Sponsored by:

BD

bio connect life sciences

BIOKÉ 15 YEARS

fwo

IncuCyte® A SARTORIUS BRAND

BCCM · GeneCorner

LUMC

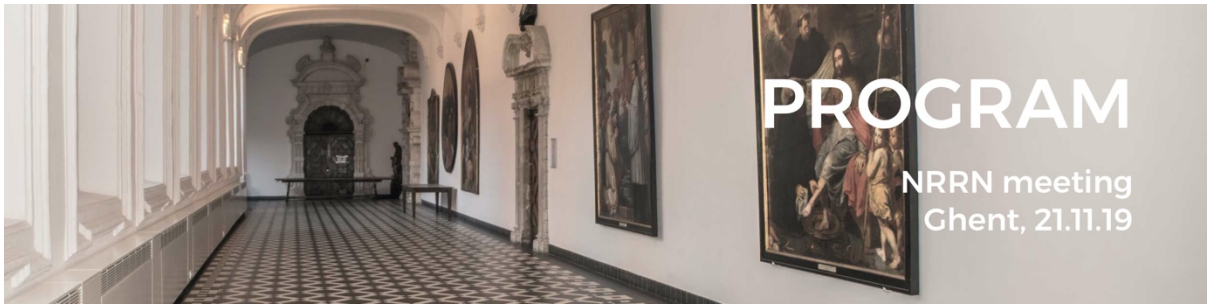
PerkinElmer For the Better

Promega

tebu-bio Innovative Lab Services & Reagents

UNIVERSITEIT GENT

ESSEN BioScience



09h00 - 09h55	Registration, poster mounting, booth set-up
09h55 - 10h05	Welcome by Karolien De Bosscher and co-organizers
10h05 - 10h45	**Bart Deplancke**, Resolving the regulatory network underlying PPARγ-driven adipogenesis
10h45 - 11h00	**Roy Eerlings**, Developing an artificial nuclear receptor, the synthetic yeast approach
11h00 - 11h35	Coffee break and poster/sponsor booth viewings
11h35 - 12h10	**Bas Van Steensel**, Large-scale perturbation approaches to study gene regulation
12h10 - 12h25	**Lisa Butler**, Fatty acid elongation is a novel androgen receptor-regulated therapeutic vulnerability in prostate cancer
12h25 - 12h40	**Fatma Özgün**, Characterization of androgen receptor variant 7 dimerization in prostate cancer
12h40 - 12h55	**Jonathan M. Preuss**, Augmented acute lung injury and dysregulated immune response of GRdim mice under intensive care management in endotoxemia
12h55 - 14h10	Lunch and poster presentations/sponsor booth viewings
14h10 - 14h50	**Jerome Eeckhoute**, Nuclear receptor control of hepatic molecular identity
14h50 - 15h25	**Saskia van Mil**, FXR controls hepatic lipid accumulation via isoform selective DNA binding
15h25 - 15h45	Short coffee break
15h45 - 16h25	**Henriette Uhlenhaut**, Essential functions of the GR require DNA binding
16h25 - 17h10	**Keynote: Vincent Giguère**, New paradigms in metabolic regulation by nuclear receptors
17h10 - 18h30	Closing remarks and Poster prizes, reception and networking opportunities


POSTER ABSTRACTS
NRRN meeting
Ghent, 21.11.19

1. Butler Lisa

Fatty acid elongation is a novel androgen receptor-regulated therapeutic vulnerability in prostate cancer

Margaret M. Centenera[1,2], Jelle Machiels[3], Zeyad D. Nassar[1,2], Irene Zinonos[1], Adrienne Hanson[4], Katarzyna Bloch[3], Elizabeth D. Williams[5], Andreas Evdokiou[1], Wayne D. Tilley[1,4], Luke A. Selth[1,4], Johannes V. Swinnen[3] and Lisa M. Butler[1,2]

[1] School of Medicine and Freemasons Foundation Centre for Men's Health, University of Adelaide, Australia; [2] South Australian Health and Medical Research Institute, Adelaide, Australia; [3] Laboratory of Lipid Metabolism and Cancer, LKI Leuven Cancer Institute, KU Leuven, Belgium; [4] Dame Roma Mitchell Cancer Research Laboratories, Discipline of Medicine, University of Adelaide, Australia; [5] Translational Research Institute, Queensland University of Technology, Brisbane QLD 4001, Australia.

