Call for 8 Early Stage Researcher PhD fellowships in EDUCational Program on Factor VIII Immunogenicity

Introduction

EDUC8 is an Innovative Training Network (ITN) funded by the European Union Horizon 2020 Programme. The EDUC8 training network represents a novel, pioneering platform for studying the immunogenicity of therapeutic pro-coagulant factor VIII (FVIII) in patients with haemophilia A (HA). It includes leading scientists from academia and industry. The EDUC8 training network offers a total of **8 doctoral research projects** (PhD projects) for 8 early stage researchers (ESRs) across **8 institutes in Europe**. The research topics range from fundamental to translational science, and aim at identifying drug- and patient-related risk factors for the development of neutralizing anti-FVIII antibodies, and at developing innovative protocols for inducing tolerance to FVIII. Successful applicants will receive training in **basic immunology, haemostasis research and bioinformatic approaches, clinical haemostasis, project management, entrepreneurship, healthcare economics, ethics and marketing**. The interaction between biologists, clinical experts, healthcare professionals, industry and patients' organizations is central to EUDC8. It will expose ESR to complex issues in bench-to-bedside research from different perspectives. Graduates from EDUC8 will be highly skilled, entrepreneurial and broadly-trained experts, equipped with innovative and beyond state-of-the-art proficiency on the exponentially expanding area of "**immunogenicity of biotherapeutics**".

Research projects

The research activities implemented in EDUC8 have the following objectives:

- To understand the immunogenicity of FVIII from drug and patients' perspective
- To develop innovative methods to predict patients at risk of developing ADA in response to treatment
- To develop pioneering therapeutic approaches to prevent the development of FVIII inhibitors in patients with HA or to eradicate ongoing neutralizing immune responses

The 8 Early Stage Researchers (ESR)' projects are in the following table.

ESR	TITLE OF THE PROJECT	HOST INSTITUTION	SHORT NAME	SUPERVISOR	EXPECTED START DATE
1	Induction of neonatal Fc receptor-mediated tolerance to therapeutic factor VIII in HA	National Institute of Health and Medical Research (France)	INSERM	Sebastien Lacroix-Desmazes sebastien.lacroix-desmazes@crcjussieu.fr	October 1st, 2020
2	Delineation of HLA-DP4-restricted FVIII peptidome	Stichting Sanquin Bloedvoorziening-Sanquin (The Netherlands)	Sanquin	Jan Voorberg j.voorberg@sanquin.nl	October 1st, 2020
3	High-throughput B-cell epitopes profiling	University of Milan (Italy)	UMIL	Flora Peyvandi flora.peyvandi@unimi.it Roberta Palla roberta.palla@unimi.it	October 1st, 2020
4	Tolerance induction towards FVIII using CAR-transduced Tregs	Goethe University (Germany)	GUF	Christoph Königs Christoph.Koenigs@kgu.de Stephan Schultze-Strasser Stephan.Schultze-Strasser@kgu.de	October 1st, 2020
5	Intracellular fate of endogenous FVIII variants and association with MHC	University Clinic Bonn (Germany)	UKB	Johannes Oldenburg johannes.oldenburg@ukbonn.de Heike Singer heike.singer@ukbonn.de	October 1st, 2020
6	Induction of tolerance to therapeutic factor VIII in HA by modification with α2,3 sialic acid	DC4U BV (The Netherlands)	DC4U	Yvette van Kooyk y.vankooyk@vumc.nl Ineke Rijnhout ineke.rijnhout@dc4u-technologies.nl	October 1st, 2020
7	Induction of tolerance to therapeutic FVIII by harnessing the tolerogenic potency of the liver	Topas Therapeutics GmbH (Germany)	Topas	Sabine Fleischer fleischer@topas-therapeutics.com	October 1st, 2020
8	Monitoring FVIII-specific T cell responses in healthy individuals and in HA patients	Commissariat à l'énergie atomique et énergies alternatives (France)	CEA	Bernard Maillère bernard.maillere@cea.fr	October 1st, 2020

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 859974.

Training Programme

All the selected students will be involved in a highly stimulating training programme, both at the local and at the network-wide level.
The training programme comprises:

1) The implementation of the individual research project at the host institution. The research project will involve collaborations with other EDUC8 institutions, to be implemented through secondments.
2) Each researcher will be involved in local training sessions.
3) Joint scientific courses and meetings will be organised by the EDUC8 consortium, together with short courses for transferable skills training.
5) Enrolment in PhD programmes of the following universities:

ESR	HOST INSTITUTION	UNIVERSITY RELEASING THE PhD
1	INSERM	Sorbonne Université (France)
2	Sanquin	AMC/University of Amsterdam (The Netherlands)
3	UMIL	University of Milan (Italy)
4	GUF	Goethe University (Germany)
5	UKB	University Clinic Bonn (Germany)
6	DC4U	VU University (The Netherlands)
7	Topas	Goethe University (Germany)
8	CEA	Université Paris- Saclay

Recruitment

The ESRs will be contractually employed for 36 months by the recruiting organisation and will be covered under the related national social security scheme. ESRs will receive a Monthly Living Allowance plus a Mobility Allowance (where applicable) compliant with the applicable EC Marie Skłodowska - Curie Actions - ITN (https://ec.europa.eu/research/participants/data/ref/h2020/wp/2018-2020/main/h2020-wp1820-mscaen.pdf page 89 and 92)

Eligibility Rules

At the time of recruitment applicants must fulfil the following rules:

Experience:

- Applicants must be in possession of the degree (usually the Master Degree) which would formally entitle them to embark on a doctorate, either in the country in which the degree was obtained or in the country in which the researcher will be recruited. In case the degree has not been obtained yet, it is necessary to send a declaration of the university stating that the degree will be obtained before the expected starting date
- Applicants must be in the first 4 years of their research careers (full-time equivalent research experience) at the signature of the contract (measured from the time the Master's degree has been obtained).
- Eligible applicants must not hold a Doctoral degree already.

