

# Blood Outgrowth Endothelial Cells (BOECs) and Megakaryocytes (MKs) for in vitro studies of hemostatic and secretory function

No registrations found.

|                              |                  |
|------------------------------|------------------|
| <b>Ethical review</b>        | Positive opinion |
| <b>Status</b>                | Recruiting       |
| <b>Health condition type</b> | -                |
| <b>Study type</b>            | Interventional   |

## Summary

### ID

NL-OMON24571

### Source

Nationaal Trial Register

### Brief title

2020-BOEC-MK

### Health condition

Bleeding disorders

## Sponsors and support

**Primary sponsor:** Erasmus MC

**Source(s) of monetary or material Support:** NWA.1160.18.038 & LSBR bk-18729 - 1707

## Intervention

## Outcome measures

### Primary outcome

1. To establish optimized approaches for the isolation and characterization of BOECs, CD34+-

derived MKs, iPSC- ECs and iPSC-MKs.

2. Dissect the cellular mechanisms that control endothelial and platelet (secretory) function, thereby understanding the pathophysiology of bleeding disorders and the genetic control of VWF secretion using BOECs, platelets, CD34+-derived MKs, iPSC- ECs and iPSC-MKs.

3. To develop in vitro methods to introduce or correct (disease-causing) mutations in regulators

of endothelial or platelet function in BOECs or iPSC-derived ECs/MK using CRISPR-mediated genome engineering.

## Secondary outcome

The study is a basic science, experimental in vitro study that requires the sampling of venous peripheral blood of the participants.

# Study description

## Background summary

Rationale:

Hemostasis is critically dependent on Von Willebrand factor (VWF), a multimeric adhesive plasma protein that is crucial for mediating platelet adhesion to sites of vascular damage and that acts as a chaperone for coagulation factor VIII (FVIII) in plasma. The bulk of plasma VWF is synthesized by endothelial cells (ECs) where it is stored in and released from Weibel-Palade bodies (WPBs). Platelets also store and secrete VWF from alpha-granules after activation, which contributes to thrombus formation. The molecular mechanisms that control secretion of VWF from ECs and platelets are poorly understood. Abnormalities in the (secretory) function of platelets and endothelial cells can lead to bleeding, such as observed in platelet storage pool disorders (SPD), Von Willebrand disease (VWD) or in individuals with "low VWF".

Problem definition: In ~30% of type 1 VWD patients and individuals with low VWF (defined as VWF levels <50 IU/dL) no VWF mutations are found. Another problem is that in around 50%

of bleeding patients referred to tertiary centers the underlying mechanisms that cause clinically

relevant bleeding problems cannot yet be identified, so-called bleeding of unknown cause (BUC). Desmopressin is administered therapeutically to improve platelet function and raise VWF in plasma by inducing WPB exocytosis from endothelial cells. In ~25% of patients desmopressin fails to trigger (sufficient) release of VWF/FVIII for reasons largely unknown.

Research hypothesis: The main hypothesis of this study is that defects in components of the secretory machinery of VWF are causative for reduced VWF levels and lack of desmopressin response. Also, defects in this machinery may be causative in patients with BUC. To identify new determinants of VWF levels and vascular health we will study secretory mechanisms in

ECs and platelets of individuals with abnormalities in hemostatic and/or secretory function. For this we will establish ex vivo (patient-derived) cellular model systems of endothelial and platelet secretion using blood outgrowth endothelial cells (BOECs), platelets, CD34+-derived megakaryocytes and induced pluripotent stem cell (iPSC)-derived endothelial cells (iPSC-ECs) and megakaryocytes (iPSC-MKs).

## **Study objective**

The main hypothesis of this study is that defects in components of the secretory machinery of VWF are causative for reduced VWF levels and lack of desmopressin response. Also, defects in this machinery may be causative in patients with BUC. To identify new determinants of VWF levels and vascular health we will study secretory mechanisms in ECs and platelets of individuals with abnormalities in hemostatic and/or secretory function. For this we will establish ex vivo (patient-derived) cellular model systems of endothelial and platelet secretion using blood outgrowth endothelial cells (BOECs), platelets, CD34+-derived megakaryocytes and induced pluripotent stem cell (iPSC)-derived endothelial cells (iPSC-ECs) and megakaryocytes (iPSC-MKs).

## **Study design**

A maximum of seventy milliliters of blood will be drawn for the isolation of PBMCs, platelets, plasma and DNA. Several parameters will be measured in plasma to characterize the hemostatic and angiogenic profile. BOECs, iPSC-ECs/MKs and CD34+-derived primary MKs will be characterized in vitro. Protein expression profiles will be determined by whole proteome mass spectrometry. DNA will be genotyped for SNPs and mutations in VWF and VWF associated genes.

## **Intervention**

The intervention in this study is venous blood sampling. A maximum of seventy milliliters of blood will be drawn for the isolation of PBMCs, platelets, plasma and DNA.

## **Contacts**

### **Public**

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## Scientific

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## Eligibility criteria

### Inclusion criteria

#### Patients

- ☐ Prior diagnosis of VWD, low VWF, inherited platelet function defects, bleeding of unknown cause or genetic disorders in which dysfunction of the secretory pathway has been implicated in disease pathogenesis.
- ☐ For patients  $\geq 16$  years old; written informed consent
- ☐ For patients 12-15 years old; written informed consent from both the patient and their parent(s)/legal guardian(s)
- ☐ For patients  $< 12$  years old; written informed consent from their parent(s)/legal guardian(s)

#### Family members

- ☐ Age 18 years or older
- ☐ Family member of a patient with VWD, low VWF, inherited platelet function defects, bleeding of unknown cause or genetic disorders in which dysfunction of the secretory pathway has been implicated in disease pathogenesis.
- ☐ Family members may or may not be affected.

#### Healthy controls

- ☐ Age 18 years or older
- ☐ Not previously diagnosed with VWD, low VWF, inherited platelet function defects, bleeding of unknown cause or genetic disorders in which dysfunction of the secretory pathway has been implicated in disease pathogenesis.

### Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- ☐ Unable to give written informed consent.
- ☐ Use of medication that can compromise platelet function or hemostasis.

## Study design

### Design

|                     |                         |
|---------------------|-------------------------|
| Study type:         | Interventional          |
| Intervention model: | Parallel                |
| Allocation:         | Non controlled trial    |
| Masking:            | Open (masking not used) |
| Control:            | N/A , unknown           |

### Recruitment

|                           |             |
|---------------------------|-------------|
| NL                        |             |
| Recruitment status:       | Recruiting  |
| Start date (anticipated): | 11-08-2020  |
| Enrollment:               | 300         |
| Type:                     | Anticipated |

### IPD sharing statement

**Plan to share IPD:** Undecided

## Ethics review

|                   |                  |
|-------------------|------------------|
| Positive opinion  |                  |
| Date:             | 24-09-2020       |
| Application type: | First submission |

## Study registrations

### Followed up by the following (possibly more current) registration

No registrations found.

## Other (possibly less up-to-date) registrations in this register

No registrations found.

## In other registers

| Register | ID                              |
|----------|---------------------------------|
| NTR-new  | NL8923                          |
| Other    | METC Erasmus MC : MEC-2020-0214 |

## Study results