Dysregulated lipid metabolism is a hallmark of prostate tumorigenesis. A key challenge in the management of prostate cancer is the therapy resistance that ensues upon androgen deprivation therapies, highlighting the need for novel targets. At the core of androgen-regulated prostate cancer cell biology is increased synthesis, uptake and utilisation of lipids, which are required for signalling processes and membrane function. While androgen signalling is a major driver of both lipid metabolism and tumorigenesis in prostate cancer, the precise influence of androgens on cellular lipid composition remains poorly understood. Using mass spectrometry-based lipidomics, this study revealed striking androgen-regulated changes in phospholipid fatty acyl chain length in prostate cancer cells and patient-derived explants. Potent and direct AR-mediated induction of ELOVL Fatty Acid Elongase 5 (ELOVL5), an enzyme that catalyzes fatty acid elongation, was demonstrated in prostate cancer cells, xenografts and clinical tumors. ELOVL5 is the predominant ELOVL expressed in clinical prostate cancer, and is upregulated at the mRNA and protein level compared to non-malignant prostate. ELOVL5 depletion by siRNA reversed the androgen-induced elongation phenotype, markedly altered membrane fluidity and mitochondrial function, and significantly attenuated prostate cancer cell viability and 3D growth, and in vivo tumor growth and metastasis. These findings identify acyl chain elongation and, specifically, ELOVL5 as a novel therapeutic vulnerability for prostate cancer. More broadly, this study demonstrates that by retuning the lipid composition of cell membranes, enzymes involved in phospholipid chain length are not only directly androgen regulated, but can significantly influence key tumorigenic properties of prostate cancer cells.

2. Buurstede Rob

Applying selective glucocorticoid receptor modulators to identify genes involved in memory consolidation

Rob Buurstede[1], Marcia Santos da Silva[1], Eduardo Umeoka[2], Haillang Mei[3], R.A. Sarabdjitsingh[4], Harm Krugers[2], Hazel Hunt[5], Marian Joëls[4] and Onno Meijer[1]

[1] Department of Medicine, Division of Endocrinology, Leiden University Medical Center, The Netherlands; [2] Swammerdam Institute for Life Sciences, Faculty of Science, University of Amsterdam, The Netherlands; [3] Sequence Analysis Support Core, Leiden University Medical Center, The Netherlands; [4] Translational Neuroscience, Brain Center Rudolf Magnus, University Medical Center, The Netherlands; [5] Corcept Therapeutics, Menlo Park, United States of America

Besides the well-known anti-inflammatory effects of glucocorticoids (GCs), these stress hormones play a key role in memory consolidation. In order to remember and in the future avoid re-encountering dangerous situation alike, GCs secreted during the stressor activate Glucocorticoid Receptors (GRs) in the brain to enhance memory consolidation. Blocking GR activation by administration of Mifepristone leads to memory impairment, confirming the role of the GR in the process. This project aims to identify the specific genes involved in GC enhanced memory consolidation. Selective GR Modulators (SGRMs) were utilized as an additional layer to select the genes involved.

Two cohorts of mice were subjected to a Cued-Fear conditioning (CFC) paradigm, followed by subcutaneous injection with either vehicle, Corticosterone, Mifepristone and SGRMs CORT108297 or CORT118335. The effect of treatment on memory consolidation was assessed by percentage freezing during retrieval in cohort I, with an increase in freezing as a measure of enhanced memory. Cohort II was sacrificed 3 hours after injection to assess the effect of acute treatment on gene expression levels. The dorsal right hippocampus was dissected and processed for RNA-sequencing to acquire transcriptome data.

The established effects of Corticosterone and Mifepristone on memory consolidation were recapitulated in the CFC paradigm in mice, evident from increased freezing after Corticosterone and decreased freezing after Mifepristone treatment. CORT118335 showed a similar effect as Mifepristone, while CORT108297 did not affect the freezing behaviour of the mice. The treatment effect of these compounds on behaviour will be combined with the transcriptome data from the parallel cohort and used as a filter to identify the genes involved in enhanced memory consolidation.

3. Clarisse Dorien

Inhibiting the mineralocorticoid receptor enhances glucocorticoid-induced apoptosis in multiple myeloma cells

Dorien Clarisse[1,2], Philip Vlummens[3], Karlien Van Wesemael[1,3], Jan Tavernier[1,2], Ilse M. Beck[4], Fritz Offner[2,3] and Karolien De Bosscher[1,2]

[1] VIB-UGent Center for Medical Biotechnology, Department of Biomolecular Medicine, Ghent University, Ghent, Belgium; [2] Cancer Research Institute Ghent (CRIG), Ghent, Belgium; [3] Department of Internal Medicine and Pediatrics, Ghent University Hospital, Ghent, Belgium; [4] Department of Health Sciences, Odisee University College, Ghent, Belgium.

