Gene therapy for PNP deficiency protocol

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ABSTRACT

Purine nucleoside phosphorylase (PNP) is a key enzyme required for the degradation of purine nucleosides into uric acid or their salvage into nucleic acids. Patients who are deficient in PNP suffer from progressive T cell immunodeficiency, with increased susceptibility to infections, autoimmunity, and neurologic abnormalities. In the absence of successful treatment to restore immune function, these patients rarely survive to adulthood. Hematopoietic stem cell transplantation is the only known cure for PNP deficiency. Use of an HLA-matched donor is preferable as the outcome with alternative donors have been variable; however, this option is rarely available.

Gene therapy represents a therapeutic option that bypasses the need for a donor, and thus associated complications. Although first generation γ-retroviral vectors have been successful in some immunodeficiencies, in others, evidence of insertional mutagenesis prompted a halt in their use. More recently, the introduction of safer lentiviral vectors holds promise in offering a viable option to treat immunodeficiency.

Here, we present a clinical trial protocol utilizing self-inactivating lentiviral vectors to treat PNP deficiency. Patients will be evaluated up to 3 years post-transplantation to determine the safety of lentiviral-treated stem cell infusion, as well as the extent of immune reconstitution.

Statement of novelty: This protocol describes the novel treatment of PNP deficiency using lentiviral-based gene therapy.

Study summary

Purpose

This is a Phase I/II clinical trial to study the safety and efficacy of gene therapy in purine nucleoside phosphorylase (PNP) deficiency. $CD34^+$ stem cells derived from PNP-deficient patients will be transplanted after the transduction of normal PNP cDNA using lentiviral vector.

The primary outcome is to determine the safety of infusing lentiviral-treated stem cells, while the secondary outcome will be assessment of reconstitution of mature lymphocytes carrying the normal PNP gene.

Rationale

Profound T cell immunodeficiencies are life-threatening conditions which require urgent assessment and consideration of curative measures, such as hematopoietic stem cell transplantation. Many of these infants, but not all, can be identified shortly after birth by screening for T cell receptor excision circle (TREC) levels (Buckley 2012), an indication of thymic function and T cell development (Douek et al. 1998). The most studied subgroup of these patients are infants born with severe combined immunodeficiency (SCID). Prior to the implementation of newborn screening (NBS), and in jurisdictions where NBS is unavailable, infants with SCID typically present in the first year of life with viral or fungal lung infections, chronic diarrhea, and failure

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to thrive (McWilliams et al. 2015). Immune evaluation in these patients reveals profound T cell lymphopenia according to the underlying genetic condition afflicting these patients (Shearer et al. 2014).

Over the past two decades there has been a growing recognition that profound T cell immunodeficiency (combined immunodeficiency, CID), unlike typical SCID, can result in significant residual immune function and sometimes normal numbers of circulating lymphocytes, as well as variable in vitro and in vivo immune function (Roifman et al. 2012). Some of these patients may present clinically like SCID while others can present later in childhood with recurrent infections, autoimmune manifestations, or atopic features. Unfortunately, many of these conditions cannot be detected by TREC-based NBS. The treatment of choice for SCID and for most cases of CID is hematopoietic stem cell transplantation (Pai et al. 2014). Use of an human leukocyte antigen (HLA)-matched related donor has previously been reported to result in the best outcome (Caillat-Zucman et al. 2004). In the absence of such a donor, matched unrelated donors (Dalal et al. 2000; Grunebaum et al. 2006) and sometimes mismatched related donors have also been used successfully (Beatty et al. 1985; Haddad et al. 1998).

PNP deficiency (OMIM #613179) is an autosomal recessive progressive immunodeficiency characterized by susceptibility to opportunistic infection, neurologic abnormalities, and autoimmunity (Markert 1991). The onset may be delayed beyond infancy. Immune evaluation varies from patient-to-patient as well as over time in the same individual. T cell lymphopenia is progressive, while in vitro function as well as TREC levels may be low or within normal range. This CID is caused by mutations in the PNP gene at 14q13.1, which encodes a key enzyme involved in the purine salvage pathway (George and Francke 1976). The features observed in this immunodeficiency are likely caused by the consequent accumulation of purine metabolites (Mitchell et al. 1978; Ullman et al. 1979).

The only known curative treatment for PNP deficiency is hematopoietic stem cell transplantation, preferably with an HLA-matched related donor (Delicou et al. 2007). Experience with alternative donors has been inconsistent, with only few reports suggesting successful engraftment after matched unrelated cord or bone marrow transplants, albeit after repeated transplants (Grunebaum et al. 2013; Brodszki et al. 2015).

