Blood 144 (2024) 5409–5410

The 66th ASH Annual Meeting Abstracts

ONLINE PUBLICATION ONLY

301.PLATELETS AND MEGAKARYOCYTES: BASIC AND TRANSLATIONAL

Human Gene Therapy Optimized for the Platelet Bleeding Disorder Glanzmann Thrombasthenia

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Introduction: Inherited platelet disorders cumulatively affect ≈1:20,000 people worldwide.Glanzmann Thrombasthenia (GT) is among these due to defects in genes encoding integrin αIIbβ3 subunits that prevent platelet aggregation, leading to recurrent mucocutaneous bleeding episodes and increased risk of morbidity and mortality from gastrointestinal or intercranial hemorrhage. GT is commonly treated with platelet transfusion, although patients often experience immune-mediated destruction of donor platelets. Antifibrinolytic agents and rFVIIa are also used to treat uncontrolled bleeding but require frequent administration. Allogeneic bone marrow transplant (BMT) has been shown to correct the GT phenotype; however, BMT can cause graft vs host disease and excludes patients without a BMT match. Thus, an effective long-term treatment to control bleeding in human GT is essential.
Objective: To develop a safe and clinically relevant gene replacement strategy using autologous hematopoietic stem cells (HSC) genetically-modified with a lentivector (LV) under the transcriptional control of a megakaryocyte-specific gene promoter driving *ITGA2B* or *ITGB3* expression in platelets for long-term hemostasis in GT patients.
Methods: Over the past 25 years, preclinical studies have collectively shown the proof-of-principle for using a retroviral gene transfer vector encoding the *ITGA2B* megakaryocyte-specific gene promoter to drive αIIb and β3 synthesis, leading to a viable αIIbβ3 complex on the platelet surface. This resulted in a functional fibrinogen receptor with potential for improved hemostasis as reported with megakaryocytes derived from human GT HSC *ex vivo*, and platelets derived from HSC of murine and canine models of GT *in vivo*. Remarkably, only 10% of *ITGA2B* altered platelets expressing 7% of normal αIIbβ3 levels were sufficient to improve long term hemostasis in GT dogs with reduced-intensity conditioning. Herein, engineering studies were performed with state-of-the-art transduction conditions to test the ability of our LV to achieve optimal *ITGA2B* transduction efficiency and production of sufficient αIIbβ3 levels on the surface of a human promegakaryocyte cell line (Dami), human (non-GT) HSC, and canine GT HSC to create a GMP-compliant transduction protocol acceptable for treating human GT.
Summary: Engineering studies using a clinically relevant protocol observed improved transduction efficiencies with *ITGA2B-LV* gene transfer into target cells. LV proviral sequence analysis detected in transduced Dami cells, with no base substitutions, deletions, or insertions identified. Thus, the provirus sequence was successfully assembled *de novo* as expected. Immunocytometry with antibodies to αIIb, β3, and αIIbβ3 revealed that *ITGA2B-LV* transduced Dami cells displayed significantly increased mean fluorescence intensity (MFI), representing ~2x normal receptor levels over control samples. Megakaryocytes derived from human HSC showed a 1.5x increased MFI receptor levels over controls. This outcome reveals that ectopic αIIb can compete with endogenous αIIb to form a complex with endogenous β3 to produce a viable αIIbβ3 complex on the surface of human megakaryocytes *in vitro*. Interestingly, megakaryocytes derived from HSC of GT dogs showed a 4x MFI increase in αIIbβ3 levels over controls indicating highly efficient transduction and expression leading to assembly of a viable αIIbβ3 complex on the megakaryocyte surface. Trypan blue analysis showed that megakaryocytes obtained *in vitro* were comparable in number for each sample, indicating that LV did not affect cellular viability. Validation testing including gene insertion analysis and αIIbβ3 functionality are ongoing.

Conclusion: The results of this study show that preclinical data can be translated into a GMP-compliant manufacturing strategy for genetically-modification of HSC with an LV encoding the platelet-specific integrin αIIbβ3. This outcome suggests the potential feasibility to initiate a clinically relevant *ITGA2B-ITGB3* gene replacement protocol, which should be compatible with a reduced-intensity conditioning HSC infusion regimen in GT patients *(adapted from our recent FDA approved clinical protocol for platelet-derived FVIII for Hemophilia A NCT03818763)*. This strategy should lead to the ability to improve hemostasis safely and efficiently for GT and other inherited platelet bleeding disorders.

Disclosures No relevant conflicts of interest to declare.

https://doi.org/10.1182/blood-2024-199243