Glucocorticoids (GCs) are the cornerstone of multiple myeloma (MM) treatment as they are present in all treatment stages. These steroidal hormones activate the glucocorticoid receptor, a nuclear receptor (NR) and transcription factor, hereby effectively inducing apoptosis of malignant myeloma cells. Unfortunately, prolonged GC treatment evokes detrimental side effects and leads to GC therapy resistance, which is still poorly understood. Here, we examine whether the cross-modulation of GR and the closely related mineralocorticoid receptor (MR) decreases GC therapy responsiveness in MM. We find that GCs downregulate MR mRNA and protein levels in GC-sensitive MM cells in a GR dependent manner. Mechanistically, GCs decrease MR mRNA stability, most probably by upregulation of tristetraprolin, a mediator of mRNA decay. We further show that MR knockdown promotes GC-induced cell killing and that MR and GR can endogenously interact in myeloma cells upon GC treatment. Data mining of public MM datasets further shows that half of newly diagnosed MM patients have high MR expression levels, with variations among the molecular subgroup of the patient. Finally, combining lower doses of GCs with an MR antagonist enhances GC induced cell death in a proportion of MM cell lines and patient-derived MM cells from various disease stages. Taken together, these results indicate that MR blockade in combination with lower doses of GCs is a promising strategy for MM treatment that may also improve the quality of life of patients.

4. Clemente Maria

Cofactor FHL2 is downregulated in early adipocyte differentiation and inhibits PPARγ activity

Maria P. Clemente, Jayron Habibe, Khang Tran, Mariska Vos, Janny van den Burg, Vivian de Waard, Carlie J. de Vries

Medical Biochemistry Department, Amsterdam UMC (location AMC), Amsterdam, The Netherlands.

Background
The LIM-only protein Four and a Half LIM-domain 2 (FHL2) is a known cofactor of nuclear receptors among which Nur77, LXR, ER and AR. FHL2 establishes a platform for protein interactions thus regulating cellular signal transduction pathways. Based on our observation of a strong FHL2 downregulation at early adipocyte differentiation, we hypothesize a possible interaction with the adipogenic master regulator and nuclear receptor, PPARγ.

Methods and results
High expression of FHL2 is found in the 3T3-L1 mouse fibroblast cell line as well as in the stromal vascular fraction (SVF) of C56BL/6 mice white adipose tissue. We demonstrated in both cell systems that in vitro differentiation results in a marked downregulation of FHL2 at gene and protein level. Preadipocytes isolated from FHL2-deficient mice have higher expression of adiponectin and start accumulating lipid droplets earlier than wild-type cells. Co-immunoprecipitation experiments showed that FHL2 interacts with PPARγ and luciferase reporter assay revealed inhibition of PPARγ transcriptional activity in the presence of FHL2. Challenging FHL2-deficient mice with a high-fat diet results in reduced gain of body weight and smaller white adipose tissue depots compared to wild-type mice.

Conclusion
We have shown that FHL2 is a novel player in the adipocyte differentiation process and through its interaction with PPARγ has an inhibitory effect in cultured preadipocytes. In mice, FHL2 deficiency protects against excessive weight gain in response to a high-fat diet.

5. Cornil Charlotte

Role for the Membrane Estrogen Receptor alpha in the sexual differentiation of the brain

Charlotte A. Cornil[1], Badr Khbouz[1], Catherine de Bournonville[1], Lucas Court[1], Mélanie Taziaux[1], Rebeca Corona[1 †], Jean-François Arnal[2] and Françoise Lenfant[2]

[1] GIGA Neurosciences, University of Liège, Belgium; [2] Inserm U1048-I2MC- Equipe 9, Institut des Maladies Métaboliques et Cardiovasculaires, Toulouse, France.
[†] Current address: Departamento de Neurobiología Celular y Molecular, Laboratorio de Neuroanatomía Funcional y Neuroendocrinología, Instituto de Neurobiología, UNAM, Campus Juriquilla, Querétaro, MÉXICO

Estrogens exert pleiotropic effects on multiple physiological and behavioral responses. Male and female sexual behavior in rodents constitutes some of the best characterized responses activated by estrogens in adulthood and largely depend on ERα. Evidence exists that nucleus- and membrane-initiated estrogen signaling cooperate to orchestrate the activation of these behaviors both in short- and long-term. However, questions remain regarding the mechanism(s) and receptor(s) involved in the early brain programming during development to organize the circuits underlying sexually differentiated responses. Taking advantage of a mouse model harboring a mutation of the ERα palmitoylation site, which prevents membrane ERα signaling (mERα ERα-C451A), this study investigated the role of mERα on the expression of male and female sexual behavior and neuronal populations that differ between sexes. The results revealed no genotype effect on the expression of female sexual behavior, while male sexual behavior was slightly but significantly reduced, in males homozygous for the mutation. Similarly, the number of kisspeptin- (Kp-ir) and calbindin-immunoreactive (Cb-ir) neurons in the anteroventral periventricular nucleus (AVPv) and the sexually-dimorphic nucleus of the preoptic area (SDN-POA), respectively, were not different between genotypes in females, while homozygous males showed increased numbers of Kp-ir and decreased numbers of Cb-ir neurons compared to wild-types, thus leading to an intermediate phenotype between females and wild-type males. Importantly, females neonatally treated with estrogens exhibited the same neurochemical phenotype as their corresponding genotype among males. Together, these data provide evidence that mERα is involved in the perinatal programming of the male brain.

