MEDICAL IMAGING RESEARCH GROUP

ICMI Brussels
In vivo Cellular & Molecular Imaging

Workshop

Immuno-Imaging and Molecular Therapy

April 25th - April 29th 2022

9th Edition

innovative medicines initiative

efpia

ImmuneImageO

Workshop Immuno-Imaging and Molecular Therapy

Workshop description:

Due to their low immunogenicity, high affinity/specificity and flexibility towards biotechnological engineering, antibodies are interesting vehicles for molecular diagnosis, imaging and therapy.

The workshop 'Immuno-imaging and molecular therapy' aims to bring together national and international experts in the fields of antibody-engineering, clinical and preclinical nuclear imaging, tracer design, image acquisition and reconstruction, (radio)chemistry, targeted radionuclide therapy, optical and microscopic imaging, immunogenicity and intellectual property. Altogether, a full overview of the research topic 'immunotheranostics' will be provided. In particular, a special focus will be put on nanobodies as an example of engineered antibody vehicles for non-invasive imaging and targeted therapy. This year's workshop will take place in the week of 25-29 April 2022 in a hybrid format. Lectures will be webcasted online, and timing of these lectures can be found below. The time slots are for Central European Time. The language of communications (lectures, discussions) will be English.

This year, the lectures will be complemented by presentations of researchers participating in the workshop. Everybody that performs independent research (Laboratory Technician, Research Associate, PhD student and Postdoctoral Fellow) is highly encouraged to submit an abstract in order to present their work. Four time slots of 20 min per speaker will be foreseen during the workshop (10 min presentation and 10 min discussion). The workshop aims to create an atmosphere of good interaction, discussion and networking and aims to give young researchers a chance to enhance their presentation skills by presenting in front of a small audience. When you are registered for the workshop itself, you will receive an abstract template and a link to submit the abstract (not for MSc students). The deadline for abstract submission will be **April 18th, 2022 before 5 PM.** Abstracts will be reviewed by a scientific panel of experts in the field of imaging and therapy.

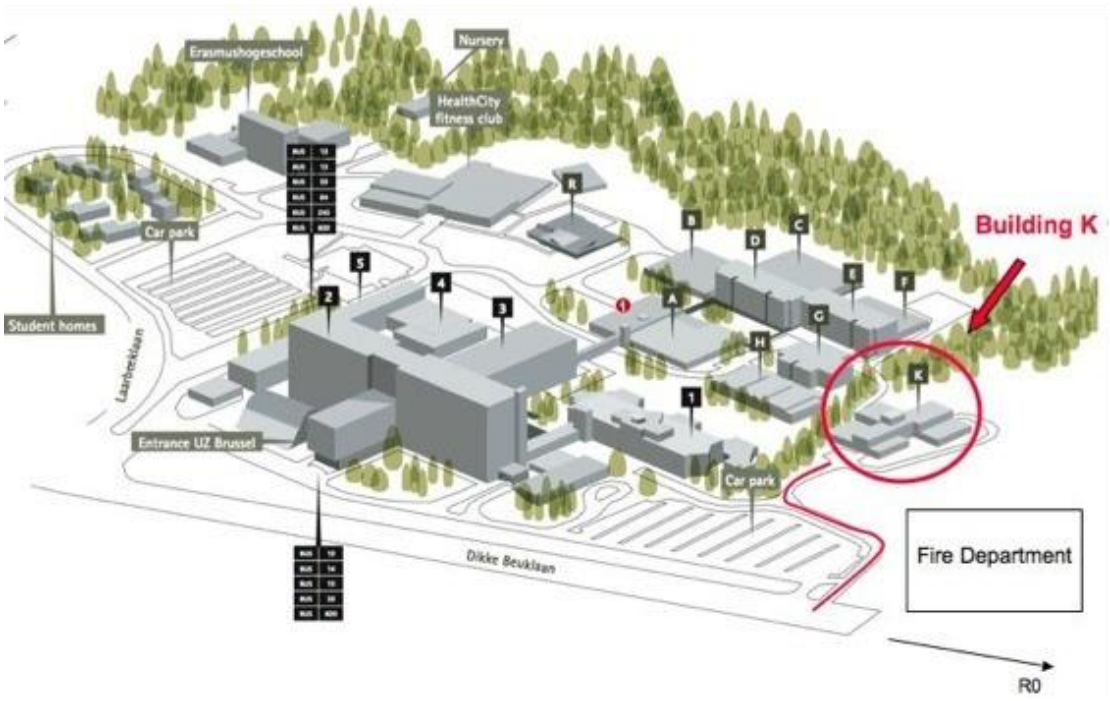
For whom?

