloreactivity. Conversely, gamma/delta T cells have high activity against intra- and extracellular pathogens (8), (9).

2. Study Design

2.1 Study Type

Interventional, Phase II/III, open-lable

2.2 Primary Purpose

To evaluate safety and efficacy of allogeneic hematopoietic stem cell transplantation from unrelated and haploidentical donors TCR alpha beta graft depletion in pediatric patients with primary immunodeficiencies.

2.3 Secondary purposes

- to evaluate overall and event-free survival (at 2 years post-HSCT)
- to estimate the risks of serious adverse events (at 1 year post-HSCT)
- to evaluate the rate of primary engraftment and risks of graft failure (at 2 years post-HSCT)
- to estimate risks and severity of acute and chronic graft versus host disease (at 1 and 2 years post-HSCT, respectively)
- to estimate risks of viral infections (at 1 year post-HSCT)
- to evaluate transplant related mortality (at 2 years post-HSCT)
- to estimate a kinetics of immune recovery (at 2 years post-HSCT)
- to estimate levels of donor chimerism (at last follow up)

2.4. Primary endpoint

1. Overall survival (OS) 2 years after HSCT, defined as time from transplantation to death of any cause

2.5. Secondary endpoints

2. Cumulative incidence of transplant-related mortality 1 year after HSCT

3. Cumulative incidence and severity of acute GVHD II-IV grade 1 year after HSCT

4. Cumulative incidence of chronic GVHD 2 years after HSCT

5. Number of participants, who reached immune recovery (CD3+, CD19+ lymphocytes subsets and intravenous immunoglobulin independence) 2 years after HSCT

6. Percentage of patients with full (more than 90%) donor chimerism among survivals at last follow-up

7. Cumulative incidence of CMV reactivation 1 year after HSCT

2.6. Inclusion Criteria

- Patients aged ≥ 1 months and < 19 years
- Patients diagnosed with Primary Immunodeficiency Diseases eligible for an allogeneic transplantation and lacking a related HLA-matched donor
- Lansky/Karnofsky score > 40, WHO > 4
- Signed written informed consent

2.7 Exclusion Criteria

- Dysfunction of liver (ALT/AST > 5 times normal value, or bilirubin > 3 times normal value), or of renal function (creatinine clearance < 30 mL / min)

2.8 Therapeutic plan

Conditioning regimen:

Drug	Total dose	Days
Treosulfan	42g/m2 (36g/m2 for patients under 1 year of age)	-5, -4, -3
Fludarabine	150mg/m2	-6, -5, -4, -3, -2
ATGAM	90mg/kg	-5, -4

Post-transplant GVHD prophylaxis – calcineurin inhibitors based in combination with methotrexate 5 mg/m2 on days +1, +3, and +6 or MMF - mycophenolate mofetil 30 mg/kg till day +60 post-HSCT.

3. Methods

3.1 Graft processing

Hematopoietic stem cell grafts will be derived from the granulocyte colony-stimulating factor -mobilized peripheral blood mononuclear cell fraction. Depletion of TCR alpha beta T cells and B cells (CD19) will be performed with CliniMACS Plus instrument (Miltenyi Biotec, Bergisch Gladbach, Germany). After depletion procedure, the numbers of nucleated cells CD34+, CD3+ and CD3+TCRab+ will be estimated in final product.

3.2 GVHD, Viral infections and Serious adverse events

Acute and chronic GVHD will be diagnosed and graded using standard guidelines (10).
For cytomegalovirus (CMV) and Epstein-Barr virus (EBV) detection in blood and other biologic liquids quantitative real- time polymerase chain reaction (PCR) analysis will be used. CMV and EBV levels will be routinely monitored weekly until CD41 T-cell recovery was above 0.3 x 10^9/L. CMV disease will be identified with the detection of CMV in affected tissue.
To grade the severity of serious adverse events, the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) will be used.

3.3 Engraftment, Chimerism and Graft Failure

Neutrophil engraftment will be registered on the first day of an absolute neutrophil count above 0.5 x 10^9/L, platelet engraft-ment on the first of 5 subsequent days of platelet counts more than 50 x 10^9/L without platelet transfusions, as well as by full donor chimerism at day +30.
Graft failure includes all cases of non-engraftment and graft rejection after demonstrated engraftment with the detection of more than 90% of the patient's chimerism in peripheral blood or bone marrow.
Whole peripheral blood and CD3+ lineage chimerism assessments will be performed monthly until day + 180 and then performed once every 3 months until day + 360. For chimerism testing in-house, a reverse transcriptase PCR system for the detection of short

tandem repeat markers will be used. To demonstrate graft failure additional analysis of bone marrow CD34+ cells will be assessed.

3.4 Immune reconstitution

For immune recovery kinetics in whole blood will be analysed monthly until day + 180 and then once every 3 months until day + 360 routinely. Patients with no demonstrated immune recovery at 1 yeas after HSCT will be monitored via individual protocols. Lymphocyte subsets analysis will be performed via flow cytometry. Routinely monitored cell lines include: CD3+, CD19+, CD3-CD16+CD56+, CD3+CD16+CD56+, CD3+TCRalpha/beta+, CD3+TCRgamma/delta+, CD3+CD45RA+CD197+.

3.5 Statistical analysis

The patients will be censored at the time of death or at the last FU in survivors (the minimum FU period in survivors was 0,5 years).

The probability of overall and event free survival (OS and EFS) will be estimated by the Kaplan-Meier method. For EFS analysis as events will be considered graft failure, deaths, severe graft dysfunctions due to mixed chimerism, severe organ damage due to HSCT complications. The probabilities of acute and chronic GVHD, graft failure, viral reactivations and transplant-related mortality will be estimated with cumulative incidence curves (considering competitive risks, e.g., graft failure or death).

4. References

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