6. De Bournonville Catherine

Involvement of nuclear vs membrane estrogen receptor in the control of male sexual behavior

de Bournonville C.[1], Arnal J.F.[2], Lenfant F.[2], Cornil CA.[1]

[1] GIGA Neurosciences, University of Liège, Liège, Belgium [2] INSERM U1048, Université de Toulouse, Toulouse, France.

Neuroestrogens are involved in many functions such as reproduction, cognition or neuroprotection. Once synthesized in the brain, they act on either nuclear or membrane receptors to regulate brain function in different time-scales, i.e. by affecting gene transcription and showing physiological effects after hours/days, or by activating intracellular signaling pathways leading to rapid (seconds/minutes) actions. Studies in several species have described rapid effect of estrogens on reproductive behavior. Interestingly, these studies usually observed effects on approach behaviors or sexual motivation, suggesting that sexual motivation and performance might be differentially controlled by membrane vs nuclear estrogen receptor respectively. In this study, a behavioral task was set up to independently assess sexual motivation and performance in mice. Then these two aspects of sexual behavior were tested in transgenic male mice carrying a mutated estrogen receptor alpha (ERα) unable to traffic to and signal from the membrane (C451A-ERα) and in control wildtypes. Our results show that C451A-ERα mice display a slight deficit of sexual performance as compared to wild type (less mounts and intromissions) but that this deficit was compensated with sexual experience. However when given the choice between an intact male and an estrous female, C451-ERα males did not show any preference for the female as opposed to wild types. Taken together, these results suggest that both nuclear and membrane estrogen receptors are required for the expression of male sexual performance, while sexual motivation seems to only require the presence of this receptor at the membrane. As this mutation is constitutive, it is not clear whether these effects depend on the developmental (organization) or adult (activation) effects of the receptors. Future studies using pharmacological tools to block membrane estrogen signaling during development or adulthood will thus be necessary to address this question.

7. de Vink Pim

PPARgamma Ligand/Coregulator Cooperativity, towards gene expression selectivity

Pim J. de Vink, Auke A. Koops, Giulia d'Arrigo, Luc Brunsveld

Department of Biomedical Engineering & Institute for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven, The Netherlands.

Nuclear receptors are ligand-regulated transcription factors that mediate the transcriptional activity of cells, cause by lipophilic endogenous hormones as well a synthetic drug ligands. The nuclear receptor ligand-binding domain (LBD) where these ligands bind, is a highly dynamic entity.

Here we present an in vitro study of the PPARgamma LBD and demonstrate a cooperative interplay between ligand and coregulator binding. The affinity of natural and synthetic ligands greatly depends on the relative concentration of coregulators and vice versa. In other word, a single shift EC50 classically used to express ligand potency greatly varies on experimental conditions.

Using 2D fluorescence polarization titrations we systematically profile ligand/coregulator profile and characterize drug potency in terms of an intrinsic affinity (KDII) of the stabilizer compound for one of the apo-proteins. Some ligands have a stronger affinity while others have a higher cooperativity, resulting in differential behavior and preference for particular coactivators. Validation was done using TR-FRET, isothermal calorimetry and a combination of X-ray crystallography and molecular dynamics showing that certain ligands reorganize the PPAR-LBD towards binding certain cofactors of such as PCG1alpha of MED1.

We believe these phenomena in general for all nuclear receptors and offers an entry into preferential transcriptional modulation.

8. de Vries Rens

Modulation of nuclear receptors through ligand architecture

Rens M.J.M. de Vries[1], Femke A. Meijer[1], Richard G. Doveston[2], Luc Brunsveld[1]

[1] Laboratory of Chemical Biology, Department of Biomedical Engineering and Institute for Complex Molecular Systems, Technische Universiteit Eindhoven, Eindhoven, The Netherlands; [2] Leicester Institute of Structural and Chemical Biology and Department of Chemistry, University of Leicester, Leicester, United Kingdom.