This workshop is targeted towards anybody interested in the broad topic of 'immune-theranostics'. Basic knowledge of radiation, biotechnology, biochemistry, medicine and engineering is required. Master students in Biology, Biomedical Sciences, Biotechnology, (Bio)Chemistry, Medicine or Pharmacy, PhD students, technologists and postdoctoral fellows working or interested in this field of research are encouraged to participate. Participants can be from any nationality and can follow the webcast from anywhere in the world.

Venue and Webcast Service:

The live workshop will be held at the Health Campus of the Free University Brussels (Vrije Universiteit Brussel), Laarbeeklaan 103, B-1090 Brussels, Belgium.The lectures will be held in the auditorium TIK, building K ground floor. Practical sessions will be held in the seminar room of the ICMI laboratory, building K ground floor (see https://mima.research.vub.be/en). More information how to reach the the campus can be found here: www.vub.be/en/campus/brussels-health-campus

All lectures (excluding practical sessions) will be streamed online in an interactive format, using Cisco WebEx service (see details below).



Workshop schedule:

Monday April 25 until Friday April 29, 2022, each day from 9AM to 5PM, unless otherwise specified. A time slot is foreseen for each lecture, see program below. The PowerPoint slides of each lecture will be digitally made available to the participants at least a day before the live lecture.

Accommodation:

The workshop participants themselves are responsible for their own accommodation. Participants seeking accommodation can be given advice.

How to participate?

Registration for the workshop will be via the link (https://docs.google.com/forms/d/e/1FAIpQLSfB6BBRd9idh5c5zsoujokDHZT4catIn-vqc2Rd0LVhuJB5DQ/viewform).

When you have registered, an acceptance mail will be sent to you, together with an abstract template, a link to submit the abstract and a link to a folder in Onedrive. The latter will be used to share essential information like lecture slides and videos of the recorded lectures.

The different lectures of the workshop will be live webcasted using the tool Cisco WebEx meetings. You can use this tool either via your browser or you can download it. But first you will have to make an account on Webex via this link (https://www.webex.com). Click on sign up (free), enter your institutional email address and a new password. You will now already be able to follow the lectures or you can choose to also download the tool via (https://www.webex.com/downloads.html). First scroll down to download the previous app **Webex meetings**, then choose on which operation system you would like to download Cisco Webex Meetings (macOS or Windows).

MacOs: Click on download for macOS, the Webex app will appear in your downloads. Click on webexapp.dmg, a pop up will appear where you have to click on Cisco Webex Meetings.pkg. Next, a window will open named 'Install Cisco Webex Meetings', click on continue 3 times and finally install Webex. When the installation has succeeded, click on close. Go to your Launchpad and the app will appear. Click on the app and sign in with your Webex account to follow the lectures.

Windows: Click on download for Windows, the Webex app will appear in your downloads. Click on Webex.msi, open the file and it will say 'Welcome to the Cisco Webex Meetings Setup Wizard, click on next. A window will pop up where you have to agree to license terms and click install. You can now sign in with your Webex account to follow the lectures.

Once you registered for the workshop and made a Webex account, you will receive invites for each webcast lecture. Simply accept the invitation (RSVP), this lecture will then become visible in your agenda and on the day of the lecture click 'Join a Webex meeting' at the specific timeslot and you will be able to follow the presentation.

For the speakers that give a lecture, Safari does not support sharing your screen during a Cisco Webex meeting, so you either have to download the tool or you will have to swith to the browsers google Chrome or Firefox.

Hence, the items you need to follow the lectures online are 1) a computer with installed Cisco WebEx meetings software (a mobile phone will also work but will be less practical if you would like to participate in the chat sessions); 2) a microphone (build-in or external); 3) a headphone

or earplugs; 4) a stable internet connection (preferentially using an ethernet cable, a stable WiFi connection will also work) and 5) a webcam (build-in or external). As this year will be a hybrid meeting, we ask each online participant to mute themselves during the presentations.

Immune-Image:

This workshop is part of the Immune-Image project that has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 831514. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA. For more information on this project, see www.immune-image.eu and www.imi.europe.eu.

Disclaimer: all communications at the workshop only represent the presenters' view and the Joint Undertaking is not responsible for any use that may be made of the information it contains.

Registration deadline and fee:

The registration deadline for this year's workshop and abstract submission is **Monday April 18, 2022**. We ask all participants, when registered, to maximally attend all lectures. The workshop is entirely free to attend. Simply register and attend the lectures.

