ONCORNET2.0 launch online

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DISSEMINATION ONCORNET2.0

The ITN Marie Curie Training Network ONCORNET (Oncogenic GPCR Network of Excellence and Training) focuses on the chemokine receptors CXCR4 and Atypical Chemokine Receptor 3 (ACKR3, formerly referred to as CXCR7). These receptors and their associated signalling cascades are involved in cancer progression and metastasis. Increased expression of CXCR4 and ACKR3 is detected in a wide range of tumours and within the tumour microenvironment and is often associated with poor prognosis. Their contribution to cancer progression, however, still remains poorly understood. As these two receptors belong to the highly druggable family of G protein-coupled receptors (GPCRs), targeting CXCR4 and/or ACKR3 function may be an attractive approach for therapeutic intervention in cancer.

As a consortium, we have developed multiple CXCR4 and ACKR3 tools for diagnostic, research and therapeutic purposes. Functional assays have been established to dissect the signalling properties of these receptors, discover their interactome, and reveal the role of receptor phosphorylation and their capacity to form oligomers in a (patho) physiological context. Multidisciplinary research and cutting-edge technologies have been employed to understand the role of these receptors in oncogenic and immunological processes. Importantly, an ONCORNET toolbox is now in place to take our research to the next level.

ONCORNET highlights

Within the first ONCORNET project, early stage researchers (ESRs) developed new unique tools to modulate and/or detect CXCR4 and ACKR3 expression. Novel modulators, including small ligands (some of which are fluorescent/radiolabelled), pepducins, adrenomedullin and nanobodies targeting these receptors, were generated and/or characterised. Compilation of experimentally validated structural and ligand data on chemokine receptors resulted in the generation of protein-ligand 3D models of CXCR4 and ACKR3 with chemokine CXCL12 and small molecules. These models helped devise synthetic strategies based on virtual screening approaches and determine the structure-activity relationships of CXCR4/ACKR3 ligands to develop novel (labelled) ligands for *in vitro* and *in vivo* studies.

Various fluorescent CXCR4 and ACKR3 ligands have been generated that serve as excellent probes in NanoBRET-based ligand binding studies and visualisation of these receptors using live-cell confocal microscopy. A suite of nanobodies and their Nanobody-Fc (Nb-Fc) derivatives, targeting multiple CXCR4 epitopes, were identified. The Nb-Fc formats demonstrated superior potencies in inhibiting GPCR signalling and HIV entry, inducing Fc-mediated antibody effector functions (ADCC and CDC) and killing CXCR4-expressing cancer cells *in vitro*.

Advanced imaging techniques using labelled receptors or ligands (small molecules, engineered nanobodies) provided insight into spatial and temporal signalling, trafficking profiles of CXCR4 and/or ACKR3 and evidence that these receptors form homo- and heteromers. Using high-resolution microscopy, CXCR4 was shown to form dynamic transient homodimers at increasing expression levels. Binding of subpocket-specific inverse agonists suppress both dimerisation and constitutive activity and may represent a new strategy to target CXCR4.

Kinetic studies using newly generated biosensors were performed to monitor changes in receptor conformation and G protein activation by different CXCR4 ligands. Proteomic analysis disclosed the interactome of ACKR3, revealing the co-localisation of novel proteins regulating ACKR3 function. ACKR3 was shown to interact with Connexin 43, inhibiting astrocytic gap junctional intercellular communication. These findings shed new light on the pathophysiological role of this receptor. Distinct residues within the C-tail of ACKR3 differentially regulate CXCL12-induced β-arrestin recruitment, ACKR3 trafficking and internalisation.

Various cancer models were established within ONCORNET. In breast cancer, CXCR4 and ACKR3 cooperate with growth factors present in the tumour microenvironment to foster oncogenic signalling. Here, GRK2 was identified as a modulator of such crosstalk in a cell type-dependent manner. Relevant organotypic 3D cultures have been set up and CRISPR-Cas technology implemented in experimental models, including for the oncogenic human papillomavirus (HPV). In addition, expression of CXCR4/ACKR3 in bone and bone marrow microenvironments of mice were assessed. The publication list on the ONCORNET highlights can be accessed via the ONCORNET2.0 website (ONCOgenic GPCR Network of Excellence and Training, available at www.oncornet.eu) with the number of publications still growing.

ONCORNET2.0 next level

After completion of the successful ONCORNET project, we were thrilled to continue our journey training the next generation of multidisciplinary scientists in drug discovery via the ONCORNET2.0 MSCA ITN funding. The ESRs of ONCORNET2.0 will, like their former colleagues in ONCORNET1.0, receive exclusive international, intersectoral and interdisciplinary 'GPCR drug discovery' training. The validated and optimised structured training programme of ONCORNET2.0 includes training in a diverse set of scientific and transferable skills (entrepreneurship, academic writing, media training, valorisation) as well as secondments, increasing the employability and career prospects of our young researchers.

