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PhD in Medical Sciences  
2016-2017

INVITATION to the Public defence of

**Nisha NAIR**

To obtain the academic degree of '**DOCTOR IN MEDICAL SCIENCES**'

**Next-Generation Gene Therapy for Hemophilia B using  
Hyperfunctional Coagulation Factor IX.**

**Wednesday 31 May 2017**

Auditorium **P. Brouwer**, 17:00

Faculty of Medicine and Pharmacy, Laarbeeklaan 103, 1090 Brussel

How to reach the campus Jette:

<http://www.vub.ac.be/english/infoabout/campuses>

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## Summary of the dissertation

Hemophilia A and hemophilia B are congenital X-linked bleeding disorders caused by mutation of gene encoding the blood clotting factor VIII (FVIII) or factor IX (FIX) on the X chromosome, respectively. The patients often suffer from recurrent bleeding and chronic damage of the soft tissues, joints and muscles. Eventually, the bleeding in the joints and the muscles results in pain and stiffness of the tissues. The bleeding could well be fatal in case of intracranial hemorrhage. The fundamental clinical treatment of hemophilia is to restore the physiological level of clotting factor systemically in the patient. The main objective of our project was to develop safe and effective next generation gene therapy strategies for clinical treatment of hemophilia B using gene delivery vehicles like Lentiviral Vectors (LV) and Adeno-Associated Viral vectors (AAV). In the first part of our study, we have shown that liver directed gene transfer of FIX transgene (optimized for codon usage along with R338L amino acid substitution) by integration-competent and integration-defective LV could correct the disease phenotype in hemophilia B mice model. This hyper-functional FIX transgene (FIX-co-R338L) increased the efficacy of gene therapy up to 15 fold *in vivo* at a low vector dose, without any detectable adverse effect. In the second part of our study, we used AAV vectors, in which we combined the synthetic hyperactive FIX transgene (FIX-co-R338L) with a hepatocyte-specific transcriptional cis-regulatory module (CRM), had led to a 11 – 15 fold increase in factor IX (FIX) level. Encouragingly, the FIX-R338L mutation boosted FIX activity up to 7-fold, resulted in supra-physiologic FIX activity (400%) with no apparent increase in thrombotic risk. In conclusion, this study shedded light on the usage of two viral vectors (LV & AAV) to develop safe next generation gene therapy strategies for hemophilia B. The gene therapy approaches resulted in long term therapeutic and supraphysiological levels of FIX activity in mouse models without any added risks. Altogether, our study represents a viable and promising strategy to improve the efficacy and safety of hemophilia B gene therapy and serves as the trailblazer for gene therapy of other such diseases.

## Curriculum Vitae

Nisha Nair was born on July 12<sup>th</sup>, 1986 in India. During her graduation in India, she obtained a Bachelor degree in Biotechnology followed by a Master degree in Stem Cell Biology & Regenerative Medicine in 2010 with highest distinction. Then, she was awarded a competitive Pre-doctoral fellowship in Biomedical Sciences at the Stamcelinstituut Leuven at KULeuven for a period of 10 months. After successfully defending her pre-doctoral thesis in 2011, she started her PhD research at the Department of Gene Therapy & Regenerative Medicine, Vrije Universiteit Brussel, under the supervision of Prof. Dr. Marinee K.L. Chuah and Prof. Dr. Thierry VandenDriessche. Her research focused on the characterization & optimization of novel gene therapy technologies in order to gain further insights into the underlying mechanisms that impact on the efficacy, safety & immune implications of gene therapy for hemophilia B. She was also engaged in several other projects beyond her immediate PhD thesis subject, the research outcomes from which have been validated in several publications. The scientific output from her research includes 7 publications in international peer-reviewed journals, 1 book chapter & 5 papers currently in revision. In addition, 2 of her first author papers from *Blood* (IF = 11.84) were selected for a commentary. Her work was also selected for an oral presentation at an international conference (ESGCT).