Contact:
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Organisation Committee:
Director: Prof Nick Devoogdt
Administration: Yoline Lauwers

Program:

Monday April 25

09u00-10u00	Prof Nick Devoogdt (VUB) *Welcome, testing webcast service and introduction round of all participants*
10u00-11u15	Prof Nick Devoogdt (VUB) *Do's and don't's in immunotracer design*
11u15-11u45	**Bio-break**
11u45-13u00	Prof Christian Van Hove (UGent) *Overview of imaging modalities, part I*
13u00-14u00	**Lunch**
14u00-15u15	Prof Christian Van Hove (UGent) *Overview of imaging modalities, part II*
15u15-15u45	**Bio-break**
15u45-17u00	Dr Timo De Groof (VUB) *Nanobody discovery and biotechnological applications*

Tuesday April 26

09u00-10u15	Prof Marleen Keyaerts (VUB/UZ Brussel) *Introduction to clinical nuclear medicine*
10u15-10u45	**Bio-break**
10u45-12u00	Prof Nick Devoogdt (VUB) *Imaging with nanobodies in preclinical models*
12u00-13u10	**Lunch**
13u10-13u30	Presentation from selected abstract
13u30-14u45	Prof Matthias D'Huyvetter (VUB) *Targeted radionuclide therapy, focus on nanobodies*
14u45-15u15	**Bio-break**
15u15-16u30	Daniëlle Vugts (VUmc) *^{89}Zr-immuno-PET imaging: from bench to bedside and back*

Wednesday April 27

09u00-10u15	Dr *Chloé Ackaert* (ImmunXperts inc, Belgium) *Immunogenicity of proteins and nanobodies*
10u15-10u35	Presentation from selected abstract
10u35-11u00	**Bio-break**
11u00-12u15	Prof Sophie Hernot (VUB) *Fluorescence applications of nanobodies*
12u15-13u30	**Lunch**
13u30-17u00	Hands-on session: only for live participants and to be chosen from one of the topics below (1) Labeling of nanobodies: fluorescent and radioactive (Dora Mugoli Chigoho, Noemi Declerck and Lukasz Mateusiak) (2) Nanobody lead selection analysis (Dr. Timo De Groof and Yoline Lauwers) (3) Visit to the nuclear medicine department and cyclotron unit of the UZ Brussel hospital: daily patient care and clinical trial procedures (Prof Marleen Keyaerts, Ordade Gondry and Sonja Van den Block) (4) Analysis of nanobody *in vivo* biodistribution data in mice (Isabel Remory)

Thursday April 28

9u00-10u15	Sabrina Oliveira (Utrecht University) *Nanobody-targeted photodynamic therapy… and beyond*
10u15-10u35	Presentation from selected abstract
10u35-11u00	**Bio-break**
11u00-12u15	Prof Marleen Keyaerts (VUB/UZ Brussel) *Clinical translation of nanobody-tracers*
12u15-13u30	**Lunch**
13u30-17u00	Hands-on session: only for live participants and to be chosen from one of the topics below (1) Labeling of nanobodies: fluorescent and radioactive (Dora Mugoli Chigoho, Noemi Declerck and Lukasz Mateusiak) (2) Nanobody lead selection analysis (Dr. Timo De Groof and Yoline Lauwers) (3) Visit to the nuclear medicine department and cyclotron unit of the UZ Brussel hospital: daily patient care and clinical trial procedures (Prof Marleen Keyaerts, Ordade Gondry and Sonja Van den Block) (4) Analysis of nanobody *in vivo* biodistribution data in mice (Isabel Remory)

Friday April 29

9u00-10u15	Erik Aarntzen (Radboudumc) *Defining the role of molecular imaging in the field of immune-oncology*
10u15-10u35	Presentation from selected abstract
10u35-11u00	**Bio-break**
11u00-12u15	Dr Jessica Bridoux (VUB) *Radiochemistry of nanobodies*
12u15-13u30	**Lunch**
13u30-14u45	Prof Geert Raes (VUB/VIB) *Intellectual property of biologics*
14u45-15u15	**Bio-break**
15u15-16u15	Discussion survey

Information about the lecturers:

Nick Devoogdt
Professor Vrije Universiteit Brussel (VUB)
Research cluster Imaging and Physical Sciences (BEFY)
In vivo Cellular and Molecular Imaging laboratory

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Nick Devoogdt was trained as a Master in Molecular Biology at the Free University of Brussels (Vrije Universiteit Brussel, VUB), where he obtained his degree with high distinction in 1997. He obtained an FWO scholarship and in 2004 obtained a PhD in Applied Biological Sciences under the promotorship of Prof De Baetselier, working on cancer genetics, cellular biology and immunity. Again, he acquired a FWO grant to continue his postdoctoral research for the periode 2004-2007. In 2004-2005 he was a visiting scientist at the National Cancer Institute in Bethesda USA, studying ovarian cancer genetics and biomarkers, after which he returned to VUB in Belgium for additional training as a postdoctoral scientist: in 2005-2006 in the field of cellular immunology and cancer genetics, in 2007 in antibody-engineering and from 2008 onwards in molecular imaging. In 2013 he became assistant professor in the small animal imaging lab ICMI and since in 2016 full professor. In 2014 he co-founded the spinoff company Camel-IDS (now called Precirix where he is still active as Sciense Advisor). In 2020 he co-founded the spinoff company AbScint as well. He is currently workpackage leader in the Immune-Image IMI consortium.