With the ONCORNET toolbox in place, we will develop new strategies for CXCR4/ACKR3 modulation (photochemistry, formatted nanobodies) and investigate their effects on oncogenic responses, to yield key new knowledge and potential leads for drug development and, potentially, commercialisation. By leveraging on the success and experience of ONCORNET and recent ground-breaking advances in the field of GPCRs (e.g. cryo-EM, CRISPR-Cas, advanced microscopy), ONCORNET2.0 brings a young, gender-balanced team of ESRs and principal investigators (PIs) together to target these oncogenic GPCRs.

In February 2020, 15 young ESRs were selected after interviews in Amsterdam with PIs from participating organisations. After selection, plans were made by the ESRs to move to their respective host institutes.

and then COVID-19 hit…

No one had foreseen what an impact this pandemic would have. The first ESRs moved to their host countries but had to start working remotely from their new homes, waiting for the labs to reopen.

A year and a half into the four-year project, ONCORNET2.0 has adapted to the new challenges and is well on its way and with a new mindset, embracing the digital world. The ONCORNET2.0 kick-off meeting was held online in November 2020 (see Figure 1). The ESRs met their peers, other consortium members and ONCORNET alumni virtually. Although not ideal, we got creative in providing a system to keep us all engaged. We established monthly ESR scientific sessions, where ESRs present their progress and plans to the consortium. More focused Zoom and Team sessions are scheduled to discuss plans amongst ESRs and PIs in more detail.

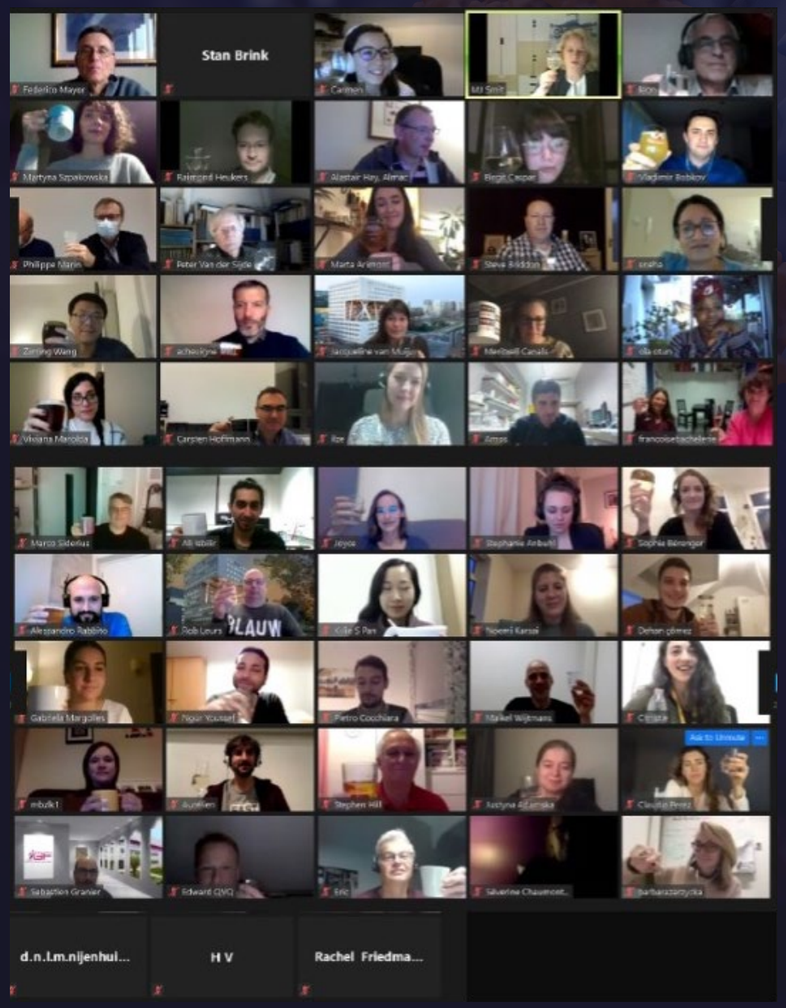

Figure 1: Online toast with ONCORNET and ONCORNET2.0 participants during the kick-off meeting in November 2020.

Additionally, technology forum sessions were introduced on a monthly basis, where more senior scientists present a particular technique or method, providing historical perspectives, current status and future expectations of given technologies. Topics presented so far include GPCR labelling strategies (GFP/SNAP/Halo/CLIP/FlAsH) (Carsten Hoffmann, UJENA), luminescence (Thomas Machleidt and Rachel Ohana, partner organisation Promega), fluorescence correlation spectroscopy (Steve Briddon, UNOTT), and molecular brightness analysis (Paolo Annibale, MDC). With the rapid progress of the GPCR-related/associated research areas and technologies, these in-depth technological sessions are much appreciated by both PIs and ESRs. This is shown by the active participation with lively discussions by the whole consortium. The participant number of these online sessions is high, which shows the interest of all consortium members.