It is fair to assume that, similar to adenosine deaminase (ADA) deficiency, the more common purine metabolism defect, transplants other than full sibling HLA-matched donors may pose a great challenge (Hassan et al. 2012). This prompted the recent effort to offer a safer alternative by using gene therapy (Fischer et al. 2013). Gene therapy for ADA deficiency, using γ-retroviral vector based gene transfer, has been studied for more than two decades and was found to be safe and frequently effective (Candotti et al. 2012). However, whether full immune reconstitution occurs as well as the durability of transduced stem cells remains unknown. In contrast, use of γ-retroviral vectors was found to induce leukemia in other conditions, leading to termination of these studies (Hacein-Bey-Abina et al. 2008; Howe et al. 2008). Lentiviral vectors might be safer as they can be self-inactivating through deletion of viral regulatory elements, as well as allowing the use of cellular promoters (Aiuti et al. 2002; Gaspar et al. 2006; Candotti et al. 2012).

We study here for the first time the safety and efficacy of lentivirus-based PNP gene transfer for the treatment of PNP deficiency. We have previously demonstrated that lentivirus transduction of the PNP gene into murine PNP-deficient stem cells was effective (Liao et al. 2008).

Principles of screening and enrollment

1. Only patients who have no HLA-matched sibling donor will be eligible for enrollment.
2. Study protocol will be described to family members by the Principal Investigator.
3. Patients' guardians will sign informed consent.
4. Patients must meet inclusion criteria.

Inclusion criteria

1. Low PNP activity in patient red blood cells and a PNP gene mutation
2. Evidence of combined immunodeficiency including lymphopenia and abnormal phytohemagglutinin (PHA) responses (stimulation index <300)
3. Older than 6 months of age
4. Absence of HLA-identical sibling donor.

Exclusion Criteria

1. Age greater than 6 years
2. Known sensitivity to Trisulfan
3. Availability of a related HLA-identical donor
4. Refusal to sign consent forms by guardians.

Study plan

Screening

1. History and physical examination
2. Renal and liver function tests
3. CBC with differential and blood film
4. Prothrombin time (PT)/Partial thromboplastin time (PTT)
5. Viral screen for HIV-1, hepatitis B, hepatitis C, cytomegalovirus (CMV), Epstein-Barr virus (EBV) and parvovirus (by DNA polymerase chain reaction)
6. Pregnancy test
7. Urine chemistry and culture
8. Immune evaluation including flow cytometry, PHA response, TCR-Vβ, TREC analysis
9. Electrocardiogram
10. Echocardiogram
11. Chest x-ray
12. Skin biopsy (growth of fibroblasts) or alternatively skin obtained during administration of central venous device.

Procedure

1. Placement of a central venous access device.

Study treatment

Stem cell harvest

Patients will be treated with erythrocyte colony stimulating factor (ECSF) at a dose of 10mg/kg for 4 days. Stem cells will be then isolated with a target of $>7 \times 10^6$/kg. Ideally, $2–3 \times 10^6$ cells will be used for gene transduction and the rest will be stored for future backup use if required.

Transduced cells will be approved for administration only if:

1. Cell count is $\geq 7 \times 10^6$ $CD34^+$ cell/kg
2. Cell viabilitiy is better than 75%
3. Endotoxic assessment
4. Bacterial and fungal stains negative
5. Culture medium shows no microbial growth.

Conditioning

Trisulfan will be given in a single intravenous administration. Trisulfan will be administered only if harvest is sufficient and $CD34^+$ cells are approved for administration. A dose of 4mg/kg will be administered intravenous 24 hours before the administration of transduced $CD34^+$ cells.

Follow up

Patients will be evaluated monthly for the first 4 months after transplantation and quarterly thereafter up to 3 years.

Follow up includes:

1. Physical exam and history
2. CBC, liver and renal function tests
3. Viral PCR tests
4. Monitoring for insertional leukemia (twice a year only)
5. Flow cytometry, PHA responses, responses to antigens, TRECs, TCR-vβ, immunoglobulins, specific antibodies
6. Analysis of vector integration site
7. PNP enzymatic activity in red cells and measurement of the frequency of cells containing inserted PNP in peripheral blood mononuclear cells (PBMC) or T cells.

Safety assessment

The records from each follow up will be used to determine adverse events. Clinically significant adverse events will be based on and compared to the National Institute of Allergy and Infectious Disease (NIAID) Pediatric AIDS toxicity evaluation to determine the grade of the adverse event.

Administration of back-up stem cells will be triggered by failure to engraft transduced cells by day +120 after autologous transplant infusion based on lack of T cell recovery.

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