Leuven, 12 juli 2013

Prof. Dr. Goffin
Decaan van de Faculteit Geneeskunde

Hooggeachte Decaan,

Graag wil ik via dit schrijven gevolg geven aan Uw oproep tot kandidaatstelling voor de vervolmakingsbeurs van de Medische Stichting Mathilde Horlait-Dapsens.

Tijdens mijn klinische opleiding als assistent Inwendige Geneeskunde – Cardiologie ben ik een doctoraatsonderzoek gestart op het Centrum voor Moleculaire en Vasculaire Biologie (CMVB), onder begeleiding van professor Peter Verhamme en professor Willy Peetermans.

In de loop van dit doctoraatsonderzoek heb ik het genoegen gehad om zowel fundamenteel wetenschappelijk onderzoek uit te voeren, als om klinische studies van nabij te kunnen volgen. Hieruit groeide mijn overtuiging dat ik ook in de toekomst de klinische praktijk graag zou combineren met wetenschappelijk werk. Het zou voor mij dan ook een unieke kans zijn om in aansluiting van mijn onderzoek aan het CMVB, mijn opleiding te kunnen verderzetten in een centrum met internationale faam op zowel klinisch als wetenschappelijk vlak.

Via de contacten van prof. Verhamme en prof. Janssens kreeg ik de mogelijkheid om na het afronden van mijn doctoraatsonderzoek een bijkomend opleidingsjaar te volgen aan de McMaster University, Hamilton, Canada (academiejaar 2013-2014). Deze befaamde universiteit heeft een uitmuntende reputatie in klinische studies en is een vermaard cardiovasculair onderzoekscentrum.

De expertise in translationeel onderzoek van McMaster University bieden dan ook een uitgelezen kans om mijn huidige onderzoek verder te zetten en uit te breiden. U vindt een uitgeschreven onderzoeksproject als bijlage van deze kandidaatstelling. Naast deze meerwaarde op wetenschappelijk vlak beschouw ik deze buitenlandse ervaring als een opportuniteit om internationale contacten aan te knopen die waardevol kunnen zijn om in de toekomst nieuwe onderzoekslijnen te genereren in het departement Cardiovasculaire Wetenschappen. Mijn aanvraag geniet dan ook de steun van mijn promotor, Prof. Verhamme, en van het diensthoofd van de dienst Hart- en Vaatziekten, Prof. Janssens.

De beurs van de Stichting zou helpen om dit jaar financieel te ondersteunen en zo deze unieke kans te realiseren. Ik dank U dan ook van harte voor het in overweging nemen van deze kandidaatstelling.

Hoogachtend,

Thomas Vanassche

The role of thrombin signalling in infectious and inflammatory conditions

Research project description at McMaster University, Hamilton, Canada
Thomas Vanassche

Introduction: State of the art and previous research

Thrombin as the link between coagulation and inflammation

The coagulation system is a conserved innate defence system that functions as an early response to tissue damage due to either injury or invading pathogens through the activation of a tightly regulated cascade of serine proteases, culminating in the generation of thrombin, the key enzyme of the coagulation cascade. The conversion of fibrinogen to fibrin by thrombin seals off leaking blood vessels, and generates a first barrier against bacterial spreading as well as a framework for the inflammatory reaction that aims at remodelling and restoring normal tissue architecture. In addition, thrombin directly activates cellular protease-activated receptors (PAR's) on platelets, endothelial cells, and leukocytes. The cellular effects of thrombin provide a positive feedback loop leading to a sustained local activation of coagulation, and initiate an inflammatory response by recruiting and activating leukocytes. The intensity of this procoagulatory and proinflammatory state is regulated by the thrombomodulin-protein C pathway. Upon binding to thrombomodulin, thrombin activates protein C, thus switching to an anticoagulant and anti-inflammatory signalling that prevents excessive vascular leakage and secondary tissue destruction. Although both the initiation as well as the regulation of this thrombin signalling are critical mechanisms for the maintenance of integrity of the host, its precise regulation in pathological conditions remains incompletely understood. The recent development of specific small molecule thrombin inhibitors has offered new possibilities to study the role of thrombin inhibition in various pathological conditions.