Mobility:

The applicants must not have resided in the country where the research training activities will take place for more than 12 months in the 3 years immediately prior to the recruitment date, and must not have carried out their main activity (work, studies, etc.) in that country.
Exceptions International Organisations: Eligible researchers must not have spent more than 12 months in the 3 years immediately prior to the date of selection in the same appointing international organisation.

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How to apply

EDUC8 will select ESR through a 2-step recruitment process.

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Candidates should submit their application for their top two preferred research projects. Applications (in English) should include:

1) an updated CV; the CV must be without gaps, in order to easily check the mobility and experience requirements. CVs that either do not clearly show the applicant's past experience, or have gaps, will be considered ineligible.
2) a letter giving reason for his/her motivation for the position;
3) at least 1 reference letter (in English) from one former supervisor and/or lecturer;
4) the scan of the degree (usually the Master Degree) which would formally entitle him/her to embark on a doctorate, either in the country in which the degree was obtained or in the country in which the researcher will be recruited. In case the degree has not been obtained yet, it is necessary to send a declaration of the university stating that the degree will be obtained before the expected starting date;
5) transcripts of records (document indicating their ranking and marks within their last year at their Master Degree as well as the courses/modules they have followed).

Applications must be in English and will be evaluated against the following criteria:
- Educational record;
- scientific quality of the applicant's CV;
- expected individual impact and benefit to the fellow and to the project.
- previous experience in the subject of EDUC8 research programme.

The closing date for applications is 30th April, 2020. Application documents should be sent by email to the relevant project supervisors by email (see individual project descriptions).

The candidates will be evaluated on the basis of the received documents and the best 8 candidates for each position will be invited for a Skype interview that will take place in the period between the **11th- 22nd May 2020**. For each position a short list will be prepared and notified to the applicants.

The 2 top candidates for each position will be invited for a virtual interview (on the **3rd or 4th June 2020**). The final selection of the ERSs will be made at the end of **interview rounds** and candidates will be informed immediately.

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ESR1 **Induction of neonatal Fc receptor-mediated tolerance to therapeutic factor VIII in HA**	
Host Institution	National Institute of Health and Medical Research (France) - **INSERM**
Primary Supervisor	Sebastien Lacroix-Desmazes
Email address	sebastien.lacroix-desmazes@crc.jussieu.fr
Planned duration	**36 months**
Subject Area	Molecular and experimental Immunology, Immunotherapy

Introduction: We have recently shown that the injection to pregnant mice of Fc-fused A2 and C2 FVIII domains leads to the transplacental delivery of A2Fc/C2Fc, induction of FVIII-specific regulatory T cells in the offspring and long-lasting tolerance to exogenous therapeutic FVIII. Transplacental delivery is mediated by the neonatal Fc receptor. Using the A2 and C2 domain of FVIII that represent 20% of the protein, the anti-FVIII immune response is reduced by 10-fold, suggesting that transplacental delivery of the entire FVIII protein should confer complete tolerance.

Aims: The general objective is to develop strategies to induce tolerance to therapeutic FVIII as early as during the foetal life. **ESR1** will generate different mutated FVIII-Fc molecules. **ESR1** will validate the transplacental transfer of FVIII-Fc mutants injected to FVIII-deficient mice. **ESR1** will confirm the induction of FVIII-specific T and B-cell tolerance.

Expected Results: The results will validate the hypothesis that the transfer of maternal proteins to babies during fetal life induces life-long tolerance and protects from resistance to treatment with the therapeutic protein equivalent. The results will reveal for the first time the maximal size of the molecular complexes that can be ferried through placenta, thus paving the way towards the design of novel strategies for immuno-intervention in babies as early as during foetal life.

Planned secondment: ESR1 will be seconded at SOBI (P1) under the supervision of Dr. Keith Wilson at Month 9 for 3 months and at Month 18 for 2 months with the following objectives: 1) cloning of FVIII-Fc mutants; 2) expression and purification of FVIII-Fc mutants; 3) Biochemical validation of FVIII-Fc mutants

Enrolment in Doctoral degree(s): ESR1 will be enrolled at Sorbonne University (P8) Doctoral School ED394: Physiologie, Physiopathologie et Thérapeutique

Project-specific selection criteria: Strong knowledge in Immunology, experience in common techniques in Molecular Biology and Cellular Immunology, experience in animal handling is a plus, main competencies include dedication, creativity, team spirit and communication skills

Recommended reading: **[1]** Gupta N et al. 2015. Regulation of immune responses to protein therapeutics by transplacental induction of T cell tolerance. Sci Transl Med 7: 275ra221. **[2]** Culina S et al. 2015. Materno-Fetal Transfer of Preproinsulin Through the Neonatal Fc Receptor Prevents Autoimmune Diabetes. Diabetes 64: 3532-3542. **[3]** Lacroix-Desmazes S et al. 2008. Dynamics of factor VIII interactions determine its immunologic fate in hemophilia A. Blood 112: 240-249. **[4]** Scott DW et al. 2019. Factor VIII: Perspectives on Immunogenicity and Tolerogenic Strategies. Frontiers in immunology 10: 3078. **[5]** Pyzik M et al. 2019. The Neonatal Fc Receptor (FcRn): A Misnomer? Frontiers in immunology 10: 1540. **[6]** Ohsaki A et. 2018. Maternal IgG immune complexes induce food allergen-specific tolerance in offspring. J Exp Med 215: 91-113.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 859974.