The traditional approach of modulating NRs is to design small synthetic molecules that interact with the ligand-binding domain (LBD) of the NR. Ligands can thereby either enhance or inhibit gene transcription. Apart from the effects on transcription, recent research shows that minor changes in the ligand scaffold can have a significant impact on the behavior of the NR. In this research, we show how small-molecules can change both the dimerization behavior of NRs and the recruitment of allosteric modulators.

The Retinoic X Receptor α (RXRα) is known as a master regulator among NRs through its ability to heterodimerize with, and thereby modulate, other NRs. We show, using a novel NanoBIT complexation assay, that small directed changes in the RXR ligand scaffold can lead to selective formation of specific hetero- and homodimers. Using our structural data and focused compound library, a model was developed to help to understand this effect of the ligand. This information can serve as a blueprint to design small-molecules that selectively target specific NRs via RXR. This makes RXR as an exciting and versatile target for NR modulation, especially when classical modulation of the partner NR is not possible.

Recently, small-molecules have been found to bind to allosteric sites of NRs. Allosteric ligands are of interest since they do not compete with the endogenous ligand of the NR and often shown an increased selectivity towards their target. We show, using X-ray crystallography and biochemical assays, that there is communication between orthosteric and allosteric ligands in the RAR-related orphan receptor γ t (RORγt). We successfully solved eleven new ternary crystal structures of RORγt in the presence of both orthosteric and allosteric ligands. These structures mechanistically show how binding of the orthosteric ligand leads to positive cooperative binding of the allosteric ligand.

9. Dubois Vanessa

Activation of the unfolded protein response triggers loss of hepatic cellular identity through downregulation of super enhancer-driven nuclear receptor expression

Vanessa Dubois[1,2], Wouter Vankrunkelsven[3], Céline Gheeraert[1], Julie Dubois-Chevalier[1], Hélène Dehondt[1], Maheul Ploton[1], Réjane Paumelle[1], Ilse Vanhorebeek[3], Lies Langouche[3], Greet Van den Berghe[3], Bart Staels[1], Philippe Lefebvre[1], Jérôme Eeckhoute[1]

[1] Université de Lille, Inserm, CHU Lille, Institut Pasteur de Lille, U1011-EGID, F-59000 Lille, France; [2] Present address: Clinical and Experimental Endocrinology, Department of Chronic Diseases, Metabolism and Ageing, KU Leuven, B-3000 Leuven, Belgium; [3] Clinical Division and Laboratory of Intensive Care Medicine, Department of Cellular and Molecular Medicine, KU Leuven, B-3000 Leuven, Belgium.

Endoplasmic reticulum (ER) stress is observed in a large variety of acute and chronic liver diseases, leading to activation of the Unfolded Protein Response (UPR). However, the extent to which UPR activation contributes to liver dysfunction as well as the underlying mechanisms remain only partially understood.

Using both in vitro approaches in murine primary hepatocytes and in vivo experiments in mice, we found that UPR activation leads to a global loss of expression of genes defining liver-identity. This effect is notably characterized by a decreased expression of key hepatic transcription factors, including an extended array of nuclear receptors (NR) instrumental for liver functions. Genes encoding these NRs display highest sensitivity to UPR activation and are driven by liver super-enhancers, which are disrupted by ER stress as judged by decreased histone acetylation and cofactor recruitment. Interestingly, concomitant UPR activation and downregulation of liver-identity NR expression was observed in livers from two different murine models of bacterial sepsis as well as in livers from critically-ill patients.

Our work establishes that, concomitantly to transcriptional induction of stress handling genes by the UPR, ER stress triggers loss of hepatic cellular identity through downregulation of super enhancer-driven NR expression. These mechanisms may contribute to hepatic failure in critical illness.

10. Eerlings Roy

Developing an artificial nuclear receptor, the synthetic yeast approach

Roy Eerlings[1], Purvi Gupta[2], Anton Gorkovskiy[3], Karin Voordeckers[3], Kevin J. Verstrepen[3], Frank Claessens[1], Arnout R.D. Voet[2], Christine Helsen[1]

[1] Laboratory of Molecular Endocrinology, KU Leuven, Belgium; [2] The Laboratory of Biomolecular Modelling and Design, KU Leuven, Belgium; [3] VIB Laboratory for Systems Biology & KU Leuven Laboratory for Genetics and Genomics, Centre for Microbial and Plant Genetics, Leuven, Belgium.

Nuclear receptors (NRs) are metazoan ligand-inducible transcription factors. They can be used as tools to induce gene expression in non-metazoan organisms, like microbes and plants. However, exposing these organisms to NR ligands has proven detrimental. Therefore, we will develop an artificial receptor (XR), inducible by a selected synthetic, non-toxic ligand by combining computational modeling of the candidate ligand in the ligand-binding pocket (LBP) with directed evolution in yeast.