Thrombin signalling in infectious diseases: S. aureus coagulases

During my doctoral research, we have demonstrated that the uncontrolled stimulation of thrombin generation by *Staphylococcus aureus* (*S. aureus*) through the secretion of coagulases contributes to the virulence of *S. aureus* in different *in vitro* as well as *in vivo* disease models. Our research demonstrated that inhibition of coagulase activity by thrombin inhibitors reduced morbidity and mortality in animal models of abscess formation, sepsis, and catheter-related infections. Furthermore, we have shown that *S. aureus* coagulases selectively modulate thrombin signalling by impairing PAR-signalling on platelets and endothelial cells. The relevance of this PAR-signalling in disease virulence has not yet been studied.

Thrombin signalling in tissue damage: ischemic necrosis and cellular remodelling.
Ischemic cellular damage such as in myocardial infarction and in ischemic stroke also triggers thrombin generation. Recent data suggest that thrombin generation contributes to neurotoxicity in the penumbra zone of cerebral infarction. By activating coagulation, platelet activation, and local inflammation, thrombin is a key determinant in the extent of myocardial damage following coronary artery occlusion. Furthermore, thrombin PAR-signalling has been shown to have direct negative inotropic and pro-arrhytmic electrophysiological effects on myocardial cells, thus contributing to reperfusion damage in rat hearts.

Project overview

1. *To study the role of thrombin inhibition in infectious diseases*
   a) preclinical: to study the modulation of endothelial cell PAR-signaling by *S. aureus*
   b) preclinical: to study the contribution of *S. aureus* thrombin activation in infective endocarditis
   c) clinical: to study the potential of inhibition of *S. aureus* coagulases by dabigatran: randomized clinical trial

2. *To study the role of thrombin inhibition in ischemic conditions*
   a) clinical: database analysis of outcome of strokes and myocardial infarctions in patients in relation to treatment with direct thrombin inhibitors

3. *To study the role of thrombin inhibition on atrial remodelling and electrical stability of cardiomyocytes*
   a) clinical: database analysis of atrial fibrillation (AF) burden and atrial dilatation in paroxysmal AF patients in relation to treatment with direct thrombin inhibitors

Concise project description and methodology, with a focus on the added value of the hosting institution and the link with the current on-going research

1. To study the role of thrombin inhibition in infectious diseases

Preclinical: disruption of thrombin signalling by pathogens in endothelial cell dysfunction and infective endocarditis

My current doctoral research focuses on the study of the inhibition of coagulase-activated thrombin (*'staphylothrombin'*) in *in vitro* and animal models of *S. aureus* disease. We have previously shown that staphylothrombin facilitates fibrin formation and platelet recruitment by *S. aureus* under flow conditions, the cornerstones of infective endocarditis (IE) vegetation growth. Furthermore, we found that staphylothrombin-mediated fibrin also enhanced the bacterial adhesion to intravascular foreign bodies, and provided a diffusion barrier to antibiotic therapy, thus reducing the bactericidal effect of vancomycin. Staphylothrombin inhibition by direct thrombin inhibitors reduced bacterial adhesion and restored antibiotic sensitivity in an in vivo model of *S. aureus* intravascular catheter infection. However, the role of staphylothrombin into growing IE lesions has not yet been studied.

To investigate the direct effects of staphylothrombin on endothelial cells, we will compare the endothelial cell response to thrombin and staphylothrombin *in vitro* in various assays. PAR-1-mediated endothelial cell activation will then be analysed via western blotting of endothelial cell lysates (degree of ERK1/2 phosphorylation) and via analysis of ICAM- and VCAM-expression. To evaluate whether the presence of bacterial coagulases disturbs the pro-inflammatory/anti-inflammatory balance of endothelial cells, we will also perform endothelial cell activation studies in the presence of the bacterial coagulases and mixtures of thrombin, thrombomodulin and APC.