ESR2
Delineation of HLA-DP4-restricted FVIII peptidome

Host Institution	Stichting Sanquin Bloedvoorziening-Sanquin (The Netherlands) - **Sanquin**
Primary Supervisor	Prof. Jan Voorberg
Email address	j.voorberg@sanquin.nl
Planned duration	**36 months**
Subject Area	Molecular and cellular immunology

Introduction: Major histocompatibility complex class II (MHCII)-restricted peptide presentation is crucial for the selection and subsequent proliferation of antigen specific CD4+ T cells. While selection of antigen-specific CD4+ T cells is beneficial in the context of vaccination, emergence of antigen CD4+ T cells following administration of therapeutic proteins like factor VIII (FVIII) is not desirable. The mechanism of uptake, processing and presentation of FVIII by antigen-presenting cells (APCs) has been the subject of intense study over the past 10 years. Until recently, our knowledge on the repertoire of FVIII derived presented on MHCII has been limited. Peptide sequences on FVIII recognized by CD4+ T cells have been identified using MHCII tetramers as well as by directly monitoring peptide-induced proliferation of CD4+ T cells. More recently, the repertoire of naturally presented peptides derived from FVIII has been identified by pulsing of immature dendritic cells with FVIII. In follow-up studies we have shown that HLA-DQ presents a distinct repertoire of FVIII peptides. As yet the repertoire of FVIII peptides presented on HLA-DP has not yet been defined. The goal of this part of the EDUC8 program is to explore the contribution of HLA-DP to FVIII peptide presentation. Using unique MHC class II dextramer technology developed by Immudex we will also asses whether HLA-DP restricted CD4+ T cells contribute to the immune response to FVIII.

Aims: The general objective of **Task 1.2.** is to delineate the HLA-DP-restricted FVIII peptidome. **ESR2** will generate naturally processed FVIII peptides upon incubation of human FVIII with immature MODCs from HLA typed healthy donors. **ESR2** will develop the protocols to purify surface exposed HLA-DP from the LPS-matured FVIII-loaded MODCs. **ESR2** will elute the peptides from HLA-DP. **ESR2** will separated the peptides using a reversed-phase C18 column and analyze them by Mass Spectrometry. **ESR2** will identify the peptides using Proteome Discoverer 1.4. In collaboration with **beneficiary B8, ESR2** will validate the binding of the identified peptides to purified HLA-DP4 by ELISA. The immunogenic nature of the peptides will be assessed by generating T-cell lines at CEA. Using the identified immuno-dominant peptides, **ESR2** will generate a revolutionary family of DNA barcoded DP4 Dextramer reagents in a secondment with Immudex (P4).

Expected Results: The expected results include i) the exhaustive delineation of HLA-DP and HLA-DP4-restricted naturally-processed peptides from FVIII, ii) the validation of DP4-associated immunodominant T-cell epitopes and the validation of FVIII-specific DP4 Dextramer for the detection of FVIII-specific CD4+ T cells in healthy donors and patients.

Planned secondment: ESR2 will have secondments at B8, under the supervision of Dr. Bernard Maillere, between M23-M24 and at P4, under the supervision of Dr. Liselotte Brix, between M32 and M40. Secondment at B8 will allow the validation of immuno-dominant HLA-DP4-restricted by ELISA and amplification of FVIII-induced T-cell lines. Secondment at P4 will lead to the generation and validation of DNA barcoded DP4 Dextramer reagents displaying the identified immuno-dominant FVIII peptides as immuno-monitoring tools for HA patients.

Enrolment in Doctoral degree(s): The PhD candidate will be enrolled in the AMC Graduate School (P7) of the Univ of Amsterdam (P9)

Project-specific selection criteria: We are looking for a candidate with a strong background in Molecular and Cellular Immunology, well-developed analytical and technical skills, a creative thinker, a team-player with excellent communication skills

Recommended reading: [1] Peyron, I., Hartholt, R. B., Pedro-Cos, L., van Alphen, F., Brinke, A. T., Lardy, N., Meijer, A. B., and Voorberg, J. (2018) Comparative profiling of HLA-DR and HLA-DQ associated factor VIII peptides presented by monocyte-derived dendritic cells. Haematologica 103, 172-178. **[2]** Sorvillo, N., Hartholt, R. B., Bloem, E., Sedek, M., ten Brinke, A., van der Zwaan, C., van Alphen, F. P., Meijer, A. B., and Voorberg, J. (2016) von Willebrand factor binds to the surface of dendritic cells and modulates peptide presentation of factor VIII. Haematologica 101, 309-318. **[3]** Sorvillo, N., van Haren, S. D., Kaijen, P. H., ten Brinke, A., Fijnheer, R., Meijer, A. B., and Voorberg, J. (2013) Preferential HLA-DRB1*11-dependent presentation of CUB2-derived peptides by ADAMTS13-pulsed dendritic cells. Blood 121, 3502-3510. **[4]** van Haren, S. D., Herczenik, E., ten Brinke, A., Mertens, K., Voorberg, J., and Meijer, A. B. (2011) HLA-DR-presented peptide repertoires derived from human monocyte-derived dendritic cells pulsed with blood coagulation factor VIII. Mol Cell Proteomics 10, M110 002246113.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 859974.