His research aims to develop novel applications in molecular and nuclear imaging and targeted therapies. His current focus is on the nanobody-technology as targeting vehicles. More in particular, his research is focused on the generation of new probes for their application (nuclear or other types of imaging a therapy) in small animal models of disease, and in a second phase on testing in patients. Hence, his preclinical and translational research is supportive and closely connected with clinically oriented research teams within the faculty of medicine and the university hospital.

Christian Vanhove
Associate professor Ghent University
Medical Imaging and Signal Processing
Head of preclinical core imaging facility UGent (INFINITY)

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Christian Vanhove graduated as a Biomedical and Clinical Engineer from Brussels University in 1990. From 1991 until 1996, he worked as a Medical Physicist at the Nuclear Medicine department of the Sint-Elisabeth hospital in Zottegem, where he was doing research and developments for the industry in a clinical environment. Research and developments were focused on all aspects of medical image processing, including image reconstruction, image registration and image quantification. In 1996, he moved to the Nuclear Medicine department of the Brussels University Hospital. As a Medical Physicist Expert, he continued his research in the field of medical image processing and obtained his PhD in Medical Science in 2004. During his PhD, a new algorithm for the automatic segmentation and quantification of gated blood pool SPECT images, in both humans and small animals, was developed and validated. In 2005, he was one of the initiators of the small animal imaging lab of Brussels University and worked as a postdoctoral researcher at this preclinical imaging facility. During this period his research was focused on quantitative pinhole SPECT. Since February 2011, he joined the Medical Imaging and Signal Processing (MEDISIP) research group of the Faculty of Engineering of Ghent University, where he is responsible for the INnovative Flemish IN-vivo Imaging TechnologY (INFINITY) lab. This lab focuses on multimodal in-vivo imaging strategies and serves as a core facility for preclinical imaging within Ghent University. The major research domains of INFINITY are the evaluation of pathophysiology in neurological diseases, cancer and inflammation. Christian Vanhove is associate professor and lecturer in molecular imaging.

Marleen Keyaerts
Associate professor Vrije Universiteit Brussel –
In vivo cellular and molecular imaging laboratory
Head of clinics UZ Brussel – Department of nuclear medicine

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Marleen studied medicine at the Vrije Universiteit Brussel and performed her master thesis at the nuclear medicine department of the UZ Brussel on the biodistribution of a new radiopharmaceutical in healthy subjects. She immediately started her PhD at the ICMI lab after graduation, which was completed in 2011. In her PhD project, Marleen investigated bioluminescence imaging for the assessment of tumor burden. When she moved back to the clinic to complete her clinical training in nuclear medicine, a first nanobody lead compound was ready for first in human testing. Marleen was strongly involved in this phase I clinical trial using anti-HER2 Nanobody PET/CT imaging in breast carcinoma patients and she is currently primary investigator in multiple phase II trials involving nanobody-based imaging.

Timo De Groof
Postdoctoral Scientist at Vrije Universiteit Brussel
Research cluster Imaging and Physical Sciences (BEFY)
In vivo Cellular and Molecular Imaging laboratory

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Timo De Groof studied Biochemistry and Biotechnology at the University of Ghent where he graduated in 2015. During his master studies, he specialized both in Biomedical Biotechnology and Structural Biology/Biochemistry. During his last year of studies, De Groof performed research in the biopharmaceutical company Argenx where he gained experience on the identification and characterization of llama-derived antibodies in inflammatory diseases and oncology.
From 2015 to 2019, De Groof performed his PhD in the Medicinal Chemistry group at VU University Amsterdam under the supervision of prof. dr. Martine Smit where he focused on development and characterization of nanobodies targeting viral G protein-coupled receptors while also focusing on different applications of these nanobodies as therapeutics and research tools.
From September 2019, De Groof started working as a postdoctoral researcher in the In Vivo Cellular and Molecular Imaging (ICMI) group under the supervision of prof. dr. Nick Devoogdt. As part of the Innovative Medicine Initiative project “Immune Image”, he is currently focusing on the development of nanobody-based immunotracers for non-invasive imaging of immune cells during immunotherapy. Moreover, De Groof supervises, in collaboration with prof. Devoogdt, the immunization and nanobody generation process for the different projects within the ICMI group.