We have previously evaluated and validated a mouse IE model, in which Balb/c mice are inoculated with *S. aureus* 24 hours after the implantation of an ethylon wire in the right carotid artery, up to the aortic valve. Following the induction of IE, we will compare real-time bacterial load using bioluminescence technology, animal survival, and histology of IE vegetations between different treatment regimens consisting of either pharmacological staphylothrombin inhibition or a vaccination strategy against the *S. aureus* coagulases will be evaluated. The technology and expertise have been developed during my doctoral research, and are available at the hosting institution.

Clinical application: inhibition of S. aureus induced thrombin activation in bacteraemia patients

The therapeutic potential of inhibition of *S. aureus* induced thrombin activation was consistently demonstrated, both by our group and by collaborating investigators, in a range of animal models of *S. aureus* disease. Staphylothrombin inhibition led to increased antibiotic efficacy, decreased bacterial load, and reduced parameters of infection and mortality. In

combination with the expertise of the CMVB with translating preclinical findings to clinical trials, this has led to the design of a pilot randomized clinical trial of staphylothrombin inhibition versus standard thromboprophylaxis in patients with *S. aureus* bacteraemia.
The hosting institution is a world-renowned centre of excellence in the design and conduction of clinical trials. The expertise of this group will be a strong support in the follow-up of this clinical trial. Furthermore, as the hosting institution is the coordinating centre in the large clinical trials with the direct thrombin inhibitors (DTI), this offers the opportunity to use the ample datasets of thousands of DTI-treated patients for epidemiological studies on the link between thrombin inhibition and the evolution of bacterial infections.

2. To study the role of thrombin inhibition in ischemic conditions

Clinical (epidemiological)
Preclinical data support a role for thrombin in the mediation of neurovascular injury, microglial activation with secondary oxidative stress response, and neuronal apoptosis during and following ischemia. The RE-LY study, coordinated at McMaster University, showed a significant reduction in ischemic stroke in patients with atrial fibrillation treated with thrombin inhibitors compared to warfarin, as well as a decrease in intracranial bleeding. The impact of treatment on the extent and clinical outcome of strokes in high-risk patients has not yet been studied, and the clinical trial databases of the host institution offer a unique source of information for these studies.
In contrast, whereas thrombin inhibition has theoretical beneficial effects in myocardial ischemia, there was a trend towards increased numbers of myocardial infarction in patients treated with dabigatran. A detailed study of the clinical events in the RE-LY trial may improve the understanding of the benefits or shortcomings of thrombin inhibition in patients with acute coronary syndromes.

3. To study the role of thrombin inhibition on atrial remodelling and electrical stability of cardiomyocytes

Clinical (epidemiological)
PAR-receptor activation on atrial cardiomyocytes contributed to electrical instability and induction of arrhythmias in preclinical studies. In this regard, we will study whether the anticoagulation by thrombin inhibitors has an effect on the AF burden in patients with paroxysmal atrial fibrillation. Furthermore, the study of the evolution of echocardiography parameters of atrial function, such as atrial dilatation, can be used as a clinical marker for reverse remodelling.
Furthermore, on-going registries of patients with AF who are electrically reconverted to sinus rhythm also allow to quantify AF burden in relation to thrombin inhibition.

Fit within current research project

As outlined above, the planned research fellowship at McMaster University will be a perfect opportunity to translate and extend my current preclinical research to a clinical study. In agreement with my promoter, Prof. Verhamme, I will remain closely involved with the recently started trial. The input and experience from McMaster University in clinical trial management in general, and in clinical trials with thrombin inhibitors more specifically, will be of immediate value to the conduction of this trial.

At the hosting institute, I will have the opportunity to continue working on the preclinical work, as it is currently ongoing at the Center for Molecular and Vascular Biology. This will ensure that there is a continuity with the ongoing work, and that the technology and expertise that has been built up remains of use to the CMVB.