ESR3 **High-throughput B-cell epitopes profiling**	
Host Institution	University of Milan (Italy) - **UMIL**
Primary Supervisor	Prof. Flora Peyvandi - Roberta Palla
Email address	flora.peyvandi@unimi.it - roberta.palla@unimi.it
Planned duration	**36 months**
Subject Area	Bioinformatic applied to immunology

Introduction: The anti-FVIII immune response is characterized by the production of polyclonal IgG directed against multiple epitopes located on the A2, A3-C1 and C2 domains of FVIII. UMIL documented that pre-existing anti-FVIII antibodies are an important risk factor for the development of FVIII inhibitors. A controversy has prevailed over the last two decades on the higher immunogenicity of therapeutic recombinant FVIII (rFVIII) as compared to plasma-derived FVIII (pdFVIII). The prospective randomized SIPPET study, substantiated by a recent observational open cohort, has shown that rFVIII concentrates are indeed associated with an 87% higher incidence of inhibitor development than pdFVIII products. Nonetheless, some cohort studies report contradictory results and the mechanisms underlying the higher immunogenicity of rFVIII over pdFVIII remain unknown. This task will investigate differences in epitope profiles between patients who have developed FVIII inhibitors under pdFVIII or rFVIII. Our preliminary data reveal distinct immuno-profiles in plasma obtained pre- and post-treatment from patients with or without inhibitors

Aims: **ESR3** will exploit a high-throughput random peptide screening strategy (Mimotope Variation Analysis - MVA, developed by PROTOBIOS), which combines peptide display library and next-generation DNA sequencing. Using the MVA technique, ESR3 will compare the arrays of B-cell epitopes recognised by antibodies from HA patients before and after treatment with therapeutic FVIII, as function of their inhibitory status. **ESR3** will also compare the arrays of B-cell epitopes recognised by antibodies from inhibitor-positive HA patients treated with plasma-derived or recombinant FVIII products. **ESR3** will develop ELISA and exploit the SIPPET cohort to evaluate the predictive value on the development of FVIII inhibitors of the recognition of particular sets of peptides by antibodies in the plasma of naïve patients.

Expected Results: The project will allow a fine mapping of the existing FVIII epitopes previously identified as targets for anti-FVIII alloantibodies as well as identification of new FVIII epitopes. It will highlight, for the first time, the differences in humoral responses to rFVIII and pdFVIII products. It will also provide the first detailed comparison of the base-line differences in IgG reactivities between patients that will or will not develop FVIII inhibitors, prior to exposure to FVIII. Interestingly, the results will identify immunogens other than FVIII that predispose patients to inhibitor development. Last, the project shall lead to the development of an ELISA kit to predict the development of FVIII inhibitor in naïve HA patients.

Planned secondment: ESR3 will be allocated to P2 PB under the supervision of Dr. Kaia Palm at Month 10 for 10 months to perform MVA analysis

Enrolment in Doctoral degree(s): ESR3 will be enrolled at the Doctoral School in Medicina Traslazionale (Translational Medicine) in the Department of Pathophysiology and Transplantation of the University of Milan.

Project-specific selection criteria: • Master degree in Bioinformatics, Computational Biology, Biostatistics or in a related discipline. • Strong background in statistics and excellent programming skills (R or Python). • Good knowledge of Linux/Unix system. • Experience with the analysis of NGS data (e.g. RNA-seq, WES, WGS) • Preferred experience in using High Performance Computing facilities. • Preferred experience in managing Phage display experiments or proteomics data and in using software for macromolecules visualization (PyMol/Chimera). • Good problem-solving skills, strong attitude to be up-to-date about the most recent discoveries in the field and to develop novel analytical pipeline. • Ability to work independently and as part of a team.

Recommended reading: **[1]** Peyvandi F et al. A Randomized Trial of Factor VIII and Neutralizing Antibodies in Hemophilia A. N Engl J Med. 2016 May 26;374(21):2054-64 and related publications. **[2]** Bifang He et al. SAROTUP: a suite of tools for finding potential target-unrelated peptides from phage display data. Int J Biol Sci 2019; 15(7):1452-1459. **[3]** Mayrose I et al. Pepitope: epitope mapping from affinity-selected peptides. Bioinformatics 23(23):3244-3246. **[4]** Krejci A et al. Hammock: a hidden Markov model-based peptide clustering algorithm to identify protein-interaction consensus motifs in large datasets. Bioinformatics. 2016 Jan 1;32(1):9-16. **[5]** Steinegger M et al. Clustering huge protein sequence sets in linear time. Nat Commun. 2018 Jun 29;9(1):2542. **[6]** Kahle J et al. Epitope mapping via selection of anti-FVIII antibody-specific phage-presented peptide ligands that mimic the antibody binding sites. Thromb Haemost. 2015 Feb;113(2):396-405. 113.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 859974.

ESR4 **Tolerance induction towards FVIII using CAR-transduced Tregs**	
Host Institution	Goethe University (Germany) - **GUF**
Primary Supervisor	Prof. Christoph Königs - Stephan Schultze-Strasser
Email address	Christoph.Koenigs@kgu.de - Stephan.Schultze-Strasser@kgu.de
Planned duration	**36 months**
Subject Area	Molecular and experimental Immunology, Immunotherapy

Introduction: We have recently shown that human chimeric antigen receptor (CAR)-transduced regulatory T cells (Tregs) are able to inhibit the activation of FVIII-specific helper T cells *in vitro*, resulting in the inhibition of differentiation of murine FVIII-specific memory B cells into antibody-secreting cells. So far the structure of CARs has been optimized for cytotoxic T cells, further studies on the effectiveness of CARs with different intracellular and extracellular domains for activation and phenotypic stabilization of Tregs are important to promote the use of CARs in this context.

