Personalised CRISPR-Cas9 mediated gene therapy for the treatment of VWD in patient derived ECFCs

Introduction

Von Willebrand Disease (VWD) is the most commonly inherited bleeding disorder. Patients with VWD have qualitative and or quantitate abnormalities with von Willebrand Factor (VWF); and subsequently have diminished haemostatic capabilities. VWF is primarily produced by endothelial cells as a large multimeric protein; which is stored in unique endothelial specific organelles. Common treats such as Desmopressin (DDAVP) aims to stimulate release of VWF from these organelles into the bloodstream; however some patients don't respond and instead rely on ongoing factor concentrates. Gene therapies can provide alternative treatment options, and are already being trialled for other bleeding disorders, such as a Haemophilia. Here we are exploring the potential of utilising gene therapies in VWD using patient derived ECFCs.

Patient derived endothelial colony forming cells (ECFCs) provide a platform to assess the potential of CRISPR-Cas9 in the treatment of VWD; and can provide insight into underlying mechanisms of pathogenic VWD mutations. The main drawback of patient derived ECFCs is their limited growth potential combined with the high variability between donors with respect to growth and isolation success. This can be an issue for VWD type 3, as it is the rarest form of VWF, and so the pool of patients to isolate from can be limited.

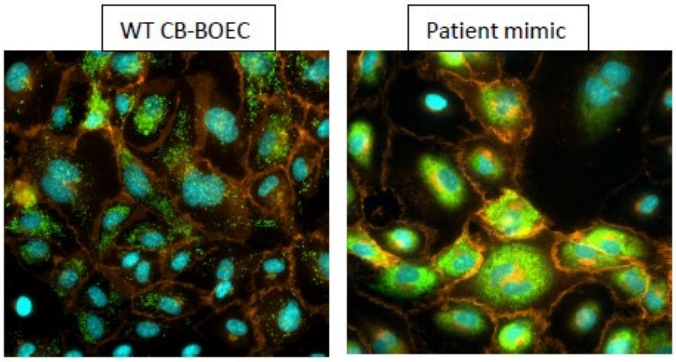
Cord Blood-ECFCs (CB-ECFCs) have strong proliferative capabilities whilst retaining a clear VWF profile; making them an attractive system to study VWD when compared to overexpression systems in other cell types; which cannot fully process VWF.

The project

The aim of this project will be to utilise CRISPR-Cas9 technologies to generate various VWD mutations in CB-BOECs (patient mimics); and then characterise the VWF profile. Through an adenine base editing lentiviral approach, we were able demonstrate this concept by introducing a p.M771V substitution with ~70% efficiency. This material was compared to patient material to verify similarity in aberrant VWF profile (Figure 1 below).

This was further built upon by a student who examined multiple mutations with various gRNAs; and was able to successfully introduce other mutations at around ~20% . This project will build upon this by generating the same mutations with a more optimised approach; and add further mutations to the pool to assess how robust this approach is. Furthermore correction gRNAs can be screened on this material as a proxy for patient material. Outlined below are some of the main techniques involved in the project.

- Culture of primary human endothelial cells
- Molecular cloning
- Lentiviral generation
- CRISPR-Cas9 base editing
- ELISAs
- Multimer Assays
- Microscopy



University students with an interest in bleeding disorders and or gene therapies are encouraged to contact Alastair Barraclough (a.barraclough@sanquin.nl, a.n.barraclough@amsterdamumc.nl)

Masters Student (University or HBO)
Duration: At least 6 months