Jessica Bridoux
Postdoctoral Scientist at Vrije Universiteit Brussel
In vivo cellular and molecular imaging laboratory

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Jessica studied Engineering Chemistry at the National Engineering School of Caen (Ecole Nationale Supérieure des Ingénieurs de Caen, ENSICAEN, Caen, France) and obtained her diploma in 2016, as well as a Master in Organic Chemistry at the university of Caen, France. She worked for 6 months at PCAS (Limay, France), where she developed and optimized the synthesis process of an active pharmaceutical ingredient and learned about GMP manufacturing. In October 2016, Jessica started her PhD project funded by the Horizon 2020 Marie Curie Actions (PET3D) at ICMI, under the supervision of Prof. Dr. Catarina Xavier and Prof. Dr. Vicky Caveliers. She worked on the development of a radiolabeled Nanobody against the human PD-L1 receptor for PET immune-imaging. In the frame of this project, she spent 2 months at the Karolinska Institute, Stockholm, Sweden, where she focused on click-chemistry mediated radiofluorinations. After her PhD, she will stay at ICMI to focus on the clinical translation of radiofluorinated Nanobodies.

Chloé Ackaert
Senior Scientist at ImmunXperts

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Chloé studied Pharmacy at the Katholieke Universiteit Leuven and performed her master thesis at the Hôpital Bichat in Paris, the CHU in Charleroi and the UZ Antwerpen on Enzyme Replacement Therapy in Fabry disease patients. She did an internship in the laboratory of Therapeutic and Diagnostic Antibodies (KUL) on the Immunogenicity of anti-TNFα mAbs in IBD patients, followed by a PhD on the immunogenicity of modified allergens at the University of Salzburg in Austria. After her PhD, she was involved in the start-up of the CRO ImmunXperts, offering integrated immunogenicity services. Since 2015, Chloé worked in the Camel Antibody group within the Cellular and Molecular Immunology lab (VUB) where she focussed on studying the immunogenicity of Nanobodies. She performed the anti-drug antibody measurements in the phase I clinical trial using anti-HER2 Nanobody for PET/CT imaging in breast carcinoma patients and assessed the immunogenicity risk of several Nanobodies by *in vitro* methods. As of October 2018, she joined the team of ImmunXperts again, as a senior scientist for projects dealing with assessment of (un)wanted immunogenicity of biopharmaceuticals in development.

Sophie Hernot
Assistant Professor Vrije Universiteit Brussel – In vivo Cellular and Molecular Imaging Laboratory

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Sophie Hernot obtained her Master of Science in Bio-engineering from the Vrije Universiteit of Brussels. She completed her PhD thesis in Medical Sciences in 2011 on the use of microbubbles (ultrasound contrast agents) as well as Nanobodies for molecular imaging and drug delivery applications. As post-doctoral researcher in the Laboratory of In vivo Cellular and Molecular Imaging (ICMI, VUB), her focus was initially on cardiovascular molecular imaging. Radiolabeled Nanobodies targeting markers of inflamed atherosclerotic plaques were optimized and their potential for the non-invasive imaging of atherosclerosis was demonstrated. As Tenure Track Professor since 2020, her research interests gradually shifted towards fluorescence molecular imaging in surgical and interventional applications. In this context, near-infrared fluorescently labeled Nanobodies are designed and preclinically validated as tools for image-guided surgery, with the aim of translating them to the clinic.

Matthias D'Huyvetter
Assistant professor Vrije Universiteit Brussel
FWO postdoctoral researcher
In vivo Cellular and Molecular Imaging laboratory

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Matthias D'Huyvetter (1986) is an assistant professor (0.1ZAP) at the Vrije Universiteit Brussel (VUB) and a postdoctoral fellow of the Research Foundation Flanders-FWO. He graduated in 2009 as Master in Biomedical Sciences at the University of Antwerp. In 2014, he received his PhD in Medical Sciences at the VUB for his work on radiolabeled single-domain antibody fragments as theranostic drugs for cancer treatment. During his PhD, he spent one year at Duke University, North-Carolina USA, under the guidance of Michael Zalutsky. Also in 2014, he co-founded the VUB spin-off company Camel-IDS. In 2019, he obtained the degree of executive Master in Business Administration at Antwerp Management School.

His academic research involves the design and characterization of novel theranostic agents in the field of targeted radionuclide therapy, with a dominant focus single-domain antibody fragments as targeting vehicles and alpha- and beta-particle emitters as toxic payload.