Aims: Our main objective is to optimize chimeric antigen receptors (CARs) established in the group for their use in regulatory T cells (Tregs) using the model of HA. CARs have initially been used and optimized for their use in cytotoxic T cells. **ESR4** will clone CAR constructs in lentiviral vectors with different intracellular domains and FVIII-specific scFvs. **ESR4** will transduce Tregs from healthy donors and from FVIII-KO mice with the lentiviral vectors. **ESR4** will analyse the phenotype of transduced Tregs in the absence and presence of FVIII stimuli and during prolonged periods of in vitro culture. The different CAR constructs will be analysed for their potential to activate Tregs and stabilize their regulatory phenotype. **ESR4** will optimise the conditions for Treg cultivation. The inhibitory potential of the most potent CAR candidates will be analysed in in vitro anti-FVIII responses. **ESR4** will examine the mechanisms underlying T-cell inhibition, concentrating on cytokine involvement, expression of costimulatory receptors as well as markers of T-cell activation and exhaustion. **ESR4** will validate the suppressive effect of human or mouse regulatory CAR T cells: i) in vitro on the re-stimulation of splenic FVIII-specific B cells of FVIII-challenged FVIII-KO mice by ELISPOT, ii) in the case of human CAR Tregs, in RAG-KO mice reconstituted with splenocytes of FVIII-challenged FVIII-KO mice, and iii) in the case of mouse CAR Tregs in naïve mice prior to injection of FVIII (preventive approach) and in FVIII-challenged FVIII-KO mice (therapeutic approach).

Expected Results: The ideal CAR composition for regulatory T cells should differ from that currently used in cytotoxic T cells. Therefore, the potential of regulatory T cells transduced with different CAR candidates will be analysed in vitro and in vivo using both human and murine systems. With this, we will decipher essential parameters including the need for the presence of co-stimulatory CAR domains or the required affinity of the scFv. This will pave the way to the translation of CAR-transduced regulatory T cells to HA patients and other diseases.

Planned secondment: ESR4 will be seconded at BT (P3), under the supervision of Dr.Jörg Schüttrumpf, at month 8 for two months to clone CAR constructs with different intracellular domains and FVIII-specific scFvs; at month 12 for 3 months to study the inhibitory capacity of CAR-transduced Tregs on FVIII-specific effector T cells; and at month 21 for 3 months for the analysis of cytokine profiles of activated CAR Tregs and suppressed effector T cells.

Enrolment in Doctoral degree(s): ESR4 will be enrolled at the Goethe University to obtain a PhD and affiliated to the Goethe Research Academy for Early Career Researchers – GRADE

Project-specific selection criteria: Strong interest and knowledge in immunology, experience in molecular biology and cell culture techniques, basic experiences in animal handling would be beneficial; main competences include a high motivation to learn and the ability to work in a team.

Recommended reading: **[1]** Yoon J et al. FVIII-specific human chimeric antigen receptor T-regulatory cells suppress T- and B-cell responses to FVIII. Blood 2017 Jan 12;129(2):238–45. **[2]** Schmidt A et al. Regulatory T cells and their potential for tolerance induction in haemophilia A patients. Hamostaseologie 2016;36(Suppl. 2):S5–S12. **[3]** Scott DW, Pratt KP. Factor VIII: Perspectives on Immunogenicity and Tolerogenic Strategies. Front Immunol. Frontiers; 2019;10:3078.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 859974.

ESR5 **Intracellular fate of endogenous FVIII variants and association with MHC**	
Host Institution	University Clinic Bonn (Germany) - **UKB**
Primary Supervisor	Prof. Johannes Oldenburg - Heike Singer
Email address	johannes.oldenburg@ukbonn.de - heike.singer@ukbonn.de
Planned duration	**36 months**
Subject Area	Molecular and experimental Haematology, Proteomics

Introduction: The prevalence of inhibitor development shows great variation with the type of mutation in the F8 gene responsible for HA: 88% and 25% for multi-domain and single domain large deletions, respectively, 31% for nonsense mutations and 22% for intron 22 inversions (INV22). In particular, preterminal stop codons (PTCs) located in the FVIII light chain (A3-C1-C2 domains) are associated with a higher risk to develop inhibitors as compared to PTCs in the FVIII heavy chain (A1-A2-B domains). Our preliminary data document the presence of significant amounts of intracellular FVIII in iPS-derived vECs from HA patients with INV22 (P-I22I) or light chain-located PTC (R1941X).

Aims: The general objective is to decipher whether HA-causing non-sense mutations in different domains of the FVIII molecule alter the intracellular trafficking of FVIII and its association with MHCI and MHCII molecules, thus impacting on the establishment of tolerance to FVIII. ESR5 will differentiate pluripotent stem (iPS) cells from healthy individual and HA patients with different null and non-sense mutations into vascular endothelial cells (vECs). ESR5 will compare the amounts and location of endogenous FVIII using assays for FVIII antigen. ESR5 will co-stain FVIII with different proteins known to be involved in the classical secretory pathway and other degradation pathways, i.e., ubiquitin-proteasome pathway, autophagy and lysosomal degradation. In a secondment with P3, ESR5 will analyse the FVIII peptide repertoire presented by MHCI and MHCII of vECs derived from healthy individuals and from HA patients with different mutations. In parallel, ESR5 will incubate vECs from different individuals with HLA-matched CD4+ T-cell lines generated by B8.