The planned fellowship thus gives me the opportunity to gain international experience and connect with a strong scientific network while continuing the research work that I have done in the previous years. Furthermore, the connection with the clinical research will add both to the scientific value of the current research as well as to my own training as a junior researcher in translational medicine.

Besides my promotor, my co-promotors Prof. Peetermans and Prof. Hoylaerts fully support this application. As I also have a commitment to my clinical training, the opportunity to participate in the clinical department at McMaster University is of great value to me. The head of the department of Cardiology, Prof. Janssens, also supports this international experience.

Personal Motivation

I consider the opportunity of a one-year research fellowship at McMaster University as a unique experience for different reasons. Through my previous years as a doctoral research student, the study of the disturbance of thrombin signalling by pathogens from a fundamental research perspective has been a fascinating challenge, and has strongly motivated me to pursue a future career that offers the opportunity to combine clinical work with research.

McMaster University is recognized as a world-leading centre in the conduction of clinical trials in cardiovascular medicine, and has the largest experience worldwide in clinical trials with the newly available thrombin inhibitors. I am convinced that the tremendous expertise of the research group of Prof. J. Eikelboom will not only be a exceptional support for the first clinical study that we have initiated as a result of my previous preclinical research, but will also give me the possibility to study the largest available database of patients treated with thrombin inhibitors to explore relations between thrombin signalling modulation and clinical outcomes.

The research division at McMaster University is embedded in a clinical department, allowing me to participate in different cardiovascular clinical care programs. Thus, this clinical research fellowship will also be beneficial for my future participation in clinical care programs at the UZ Leuven.

During this one-year stay, I will be interacting and collaborating with colleagues from all over the world, and building an international network that will be useful for academic projects upon my return. Indeed, also in the future, it is my ambition to pursue an academic career in cardiovascular medicine. This is supported by prof. Janssens, chairman of the department of cardiovascular diseases.

I truly believe that a one-year stay at McMaster University will be a unique opportunity to broaden my scientific, clinical, and personal horizon, bridging my doctoral research with a future academic career.

Center for Molecular and Vascular Biology

KATHOLIEKE UNIVERSITEIT LEUVEN

Leuven, 12 juli 2013

Prof. Dr. Jan Goffin
Decaan Faculteit Geneeskunde

Hooggeachte Decaan,

Het is een goede gewoonte dat toekomstige stafleden van de dienst Hart- en Vaatziekten zich specialiseren in een buitenlands topreferentie-centrum.

Thomas Vanassche was een uitmuntend student en hij werd ook uitstekend geëvalueerd als GSO. Zijn PhD-onderzoek wordt gepubliceerd in toptijdschriften en krijgt internationale waardering; ook 2 *Young Investigator Awards* zijn hiervan getuige.

De McMaster-universiteit in Hamilton is wellicht het meest wereldvermaarde onderzoekscentrum voor cardiovasculaire klinische studies in het domein van arteriële en veneuze trombose. Op unieke wijze wordt er wetenschappelijk onderzoek verweven met klinische zorg. Een gespecialiseerde opleiding met aandacht voor zowel klinische zorg als onderzoek, is een logische volgende stap in de opleiding van Thomas die ik als promotor van zijn PhD-thesis voluit ondersteun.

Thomas is een uitstekende kandidaat voor een beurs van de Medische Stichting Mathilde Horlait-Dapsen.

Met de meeste hoogachting,

Prof. Dr. Peter Verhamme
Centrum voor Moleculaire en Vasculaire Biologie, KULeuven
Afdeling Bloedings- en Vaatziekten, Hart- en Vaatziekten, UZ Leuven

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Leuven
11 juni 2012

Onderwerp: beursaanvraag Dr. T. Vanassche bij Medische Stichting M. Horlait-Dapsen

Hooggeachte Decaan,

Het is voor mij een bijzonder genoegen om de kandidatuur van Dr. Thomas Vanassche voor een beurs van de Medische Stichting Mathilde Horlait-Dapsen te steunen. Dr. Vanassche was een briljante geneeskundestudent met een uitmuntend curriculum en een zeer uitgesproken wetenschappelijke interesse voor het cardiovasculair onderzoek.