Geert Raes
Assistant professor & valorization manager
Vrije Universiteit Brussel
Cellular and Molecular Immunology unit
Staff Scientist at VIB Inflammation Research Centre

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After obtaining a degree in bio-engineering and a PhD in tumor immunology, Geert has been staff scientist at VIB, where he has been involved in projects aimed at translating basic immunology research into industrial applications. Since 2013, he also has a partial appointment as valorisation manager at Vrije Universiteit Brussel, focusing on economic valorisation of nanobody applications in molecular imaging and therapy. Geert is co-inventor on 6 patent families. He is co-founder and IP & Legal Advisor of the VUB spin-off company Precirix and co-founder of Abscint.

Sabrina Oliveira
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Sabrina Oliveira was introduced to Utrecht University through an internship at the department of Pharmaceutical Sciences (2004) during her studies at the Faculty of Pharmacy of Coimbra University in Portugal. After graduation, she obtained an individual doctoral grant from the Portuguese Foundation for Science and Technology (FCT) to return to this department to do her PhD research on Targeted Cancer Therapies (2004-2008). She then worked as a postdoc on the development of tracers based on nanobodies for optical molecular imaging, in the group of Cell Biology, department of Biology (2008-2010) and the department of Pathology from the University Medical Center Utrecht (2010-2012). In 2012, she was awarded a VENI grant from the Netherlands Organisation for Research (NWO-STW), giving her the opportunity to start her own research line, combining her interests in cancer therapy and molecular imaging. Her main research is focused on rendering photodynamic therapy tumor-specific, by targeting the photosensitizer with nanobodies. In 2016, she received a Starting Grant from the European Research Council (ERC) to continue her line of research and start her own group. In July 2016, Sabrina was appointed Assistant Professor, and in May 2019 Associate Professor, with a shared position between the division of Cell Biology, Neurobiology and Biophysics, department of Biology and the division of Pharmaceutics, department of Pharmaceutical Sciences. In addition to developing nanobody-targeted photodynamic therapy, her interests are on using nanobodies for rendering other therapies more specific.

Daniëlle Vugts
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Danielle Vugts studied chemistry at the VU University Amsterdam, where she graduated in 2002. Hereafter she continued in the same field and did her PhD studies in the group of Prof. Dr. Romano Orru, which she completed in 2006 and combined multicomponent reaction chemistry, enzymatic synthesis with total synthesis. Next, she did a postdoctoral study on pretargeted imaging with Prof. Dr. Guus van Dongen at Amsterdam UMC, the Netherlands. Hereafter she was a Roche postdoctoral fellow, followed by assistant professor in the same group. Currently she is associate professor at Amsterdam UMC, and she is responsible for the development of all 89Zr-labeled compounds for preclinical and clinical studies. Her research interests are immuno-PET, neuroimaging as well as development of new radiolabelling methodologies for fluorine-18, carbon-11 and zirconium-89.

Erik Aarntzen
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Erik worked as a resident Internal Medicine at the Radboudumc in Nijmegen, The Netherlands, from 2008 to 2012. He defended his PhD thesis titled 'Monitoring immune responses to dendritic cell-based vaccination, with a focus on in vivo imaging' with honors in 2013. After completion of his residency in Nuclear Medicine in 2017, he now holds a permanent staff position at the Department of Medical Imaging at the Radboudumc. His research group on Immune Imaging performs several clinical studies with a strong translational focus, mainly in the field of onco-immunology. In these studies, multiple tracers, ranging from radiolabelled nanoparticles, autologous immune cells to therapeutic antibodies and small peptides are exploited to better understand the critical components of the tumor microenvironment at both local and system level.
Erik is also one of the founders and current co-chair of the European Society for Molecular Imaging (ESMI) study group Onco-Immunology and Therapy, and former board member of the European Association for Nuclear Medicine (EANM) committee Infection and Inflammation.

Information lectures:

Do's and don'ts in immunotracer design (Nick Devoogdt)

In this presentation, the concept 'molecular imaging' will be introduced and a general overview of different types of tracers will be given. A major focus will be on antibody-tracers: how does the immune system develops an efficient antibody response? what are the molecular and structural features of antibodies? What are the different techniques to generate antibodies and how can they be engineered into different types of antibody-fragments? The pharmacokinetic behavior of antibodies and engineered fragments will be explained, and their relation to molecular and nuclear imaging. The antibody pre-targeting approach will be briefly touched upon. Finally, various concepts related to tracer design will be explained, including 'affinity', 'avidity', 'enhanced permeability and retention', 'the site-barrier-effect in tissue penetration', 'specific activity' and 'antigenic sink'.
This first lecture will also try to provide links and an introduction to other lectures given during the workshop.