To determine the candidate ligand, a set of 55 compounds that differ from natural (ant)agonists was assembled. Binding requirements for (ant)agonists were based on crystal structures of the NR subfamily 3 (NR3). Via a luminescent reporter assay in HEK-293T cells, inducible by all members of the NR3 subfamily (AR, PR, GR, MR, ERα/β), 38 compounds showed to be activating or repressing on at least one NR. Toxicity of the remaining compounds was assessed via proliferation and viability assays on mammalian cell lines and yeast for which 7 compounds showed to be detrimental. From the remaining 10 NR3-non-reactive compounds, one compound will be chosen as the agonist of the XR.

With in silico techniques, the LBP of a NR3-receptor will be adapted to accommodate the candidate ligand. To evaluate the response of this XR design to this ligand, we developed a yeast strain with a dual reporter for NR3 activity. Next, we will enhance the sensitivity of the XR-LBP for the ligand and its maximal output via multiple rounds of directed evolution using survival with FACS as a read-out of XR activity in yeast.

This artificial ligand/receptor pair will have numerous applications in various domains ranging from basic microbial studies to fundamental mice research. Therefore, we will fuse the XR-LBD to a Cre-recombinase to develop a non-toxic, non-endocrine inducible Cre-recombinase system. Additionally, mammalian coregulatory-NR interactions can be studied with our novel NR dependent yeast reporter.

11. El Kharraz Sarah

Physiological relevance of N/C interaction in the androgen receptor: a new mouse model

Sarah El Kharraz[1], Christine Helsen[1], Dirk Vanderschueren[2], Frank Claessens[1]

[1] Molecular Endocrinology Laboratory, Department of Cellular and Molecular Medicine, University of Leuven, Campus Gasthuisberg, Leuven, Belgium; [2] Clinical and Experimental Endocrinology, Department of Chronic Diseases, Metabolism and Ageing, University of Leuven, Campus Gasthuisberg, Leuven, Belgium.

As all steroid receptors, the androgen receptor (AR) dimerizes during the transcription activation process. However for the AR, this dimerization is more complicated since there are three dimerization interfaces present: dimerization can occur via the ligand binding domain (LBD), the DNA binding domain and via an interaction between the aminoterminal domain (NTD) and LBD. In Van Royen et al. (2007), it was shown that mutations of the 23FQNLF27-motif in the AR NTD disrupts the interactions with the coactivator binding groove on the surface of the LBD. This interaction is called the N/C-interaction and has been studied intensely, resulting in many hypotheses on its involvement in receptor activation. Via luciferase assays in vitro, our group found for example that the deletion of the 23FQNLF27-motif has differential effects on AR activity depending on the AR binding site under investigation (Callewaert et al., 2003). Unfortunately, all the hypotheses on the relevance of the N/C interaction in the AR are based on in vitro data. To determine the in vivo relevance of the N/C interactions, we generated a CRISPR/Cas9 mouse model in which the 23FQNLF27-motif is mutated to 23AQNAA27. The generated mice are called the ARNoC mice (for no N/C interaction). The external phenotype of the male ARNoC/Y mice is indistinguishable from wild type littermates. No differences are observed in the weight of reproductive organs, and their bone phenotype remains as well unaffected by the mutation. Surprisingly, serum testosterone levels are increased in ARNoC/Y mice, indicating an effect on the HPG axis. Interference with the feedback regulation due to a defect of the AR corroborates with the overall reduced transactivation of the AR NoC on different AR response elements that is found in the in vitro luciferase reporter assays. In conclusion, by combining our in vitro data with the new generated mouse model, we are trying to reveal the role of the N/C interaction in global AR functioning.

12. Faure Mélanie

Palmitoylated nuclear estrogen receptor alpha is implicated in the control of fertility

Faure M.C.[1], Vandries L.[1], Corona R.[1], Rusidze M.[2], Arnal J.F.[2], Lenfant F.[2], Cornil C.A.[1]

[1] GIGA Neurosciences, University of Liège, Liège, Belgium; [2] INSERM U1048, Université de Toulouse, Toulouse, France.

