Platelet-Targeted FVIII "Pleightlet™" LV-HSC for Severe Hemophilia A

Pre-Clinical Research Supporting a Clinical Protocol for a First-In-Human Trial

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Objective

Hemophilia A (HA) is an inherited bleeding disorder affecting ≈1:10,000 people due to genetic defects on the X-chromosome causing a deficiency of coagulation factor VIII (FVIII). HA is commonly treated with a continuous infusion of FVIII protein replacement therapy. Although there is no cure for HA, there are several promising therapies for HA including: intravenous infusion of naked DNA, genome editing, bi-specific antibodies, and adeno-associated viral gene replacement vectors (AAV) targeting expression of FVIII in liver. Current AAV clinical trials cause FVIII secretion from liver into the plasma. This excludes (≈40%) HA patients with pre-existing antibodies to the AAV viral capsid and (≈30%) patients with inhibitory antibodies (PWIA) that neutralize plasma FVIII as well as persons with pre-existing liver disease. Thus, our goal is to develop a safe, efficient, and effective long-term treatment to control bleeding in HA patients with inhibitory antibodies (PWIA) to Coagulation FVIII.

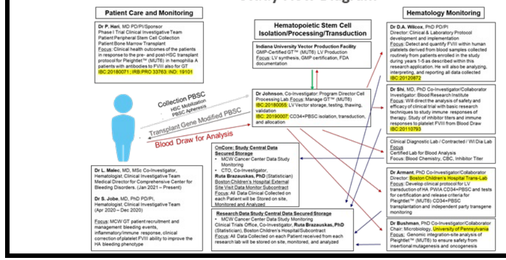
Seminal Pre-Clinical Studies: In Support Of Clinical Efficacy

1. Wilcox DA, et al. *Proc Natl Acad Sci USA.* 1999;96(17)9654-9659. 2. WilcoxDA, et al. *J Thromb Haemost.* 2003;1(12):2477-2489. 3. Shi Q, et al. *J Clin Invest.* 2006;116(7):1974-1982. 4. Shi Q, et al. *J Thromb Haemost.* 2007;5(2):352-361. 5. Du LM, et al. *Nat Commun.* 2013;4:2773.

Fig. 3 Twenty Years of Research

Previous pre-clinical studies showed that LV-HSC driven by the *(ITGA2B)* megakaryocyte-specific gene promoter permits FVIII to traffic into platelet α-granules leading to long-term improved hemostasis in human cells in tissue culture and murine and canine models of Hemophilia A *(even in the presence of inhibitory antibodies in mice and without eliciting an immune response to FVIII in mice and dogs).*

Study Flow Diagram

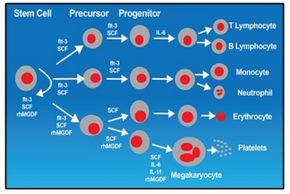


Conclusion

A clinical trial protocol (*ClinicalTrials.gov NCT03818763*) under FDA IND was successfully developed that utilizes LV-transduced autologous HSC encoding FVIII for treatment of HA PWIA. This phase 1 trial is approved for safety testing this treatment for severe HA with potential to improve hemostasis long-term even in PWIA.

Hematopoietic Stem Cell: Target for Hem A Gene Therapy

Fig. 1 Goal: For Platelets to Deliver FVIII



Flt-3, fms related receptor tyrosine kinase 3; FVIII, factor VIII; IL-6, interleukin-6; IL-11, interleukin-11; SCF, stem cell factor; SCF rhMGDF, stem cell factor megakaryocyte growth and development factor.
Figure adapted from Cheng H, et al. *Protein Cell.* 2010;1(1):54-64.

The hematopoietic stem cells (HSC) is an ideal target for gene therapy strategies because it can be safely collected, genetically modified and returned to the patient. Our goal is to develop methods for therapy for cancer by targeting gene products to platelets. As a model, we utilized the *ITGA2B* gene promoter to investigate its ability to drive megakaryocyte-specific expression of coagulation Factor VIII for efficient long-term treatment of Hemophilia A.