Overview of imaging modalities (Christian Vanhove)

In 1895 the German physicist Wilhelm Conrad Roentgen discovered the X-rays, an achievement that earned him the first Nobel Prize in Physics in 1901. These X-rays produced the first medical images in the beginning of the previous century. Nowadays, a wide range of imaging techniques are available that can be roughly sub-divided into two main categories: structural and functional imaging devices. The most commonly used structural imaging technologies are computed tomography (CT), magnetic resonance imaging (MRI) and ultrasound imaging (US). These techniques were developed to non-invasively visualize nonspecific macroscopic anatomical and physiological changes in tissues. Functional imaging modalities, such as single photon emission tomography (SPECT), positron emission tomography (PET) and optical imaging (OI) focus on the visualization of molecular/cellular targets in living subjects. These targets in functional imaging can include transporters, cell surface receptors and intracellular enzymes.
An overview will be presented of the physical basics behind the image formation process of these imaging modalities. The requirements to move from clinical to preclinical imaging will also be discussed for each modality. The presentation will start with CT that can be seen as a direct evolution of X-ray imaging. Secondly, the nuclear imaging techniques SPECT and PET will be presented, including the important evolution of combining these nuclear imaging techniques with CT and MRI. The more complex image formation process of MRI will be shortly introduced. Finally, OI will be presented, including bioluminescence and fluorescence in-vivo imaging, which are of more importance in the preclinical arena.

Radiochemistry of Nanobodies (Jessica Bridoux)

At ICMI, radiolabeled Nanobodies are a produced to be used as therapeutic or diagnostic agents. In this lecture we will discuss the techniques to radiolabel Nanobodies. We will first describe the different radionuclides, their related radiochemistry and how the different chemistries can be applied to Nanobodies. In particular, we will discuss two possible approaches: 1/ a random approach where the radionuclide is coupled onto the Nanobody's structure, or 2/ a site-specific approach where the radionuclide is coupled to the Nanobody in a controlled manner. Finally, we will focus on how to analyze the radiolabeled Nanobodies and see how the techniques can be applied clinical settings. The goal of the lecture is to understand the radiosynthesis process of Nanobodies and the specifications of the obtained radiopharmaceutical for pre-clinical or clinical applications.

Nanobody discovery and biotechnological applications (Timo De Groof)

Llama and camels have unique antibodies comprising a homodimer of heavy chain polypeptide, whereby the antigen is recognized by virtue of one single domain. A straightforward technology was developed to immunize a camelid, to clone the repertoire of antigen-binding fragments, from which the antigen-specific fragments are identified after phage display selections. The resulting recombinant, antigen-binding single-domain antibody fragments are also referred to as Nanobodies (Nbs) because of their size of 4 nm by 2.5 nm in diameter.

Nanobodies are well produced in microbial systems, very robust and highly soluble, bind their cognate antigen with high affinity and specificity. Very often the Nanobody recognizes an epitope that is difficult to target with human or mouse antibodies. The 'humanization' of a camel derived single domain antibody is straightforward. Probably, the largest advantage of Nanobodies comes from their strict monomeric behavior and the ease to tailor them into larger pluripotent constructs.

Such beneficial properties of Nanobodies over other antigen-binding fragments from conventional antibodies inspired many researchers to employ Nanobodies as a versatile tool in various innovative applications in biotechnology and medicine.

Imaging with nanobodies in preclinical models (Nick Devoogdt)

In this lecture, the focus will be on nanobodies and their evaluation by nuclear imaging in animal models of various diseases. The lecture will start with a rather detailed overview of protocols to generate good nanobody vehicles and will then look at subsequent attention points on which parameters and criteria should be considered to select a lead compound. We will look at structural features of nanobodies and how this is reflected in their amino acid sequence. A major part of this lecture will consist of an overview of recent published and unpublished applications of nanobodies in small animal nuclear imaging in pathologies ranging from oncology to autoimmune disease, macrophage-tracking, and some examples of nanobody engineering to ameliorate tracer performance.

Introduction to the clinical nuclear medicine (Marleen Keyaerts)

This presentation will give an overview of the routinely performed nuclear medicine examinations in patients, with a focus on the uptake mechanism of different tracers as well as a personal view on the future of clinical nuclear medicine. The session is intended for students and researchers in the preclinical field that would like to get a better understanding of daily practice in a nuclear medicine department in the hospital. Typical images of routinely performed scans will be discussed and interpreted.

Fluorescence applications of nanobodies (Sophie Hernot)

A general overview will be given regarding the application of fluorescence imaging for image-guided surgery. Both the technical aspects of optical imaging devices as well as the design of fluorescent tracers will be discussed. Current and emerging clinical applications will be illustrated, as well as the role the nanobodies in this field.

Clinical translation of nanobody-tracers (Marleen Keyaerts)

The clinical translation of new imaging agents will be discussed using the 68-Ga-labeled MMR and HER2 nanobodies as examples. Participants will get insight into regulatory requirements for clinical trials, including GMP compliance, clinical trial application with the competent authorities and ICH-GCP requirements. A study protocol for a phase I study will be presented and explained, as well as the obtained results.