Despite the COVID-related disruptions, the ESRs have adapted well, significant volumes of data have been acquired, and projects and collaborations are on their way. Most notably, the output of ONCORNET and the transfer of tools and assays to this consortium was important to kick start this programme.

A growing consortium: addition of partner organisations

During the first year of the project, partner organisation Luxembourg Institute of Health (LIH) and biotech company ISAR Biosciences (ISAR) joined ONCORNET2.0. A 16th PhD student from LIH was added to the ONCORNET2.0 team. Chevigné and colleagues from LIH have recently revealed a link between ACKR3 and the opioid system, providing a new and important asset to our consortium. ISAR is envisioned to play a role in the implementation of CRISPR-Cas edited stem cells in diverse research lines.

ONCORNET alumni

ONCORNET alumni also met with the next generation of ONCORNET2.0 ESRs online, and some have been involved in the first online workshop. Secondments and transferable skills were ranked as most helpful in the career development by the ONCORNET1.0 ESRs. The benefits of building extensive networks during their PhD training and inter/transdisciplinary and international secondments enabled the ESRs to see their projects in a new and broader perspective. Several of them currently coordinate and manage multidisciplinary drug discovery projects in their current roles, having learned the necessary skills to bridge different fields and organisations through their ONCORNET experience.

Outreach to the broader public

To disseminate ONCORNET2.0 activities and discoveries more widely and reach a larger, more diverse audience, the ESRs have started using the ONCORNET Twitter account. So far, some ESRs have introduced themselves and their research projects using this platform. Additionally, relevant conferences, poster- and oral presentations and (open access) publications will be posted when applicable. Blogs and vlogs are in the pipeline, so keep an eye out on the ONCORNET website!

A brighter future…?!

With the pandemic keeping the world in a tight grip, we have learned to adapt. Secondments (international research internships) by the ESRs will start in the coming year, after the reopening of the (vaccinated) world. Also, the second workshop, which is planned for mid-November 2021 at IGF in Montpellier, contains hands-on experimental activities on proteomics and structural biology. Fingers crossed that this workshop will not be hampered by further restrictions. All ESRs, PIs and supervisors wish to (finally) meet in person (again)!

For more information, please visit **www.oncornet.eu**

Project Partners

PIs from Academia:
Martine Smit, Jacqueline van Muijlwijk and Ellen Langemeijer, **(Coordinators and project manager),** Rob Leurs, Marco Siderius, Henry Vischer, Maikel Wijtmans and Iwan de Esch, **Vrije Universiteit Amsterdam (VUA);**
Steve Hill, Steve Briddon, Meritxell Canals and Laura Kilpatrick, **University of Nottingham (UNOTT);**
Philippe Marin, Sébastien Granier, Thierry Durroux and Séverine Chaumont-Dubel, **CNRS IGF, Montpellier;**
Martin Lohse, Paolo Annibale, Andreas Bock, **MDC Berlin;**
Carsten Hoffmann and Julia Drube, **University of Jena (UJENA);**
Graeme Milligan and Gerry Graham, **University of Glasgow (UGLA);**
Federico Mayor and Petronila Penela, **Universidad Autonoma de Madrid (UAM);**
Françoise Bachelerie and Geraldine Schlecht-Louf, **INSERM, Paris;**
Andy Chevigné and Martyna Szpakowska, **Luxembourg Institute of Health (LIH).**

PIs from SMEs:
Raimond Heukers and Edward Dolk, **QVQ;**
Aurélien Rizk, Mirjam Zimmermann and Susan Roth, **InterAx.**

Partner organisations:
Eric Trinquet, **Cisbio;**
Chris de Graaf, **Sosei Heptares;**
Rachel Friedman Ohana, **Promega;**
Leon Delbressine, **Learning By Simulation (LBS);**
Alastair Hay, **ALMAC Sciences Limited.**

Joining after the project started:
Andy Chevigné, **Luxembourg Institute of Health (LIH);**
Martin Lohse, **ISAR Biosciences.**

PROJECT SUMMARY

The ONCORNET2.0 (ONCOgenic GPCR Network of Excellence and Training) consortium (2020–2024) aims to consolidate an international training network of early stage researchers (ESRs) focused on drug discovery for oncogenic GPCRs. The project methods span a wide range of techniques and disciplines aimed at furthering our understanding of two receptors heavily involved in oncogenic processes.

PROJECT LEAD

The project coordinator of ONCORNET2.0 is Prof. Martine J. Smit (Professor Target and Systems Biochemistry, VUA). Martine Smit coordinated ONCORNET1.0, has received personal (NWO-Vidi/Vici) and public-private funding. Her expertise focuses on modulating and unravelling the signalling properties of human and viral chemokine receptors. She is supported by Prof. Jacqueline van Muijlwijk (educational research chair and vice dean education, VUA), Dr Ellen Langemeijer (project manager, VUA) and Prof. Steve Hill (UNOTT) as confidential advisor to support and advise ESRs.

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