Estrogens act through both nuclear and membrane-initiated signaling. Estrogen receptor alpha (ERα) is critical for reproduction, but the relative contribution of its nuclear and membrane signaling is unclear. To address this question, we characterized the reproductive phenotype of a knock-in mouse model (ERα-C451A backcrossed in the CD1 background) in which ERα's palmitoylation site is mutated resulting in a loss of the palmitoylation site allowing the translocation of estrogen receptor alpha to the membrane. ERα-C451A females present several reproductive abnormalities. While their LH response to ovariectomy does not differ from this of wildtype females, ERα-C451A females present an altered response to estrogens indicative of a dysregulated negative feedback. ERα-C451A females do not show a pre-ovulatory LH surge (positive feedback) and the associated activation of kisspeptin and GnRH neurons in response to estrogens unless they are treated with progesterone. However, when left gonadally intact and mated with a wildtype male, these females become pregnant suggesting they are able to ovulate without exogenous progesterone. Yet, they present multiple gestational defects resulting in a decreased or total absence of pups in the nest depending on the strain. Daily weighing and scheduled ultrasound confirmed that ERα-C451A females (C57Bl6) do get pregnant and carry live fetuses. However, fewer implantation sites were found and developmental arrest occurred in some embryos before embryonic day 9. Parturition also appears to occur later in these females. Therefore, although membrane ERα appears to participate in the negative feedback and in the activation of the nervous circuits underlying the LH surge, its ablation does not prevent ovulation, but induces gestational defects resulting in lower or no live birth.

13. Ferhat Maroua

Metabolic sensing by PPARγ is required for ILC2 recruitment and function

Tinhinane Fali[1,2], Tegest Aychehk[1,2], Maroua Ferhat[3], Jean-Yves Jouzeau[3], Meinrad Busslinger[4], David Moulin[3,5], Gérard Eberl[1,2]

[1] Institut Pasteur, Microenvironment & Immunity Unit, 75724 Paris, France; [2] INSERM U1224, 75724 Paris, France; [3] IMoPA, UMR7365 CNRS-Université de Lorraine, Vandœuvre-lès-Nancy, France; [4] Research Institute of Molecular Pathology, Vienna Biocenter, Campus-Vienna-Biocenter 1, Vienna, Austria; [5] CHRU de Nancy, Contrat d'interface, Vandœuvre-lès-Nancy, France.

Introduction
Group 2 innate lymphoid cells (ILC2s) has emerged as a central component of type 2 inflammation in mice and humans. This rare subset of innate lymphocytes plays critical roles in immunity against parasite infection, in the development of allergic reactions, in metabolic homeostasis and thermogenesis, as well as in tissue repair through the expression of IL-5, IL-13 and amphiregulin. These versatile roles of ILC2s are tightly controlled by complex regulatory systems (involving cytokines, neuropeptides, nutrients, cell-to-cell interactions, hormones, lipids and metabolic mediators) produced in the local microenvironment.

Objective
Herein we aim to investigate the contribution of the metabolic sensor PPAR (peroxisome proliferator-activated receptor)-γ to ILC2 function and metabolism.

Methods and Results
Our data demonstrate that PPAR-γ, highly expressed in ILC2s, is required for their recruitment, expansion and effector function (expression of the IL-5 and IL-13). Pharmacological inhibition of PPAR-γ leads to a decrease in fatty acid uptake by ILC2s, a major energy source for these cells. As a consequence, treatment of mice with a PPAR-γ antagonist efficiently blocked ILC2-dependent acute airway inflammation.

Conclusion
Together, our findings demonstrate a critical role for the metabolic sensor PPAR-γ in the regulation of type 2 immunity.

14. Gallez Anne

Inclusion of 17β-estradiol into liposome: a tool to study the molecular mechanisms of estrogen receptors

Anne Gallez[1], Claudio Palazzo[2], Isabelle Dias Da Silva[1], Brigitte Evrard[2], Agnès Noël[1], Géraldine Piel[2], Christel Péqueux[1]

[1]Laboratory of Biology, Tumors and Development (LBTD), Giga-Cancer, University of Liège, Belgium; [2] LTPB, CIRM, University of Liege, Liege, Belgium.

Encapsulation into liposomes of several types of molecules such as steroids is a growing field of research to develop innovative treatments. Nevertheless, a major obstacle remains the incomplete understanding of the impact that inclusion of drugs into liposome could have on the molecular and cellular mechanism of action of a drug. Estrogen receptors (ER) relay pleiotropic actions of estrogens in physiology and pathophysiology. At a molecular level, the activation of estrogen receptor alpha (ERα) by 17β-estradiol (E2) leads to two major pathways: (1) the genomic effects and (2) the nongenomic/membrane-initiated steroid signaling (MISS) effects related to the induction of fast signaling pathways occurring when ERα is anchored to the plasma membrane.

As a proof of concept, we evaluated the impact of the inclusion of E2 into liposome on ERα signaling pathways. Free E2 and E2 encapsulated into liposome (POPC-E2) were tested in paradigms specifically related to the genomic or to the MISS pathway.