Targeted radionuclide therapy, focus on nanobodies (Matthias D'Huyvetter)

This presentation will introduce the basics of Targeted Radionuclide Therapy of cancer and highlight different exciting applications that are now available in the clinic. In addition, an important part of the talk will focus on the use of Single-Domain Antibody fragments (sdAbs) as potential vehicles for Targeted Radionuclide Therapy using both beta$^-$- and alpha-particle emitting isotopes as toxic payload.

Intellectual property of biologics (Geert Raes)

Some basic principles of intellectual property rights as they relate to the use of biologics will be discussed, thereby using nanobodies as an example. After a brief overview of the main features and types of intellectual property rights, the focus will be on patents: advantages of patents, requirements for patentability, limitations to patentable subject matter and formulation of patent claims. Finally, the distinction between patentability and freedom-to-operate will be explained.

Immunogenicity of recombinant proteins (Chloé Ackaert)

The immunogenicity of recombinant biopharmaceuticals will be discussed with the focus on clinical impact of immunogenicity and methods available to assess the immunogenicity risk. Participants will get insight into in silico, in vitro and in vivo methods for immunogenicity risk assessment, as well as anti-drug antibody monitoring in clinical trial phases. Case studies will be discussed as illustration and the immunogenicity of the Ga-NOTA-anti-HER2 Nanobody will be presented.

Nanobody-targeted photodynamic therapy… and beyond (Sabrina Oliveira)

In an attempt to improve selectivity of photosensitizers in oncologic photodynamic therapy (PDT), antibodies have been employed to target the photosensitizers to particular receptors overexpressed on cancers' cell surface. Recently, this strategy has led to promising results and continues to be tested in the clinic (NCT03769506) using the photosensitizer IRDye700DX, that is a silicon phthalocyanine derivative, conjugated to the antibody cetuximab to target the epidermal growth factor receptor (EGFR), overexpressed in head and neck cancers. Nevertheless, antibodies (150 kDa) are known for their long half-life in the bloodstream and their relatively poor tissue distribution, which may lead to long photosensitivity in patients and limited therapeutic efficacy. As an alternative approach, we have employed nanobodies for targeting of photosensitizers. The relatively small dimensions of nanobodies combined with high binding affinities, lead to rapid tumor accumulation, homogenous distribution, and rapid clearance of unbound fractions. After our first studies targeting EGFR for head and neck cancer, we have explored this targeted PDT approach with other nanobodies targeting different tumor markers. As photosensitizer, we too employ the IRDye700DX. Overall, with nanobody-targeted PDT, cytotoxicity is selectively induced in cells which have high target expression level. Preclinical studies have shown selective tumor necrosis and significant tumor

regression after a single treatment session. This lecture will summarize these studies and discuss more recent results obtained with nanobody-targeted PDT. Finally, brief examples will be given on additional approaches using nanobodies for targeted therapy.

89Zr-immuno-PET imaging: from bench to bedside and back (Daniëlle Vugts)

In this presentation, the basics of Zr-89 radiochemistry will be covered including a) the production of zirconium-89, b) the different chelators that are suited for zirconium-89 and 3) the optimal radiochemistry conditions. Hereafter examples of preclinical and clinical studies will be discussed both for imaging in oncology and neurology.

Defining the role of molecular imaging in the field of immune-oncology (Erik Aarntzen)

Immune checkpoint inhibitors landmark an era of clinically effective immune therapies targeting checkpoint molecules like CTLA-4 and PD-1/PD-L1 to unleash cytotoxic CD8+ T-cells. Early positive clinical studies sparked an unprecedented number of combination strategies in a wide range of tumor types. Notwithstanding the positive impact of immune checkpoint inhibitors, its incremental value on overall survival after its' initial success is in sheer contrast with the exploded number of clinical trials and in health care expenditure. Thus, smarter designs of immune therapy development pipelines should be investigated to guide these developments. Ideally, these should be built on patient data, avoiding the inherent differences between mouse and human immune systems, and incorporating age, medication and co-morbidities to conceptualize the potential efficacy of novel (combination) strategies. In vivo molecular imaging has potential to become a part of such pipeline, as it offers quantitative and reproducible, longitudinal data of a functional process, on a whole-body scale and with great sensitivity. Challenging however, is to implement in vivo molecular imaging in such way that it creates maximum impact with regard to its' strengths and limitations. This lecture will introduce the current clinically available tracers to image immune cells and will discuss how these are being used as biomarkers. Based on recent literature, we will together define the role of molecular imaging in onco-immunology.