Als assistent in opleiding inwendige ziekten met de ambitie tot subspecialisatie in hart- en vaatziekten, koos Thomas voor een gecombineerd MD-PhD traject. Zijn onderzoekswerk rond thrombose, infectie en inflammatie wordt gepromoot door Prof. Peter Verhamme in onze dienst en werd door peers in het vakgebied uitstekend beoordeeld, getuige de mooie publicatieoutput en young investigator awards op het internationale forum. Zonder twijfel behoort Dr. Vanassche tot de absolute top 5% onder leeftijdsgenoten in een vergelijkbaar stadium van hun medische en wetenschappelijke loopbaan.

Bij de uitbouw van een geintegreerde dienst 'Hart- en Vaatziekten' weet U hoeveel belang ik als diensthoofd hecht aan de klinisch-wetenschappelijke uitbouw van de diverse expertises, die inherent vereist zijn binnen ons uitgebreid vakdomein. Als academisch centrum moeten we daarbij de ambitie tot blijvende kenniswerving en kennisoverdracht vooropstellen en ik verwacht van jonge medewerkers dat ze zich hier voluit kunnen in engageren. Thomas heeft alle vereiste kwaliteiten daartoe.

De ervaring leert dat een bijkomende opleiding in een gerenommeerd buitenlands centrum de beste waarborg is om intrinsieke kwaliteiten verder te ontwikkelen en specifieke expertisedomeinen in onze dienst en ons departement uit te bouwen. Daarom is de mogelijkheid tot fellowship aan de McMaster-universiteit in Hamilton, Ontario onder supervise van Prof. J. Eikelboom, voor Thomas een unieke gelegenheid om in arteriele en veneuze thrombose deze bijkomende kennis op te doen en in de toekomst hierin te excelleren.

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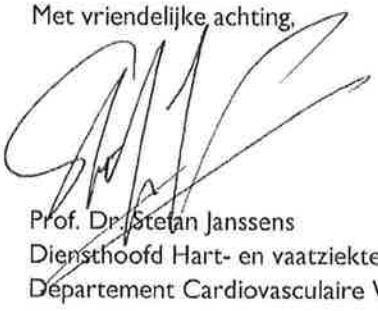
De toekenning van een beurs van de Medische Stichting Mathilde Horlait-Dapsen zou een enorm motiverende ondersteuning voor Thomas betekenen in zijn terechte ambitie voor een toekomstige academische loopbaan.

Ik kan geen betere kandidaat aanbevelen.

Met vriendelijke achting,



Prof. Dr. Stefan Janssens
Diensthoofd Hart- en vaatziekten, UZL
Departement Cardiovasculaire Wetenschappen KULeuven

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June 8, 2012

Re: Dr. Thomas Vanassche

To whom it may concern:

I am delighted to offer Dr. Thomas Vanassche a research fellowship at McMaster University for the academic year 2013/2014.

McMaster University is a vibrant multi-disciplinary environment that offers superb opportunities for education, training and research. Dr. Vanassche will work with cardiologists, stroke neurologists, vascular medicine specialists, hematologists and clinical trialists during his fellowship and will also have access to experts in epidemiology and biostatistics.

I very much look forward to seeing Dr. Thomas Vanassche at McMaster University in 2013.

Please do not hesitate to contact me should you require further information.

With kind regards,

Yours sincerely,

John Eikelboom, MBBS, MSc, FRACP, FRCPA, FRCPC
Canada Research Chair in Cardiovascular Medicine, Canadian Institutes for Health Research
Associate Professor, Department of Medicine
McMaster University