Expected Results: The results will indicate whether different non-sense mutations differentially affect the intracellular trafficking of endogenously produced FVIII, targeting it differentially to the ubiquitin-proteasome, autophagosome or lysosome pathways. A link will be established between the altered intracellular trafficking of endogenous FVIII, FVIII presentation to T cells and induction of T-cell mediated tolerance/occurrence of allo-immunisation to therapeutic FVIII.

Planned secondment: **ESR5** will be seconded at partner P3, BT, under the supervision of Dr. Dr.Jörg Schüttrumpf at M19 for 8 months to establish the methods allowing isolation of FVIII from vECs and develop protein-based analysis of FVIII: immunoprecipitation, affinity chromatography, mass spectrometry.

Enrolment in Doctoral degree(s): ESR 5 will be registered to the **B**onn **I**nternational **G**raduate **S**chool (BIGS) DrugS Phd program: Pharmacy (https://www.bigs-drugs.uni-bonn.de) of Beneficiary UKB.

Project-specific selection criteria: Strong knowledge in Immunology, experience in common techniques in Molecular Biology and Cellular Immunology, experience in animal handling is a plus, main competencies include dedication, creativity, team spirit and communication skills

Recommended reading: **[1]** Oldenburg J et al. Genetic risk factors for inhibitors to factors VIII and IX.Haemophilia. 2006 Dec;12 Suppl 6:15-22; **[2]** Gouw SC et al., F8 gene mutation type and inhibitor development in patients with severe hemophilia A: systematic review and meta-analysis. Blood. 2012 Mar 22;119(12):2922-34; **[3]** Zimmermann MA et al. Expression studies of mutant factor VIII alleles with premature termination codons with regard to inhibitor formation. Haemophilia. 2014 May;20(3):e215-21; **[4]** David D et al., Analysis of the consequences of premature termination codons within factor VIII coding sequences. J Thromb Haemost. 2003 Jan;1(1):139-46; **[5]** Caron E. Analysis of Major Histocompatibility Complex (MHC) Immunopeptidomes Using Mass Spectrometry. Mol Cell Proteomics. 2015;14(12): 3105-17. [6] Lim WC. Human Endothelial Cells Modulate CD4+ T Cell Populations and Enhance Regulatory T Cell Suppressive Capacity. Front Immunol. 2018 Mar 23;9:565113.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 859974.

ESR6
Induction of tolerance to therapeutic factor VIII in HA by modification with α2,3 sialic acid

Host Institution	DC4U BV (The Netherlands) - **DC4U**
Primary Supervisor	Prof. Yvette van Kooyk - Ineke Rijnhout
Email address	y.vankooyk@vumc.nl - ineke.rijnhout@dc4u-technologies.nl
Planned duration	**36 months**
Subject Area	Molecular cell biology and Immunology, Glycobiology

Introduction: The GlycoDCTM technology is developed by DC4U, which allows modification of antigens with an α2-3-sialic acid. α2-3-sialic acid-conjugated antigens (Sia-Ag) target dendritic cells by specific binding to inhibitory sialic acid-binding Ig-type lectin (Siglecs) receptors. Application of this technology to models antigens, such as ovalbumin or the myelin oligodendrocyte glycoprotein-derived peptide (MOG35-55), facilitated uptake by dendritic cells of the Sia-Ags, and promoted naïve CD4+ T cell differentiation to regulatory T cells in vitro and in vivo. Importantly, Sia-Ag stimulated DCs were able to dampen the functioning of established effector T cells, implying that antigen sialylation is a highly attractive strategy to inhibit ongoing inflammatory immune responses.

Aims: The goal is to exploit the GlycoDCTM technology to induce preventive or therapeutic antigen-specific tolerance to therapeutic FVIII. **ESR6** will couple recombinant human B domain-deleted FVIII as well as immuno-dominant FVIII peptides to 2,3-sialic acid (Sia-FVIII/peptides) and will generate several Sia-FVIII/peptides candidates. **ESR6** will confirm the binding of Sia-FVIII/peptides candidates to siglec-E on mouse splenic DCs and to siglec-9 on immature human MODCs, determine the efficiency of Sia-FVIII/peptides uptake and analyze the maturation profile of DCs by measuring cytokine release and phenotypic analysis by flow cytometry. **ESR6** will determine the capacity of Sia-FVIII/peptides-loaded DCs to generate *de novo* functionally proficient CD4+Foxp3+ Tregs both using mouse and human cells. Using the most promising Sia-FVIII/peptides candidates and in secondments with partner B1 and Partner B4, **ESR6** will then investigate the *in vivo* tolerogenic effect of Sia-FVIII/peptides both in preventive and therapeutic experimental protocols.

Expected Results: The results will confirm the capacity of glycosylated immunodominant FVIII-derived peptides and/or glycosylated recombinant FVIII to induce tolerance to therapeutic FVIII. The results will confirm the efficiency of binding of Sia-FVIII to the targeted siglecs, internalization efficiency, tolerogenic cytokine profile and induction of FVIII-specific Tregs.