Single-Center, Open Label, Phase I Study Of Platelet-targeted Lentiviral Gene Therapy In Adult Male Patients With Severe Hemophilia A With Inhibitors

Objective: Determine safety and feasibility of *ex vivo* lentiviral gene therapy vector Pleightlet™, as well as incidence of sustained platelet engraftment of FVIII-transduced human PBSC seen 30 days after transplant

Key inclusion criteria *

- Adult males >18 years of age with severe hemophilia A and high titer FVIII inhibitors (>5 BU)
- Diagnosis of severe hemophilia A by undetectable plasma FVIII:C by one--stage PTT-based assay and Coatest chromogenic FVIII assay
- Subject may use prophylactic therapy with FVIII bypassing agents or FVIII mimetics prior to referral for inclusion in the study

Key exclusion criteria *

- Medical contraindication to PBSC cytokine mobilization, use of GCSF, PBSC apheresis procedure or conditioning regimen
- Medically significant organ dysfunction that would prevent compliance with conditioning or would severely limit probability of survival based on clinical status

** Full eligibility criteria listed at ClinicalTrials.gov Identifier: NCT03818763*

BU, Bethesda unit; FVIII, factor VIII; GCSF, granulocyte colony-stimulating factor; FVIII:C, factor VIII procoagulant activity; PBSC, peripheral blood stem cell; PTT, partial thromboplastin time.
1. Gene Therapy Trial for Platelet Derived Factor VIII Production in Hemophilia A. https://clinicaltrials.gov/ct2/show/NCT03818763. Accessed 5/3/2021.

Clinical Trial Validation Assay Show Direct Correlation of FVIII:C vs Vector Copy Number

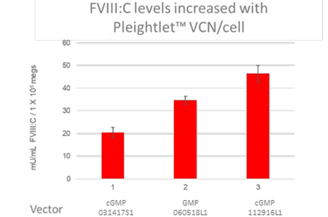


Fig. 4 Summary of Results Part I

Engineering runs were designed to test and validate the ability of GMP clinical-grade LV-HSC to undergo a clinical transduction protocol (CTP) producing human megakaryocytes that synthesize and store FVIII within α-granules for regulated-secretion of FVIII from platelets to establish hemostasis in HA PWIA. Shown is 3 results of 6 engineering runs of the protocol that validate *ex vivo* gene transfer with "Pleightlet™" into normal HSC achieved significant synthesis and storage of biologically active FVIII:C in megakaryocytes *in vitro* in a safe and efficient manner that was directly proportional to LV copy number/HSC.

For More Information Contact

ClinicalTrials.gov Identifier: NCT03818763 Open for Recruitment

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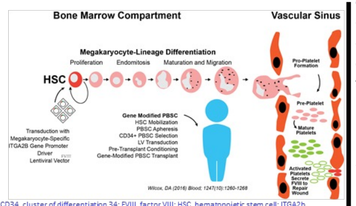
Use of Platelet FVIII Gene Therapy to Establish Hemostasis for Hem A

Fig. 2 Strategy



CD34, cluster of differentiation 34; FVIII, factor VIII; HSC, hematopoietic stem cell; ITGA2b, Integrin Subunit Alpha 2b; LV-HSC, lentiviral hematopoietic stem cell; PBSC, peripheral blood stem cell.
1. Wilcox DA. *Blood.* 2016;127(10):1260-1268.

Our strategy involves transplantation of autologous hematopoietic stem cells (HSC) transduced with a lentiviral vector (LV) encoding platelet-derived FVIII (Pleightlet™). Ectopic FVIII production and storage within platelet α-granules allows storage in an immune privileged fashion and regulated secretion of platelet-derived FVIII to directly repair broken blood vessels.

First-In-Human Protocol Outline

Safety Is The Goal Of The Pleightlet™ HSC GT Phase I Trial

Goal of our clinical protocol is aimed at treating severe hemophilia A patients with inhibitory antibodies to FVIII for highest benefit to risk ratio

- Maximize the benefit of improved hemostasis
- Reduce risk of harm from pretransplant conditioning regimen

– **Three Easy Steps In The Clinic** –

1. Bone marrow stem cells collected from patient by apheresis (outpatient)
2. Patient returns to clinic ~4 weeks (for cell transplant therapy) cells infused
3. Patient is monitored for correction of hemophilia A

FVIII, factor VIII; HSC GT, hematopoietic stem cell gene therapy.
1. Gene Therapy Trial for Platelet Derived Factor VIII Production in Hemophilia A. https://clinicaltrials.gov/ct2/show/NCT03818763. Accessed 5/3/2021

Engineering Shows Pleightlet™ HSC Acceptable for Hem A

Fig. 5 Summary of Results Part II

Results shown from 1 of 6 engineering runs validate that *ex vivo* gene transfer with the LV "Pleightlet™" into normal HSC produced genetically-modified blood stem cell product that passed all release criteria for cell viability, sterility, and absence of recombinant LV that is required to to deliver into HA PWIA.

Disclosures

- This work is supported primarily by the National Heart, Lung, and Blood Institute of the National Institutes of Health (Award Number R01HL142791). The content is solely the responsibility of the author and does not necessarily represent the official views of the National Institutes of Health
- Dr David A. Wilcox, PhD, is President and Founder of Platelet Targeted Therapeutics, LLC
- Dr. Wilcox has an equity interest and intellectual property rights in the company