Planned secondment: ESR6 will be seconded at B1 for 5 months from M29 (INSERM, HLA-DR Tg FVIII-KO mice) and B4 for 4 months from M34 (GUF, FVIII-KO mice) under the supervisions of Dr.Sébastien Lacroix-Desmazes and Dr. Christoph Königs respectively. The secondments will have the following objective: i) injection of Sia-FVIII/peptides to relevant animal models and validation of tolerance induction to therapeutic FVIII by ii) analysis of the antidrug antibody levels in plasma iii) functional coagulation assays iv) immunological studies including T and B cell phenotyping and functional assays.

Enrolment in Doctoral degree(s): ESR6 will be enrolled in the VUMC/VU University, Amsterdam (PX), The Netherlands

Project-specific selection criteria: A master degree Biomedical Sciences or related, knowledge of immunology, experience with (primary) cellular responses and molecular biology, interest in chemical immunology is a plus, flexible, team player, able to interact with scientists from different backgrounds, departments, and institutions, excellent communication skills in English (both written and verbal).

Recommended reading: **[1]** Lübbers J et al. (2018) Modulation of Immune Tolerance via Siglec-Sialic Acid Interactions. Front Immunol. **[2]** Perdicchio M, et al. (2016) Sialic acid-modified antigens impose tolerance via inhibition of T-cell proliferation and de novo induction of regulatory T cells. Proc Natl Acad Sci U S A. **[3]** Perdicchio M et al. (2016) Tumor sialylation impedes T cell mediated anti-tumor responses while promoting tumor associated-regulatory T cells. Oncotarget. **[4]** Scott DW et al. (2019) Factor VIII: Perspectives on Immunogenicity and Tolerogenic Strategies. Front Immunol. **[5]** Läubli H et al. (2019) Sialic acid-binding immunoglobulin-like lectins (Siglecs) detect self-associated molecular patterns to regulate immune responses. Cell Mol Life Sci.

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ESR7 **Induction of tolerance to therapeutic FVIII by harnessing the tolerogenic potency of the liver**	
Host Institution	Topas Therapeutics GmbH (Germany) - **Topas**
Primary Supervisor	Dr. Sabine Fleischer
Email address	fleischer@topas-therapeutics.com
Planned duration	**36 months**
Subject Area	Nanotechnology and experimental immunology

Introduction: Liver sinusoidal endothelial cells (LSEC) have the potential to regulate CD4+ and CD8+ T cell responses. LSECs can be targeted by intravenous injection of specialized peptide-coupled nanoparticles. After uptake by LSECs, the peptides are released from the nanoparticles and presented by MHCII, resulting in peptide-specific tolerization of CD4+ T cells. Based on proofs of concept of tolerance induction in preclinical T cell- and antibody-mediated diseases, Topas developed proprietary tolerizing particles (TP) to which various disease-relevant antigenic peptides can be coupled (TPC), that foster antigen-specific tolerance.

Aims: The general objective of this project is to validate the Topas nanoparticle-based approach to induce preventive or therapeutic antigen-specific tolerance to therapeutic FVIII. **ESR7** will chemically couple immunogenic FVIII peptides to Topas nanoparticles (FVIII-TPCs) and will generate several types of FVIII-TPCs. **ESR7** will characterize the TPCs using multiple analytical methods established at Topas. **ESR7** will perform *in vitro* tests to analyse the presentation of the released peptide(s) after TPC uptake by APCs and the subsequent stimulation of T cells. **ESR7** will be involved in establishing an in vitro assay able to predict the regulatory effect of peptide-coupled TPCs. **ESR7** will then test the tolerogenic effect of FVIII-TPCs in pre-clinical models of severe HA. ESR7 will validate the targeting of LSECs by FVIII-TPCs using classical *in vivo* imaging approaches. **ESR7** will then investigate the *in vivo* tolerogenic effect of FVIII-TPCs with Partner B1 and with Partner B4 depending on the animal model.

Expected Results: The results will validate the hypotheses i) that the generation of antidrug antibodies that develop in preclinical models of HA in response to the intravenous injection of therapeutic FVIII can be prevented upon intravenous injection of selected FVIII-peptides coupled to Topas proprietary nanoparticles, and ii) that ongoing anti-FVIII immune response may be abrogated using FVIII-peptides coupled to Topas nanoparticles.

Planned secondment: **ESR7** will be seconded at partner B1for 5 months from M29 (INSERM, HLA-DR Tg FVIII-KO mice) and partner B4 for 5 months from M34 (GUF, FVIII-KO mice) under the supervisions of Dr Sébastien Lacroix-Desmazes and Dr Christoph Königs respectively. Secondments will have the following objectives: i) injection of nanoparticle-conjugates to relevant animal models and validation of tolerance induction to therapeutic FVIII by ii) analysis of the antidrug antibody levels in plasma iii) functional coagulation assays iv) immunological studies including T and B cell phenotyping and functional assays.

Enrolment in Doctoral degree(s): **ESR7** will be registered at GUF (P8) Doctoral School Goethe University Frankfurt, Germany.

Project-specific selection criteria: Strong knowledge in nanotechnology and basic immunology. Hands-on experience in bioconjugation techniques, nanoparticle synthesis and characterization. Experience in immunological techniques is a plus. Excellent team spirit, communication and strong organizational skills, flexibility and creativity.

Recommended reading: **[1]** Horst AK et al. 2016. Cellular & Molecular Immunology 13, 1-16. **[2]** Carambia et al. 2015. Nanoparticle-based autoantigen delivery to Treg-inducing liver sinusoidal endothelial cells enables control of autoimmunity in mice. J Hepatology 62: 1349-1356. **[3]** Bargheer et al. 2015. The distribution and degradation of radiolabeled superparamagnetic iron oxide nanoparticles and quantumdots in mice. Beilstein J Nanotechnol 6:111-123. **[4]** Scott DW et al. 2019. Factor VIII: Perspectives on Immunogenicity and Tolerogenic Strategies. Frontiers in immunology 10: 3078.113.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 859974.

ESR8
Monitoring FVIII-specific T cell responses in healthy individuals and in HA patients

Host Institution	Commissariat à l'énergie atomique et énergies alternatives (France) - **CEA**
Primary Supervisor	Prof. Bernard Maillère
Email address	bernard.maillere@cea.fr
Planned duration	**36 months**
Subject Area	Cellular and molecular immunology

Introduction: T cells are a key component of the regulation of the immune response raised against factor VIII (FVIII) but the peptide specificity of FVIII-specific T cells remains unknown. T cell epitopes might vary from one donor to another owing to HLA polymorphism but also to the severity of the disease. FVIII is a complete foreign molecule for severe hemophilia A patients, while it is a fully self-molecule for healthy donors. Strikingly we recently revealed that a large T cell repertoire specific for FVIII exists in healthy donors demonstrating that many FVIII-specific T cells escape from central tolerance (Meunier et al, Blood Adv., 2017). The question arises to decipher and compare the fine specificity of the T cell response to FVIII in healthy donors and hemophilia A patients in the perspective to anticipate onset of FVIII inhibitors.

Aims: The general objective is to determine the specificity, the diversity and the clonal frequency of FVIII-specific T cells in healthy individuals and in patients with HA. ESR8 will derive FVIII-specific CD4+ T-cell lines from healthy donors. Upon stimulation with the identified naturally processed HLA-DR-restricted peptides, **ESR8** will identify the most frequent FVIII CD4+ T-cell epitopes by Elispot. **ESR8** will perform NGS of the CDR3 ß TcR of FVIII-specific CD4+ T-cell lines in order to estimate the diversity of FVIII-specific T cells in each individual. **ESR8** will also perform CDR3 ß TcR sequencing on CD4+ T cells from whole blood to determine the minimal frequency of FVIII-specific clones leading to in vitro T cell response. **ESR8** will be seconded at partner P4 to generate DNA barcoded Dextramer reagents displaying the identified immuno-dominant peptides. After a step of specific enrichment, **ESR8** will sort Dextramer-positive T cells from the whole blood of healthy donors to determine the TCR-specificity, TCR sequence, naïve/memory/regulatory phenotype, diversity and clonal frequency of these FVIII-specific T cells. In collaboration with clinicians in the EDUC8 consortium, **ESR8** will combine the DNA barcoded Dextramer approach to NGS and single cell analysis to characterize the FVIII-specific T cells in the blood from HA patients during the course of replacement therapy and HA patients treated with and without FVIII inhibitors.

Expected Results: The expected results include i) the identification of the most frequent FVIII T-cell epitopes; ii) the identification of CDR3 ß TcR sequences of FVIII-specific T cells and estimate of the diversity of the FVIII specific T-cell repertoire; iii) the development of FVIII-specific Dextramers as immuno-monitoring tool for the FVIII-specific T-cell response; iv) the extended characterization of FVIII-specific T cells in healthy donors and HA patients. Together, the results will provide a comprehensive analysis of FVIII-specific T cell responses in healthy donors and HA patients. The study will pave the way towards the stratification of HA patients on the basis of the frequency of FVIII-specific T-cell clonotypes, thus allowing to anticipate the risk of neutralising immune response. The project will lead to the development of new immuno-monitoring tools to follow FVIII-specific T cell responses in HA patients.

Planned secondment: ESR8 will be seconded at Immudex (P4) under the supervision of Dr. Liselotte Brix from Month 25 to 33. The objectives are i) to produce DNA barcoded Dextramer reagents by using the FVIII peptides validated in the first period of the thesis ii) to assess their functionality using FVIII-specific T-cell lines, and iii) to set up experimental conditions to perform Dextramer based NGS analysis of FVIII-specific T cells in whole blood.

Enrolment in Doctoral degree(s): ESR8 will be registered at University Paris-Saclay (UPS) Doctoral School ED569: Therapeutic innovation from fundamental to applications

Project-specific selection criteria: Strong knowledge in cellular and molecular immunology, experience in techniques in cell culture, molecular biology and protein chemistry. Personal commitment, constructive attitude with the supervisor and the team and oral and written communication skills are also requested.

Recommended reading: [1] Spindeldreher, S., et al. 2020. T cell epitope mapping of secukinumab and ixekizumab in healthy donors. mAbs 12: 1707418. **[2]** Meunier, S. et al. 2019. Impact of human sequences in variable domains of therapeutic antibodies on the location of CD4 T-cell epitopes. Cellular & molecular immunology. **[3]** Meunier, S.,et al 2017. CD4 T cells specific for factor VIII are present at high frequency in healthy donors and comprise naive and memory cells. Blood advances 1: 1842-1847. **[4]** Hamze, M., et al 2017. Characterization of CD4 T Cell Epitopes of Infliximab and Rituximab Identified from Healthy Donors. Frontiers in immunology 8: 500. **[5]** Jacquemin, M., et al. 2003. CD4+ T-cell clones specific for wild-type factor VIII: a molecular mechanism responsible for a higher incidence of inhibitor formation in mild/moderate hemophilia A. Blood 101: 1351-1358.

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