We observed that POPC-E2 formulation did not increase ERα-Src interaction, an initiating step of the MISS pathway. POPC-E2 did not increase the expression of MISS pathway target genes (TSKU, HSPB8, PMAIP1). To study the genomic pathway of ERα, we evaluated the expression of two specific genes of this pathway, progesterone receptor (PR) and pS2 (TFF1). Similarly to free E2, POPC-E2 increased the mRNA expression level of PR and pS2. In addition, POPC-E2 was able to induce uterotrophic activity by increasing uterus wet weight, epithelial proliferation and luminal epithelial height.

In conclusion, the inclusion of E2 into liposome prevented the activation of the ERα-MISS pathway, while the activation the genomic pathway was maintained. These results highlight that the encapsulation of molecule into liposomes could impact their cellular action. In addition, our results suggest that POPC-E2 could be an interesting tool to delineate the complex molecular mechanisms associated to ERα.

15. Gentenaar Max

Regional hippocampal gene expression in glucocorticoid-enhanced memory consolidation

Max Gentenaar[1], Lisa Koorneef[1], Rob Buurstede[1], Eva Viho[1], Lisa van Weert[1,3,4], Paola Colucci[4], Benno Roozendaal[4], Patrizia Campolongo[4], Onno Meijer[1]

[1] Department of Medicine, Division of Endocrinology, Leiden University Medical Centre, the Netherlands; [2] Department of Physiology and Pharmacology, Sapienza University of Rome, Italy; [3] Department of Cognitive Neuroscience, Radbound University, the Netherlands; [4] Donders Institute for Brain, Cognition and Behaviour, Radbound University, the Netherlands.

Glucocorticoids have the ability to improve the consolidation of memories after emotionally arousing events, but only if there is a concomitant release of noradrenaline. This suggest that an interaction may exist between the two associated receptors, the glucocorticoid receptor (GR) and cAMP response element binding protein (CREB), during memory formation. Using ChIP-seq, we have previously identified Fgf2, Nsmf, Gjb6 and Ssc5d to be upregulated by glucocorticoids in the rat hippocampus, yet the regions and cell types are unknown. In the present study, we investigated their regional patterns of expression in the hippocampus after a memory task under stressed and non-stressed conditions.

12-week old male rats (n=8 per group) underwent an object location memory (OLM) test or stayed at their home cage, after which they received a corticosterone (3.0 mg/kg) or vehicle injection. After three hours, the rats were sacrificed and their brains were collected for 4-plex in situ hybridization analysis.

In the dorsal CA1 region of the hippocampus, GR binding was associated with Fgf2, Nsmf and Gjb6 expression, while the OLM did not affect their expression. Expression of Fgf2, Nsmf and Ssc5d was limited to the pyramidal layer while Gjb6 was also expressed in the stratum oriens and stratum radiatum, which suggest that Gjb6 is expressed in more diverse cell types. Interestingly, Fgf2 was always co-expressed with Nsmf and Ssc5d but not with Gjb6. To conclude, corticosterone is associated with Fgf2, Nsmf and Gjb6 expression in the pyramidal layer of the CA1 region and Gjb6 was additionally expressed in the the stratum oriens and stratum radiatum. Additional research on other brain regions and target genes is needed to better assess the interaction of corticosterone and CREB in the brain.

16. Gupta Purvi

Computational design of an artificial nuclear receptor for applications in synthetic biology

Purvi Gupta, Roy Eerlings, Frank Claessans, Christine Helsen, Arnout Voet

Laboratory of Biomolecular Modeling and Design, Department of Chemistry, KU Leuven and Laboratory of Molecular Endocrinology, Campus Gasthuisberg, KU Leuven.

Nuclear receptors (NRs) are well known transcription factors activated by a ligand. Since they are not naturally present in plants and yeasts, the NRs or their domains can be used as building blocks to construct an orthogonal circuit. A drawback of this system is the use of steroid hormones as inducers for NRs which results in toxicity at high concentrations. Therefore, we aim to design an artificial nuclear receptor that recognizes a given ligand which is not toxic. The ligand binding domain (LBD) of the NR will be modified using computational protein design (CPD) methods in order to activate the NR in response to the chosen ligand. A set of putative inducers that does not activate any of the existing steroid NRs and that are non-toxic were chosen using a series of screening methods and experimental cellular assays. Crystal structures of the LBD of androgen and estrogen receptors were used as starting scaffolds for design of the artificial receptor using Rosetta Enzyme Design application and RosettaScripts. The ligand was placed inside the receptor pocket by defining geometric constraints with the residues of the active site. Characteristic hydrogen bonding interactions between natural ligand and residues in the binding pocket were retained during the design process. These generated LBD designs were docked with their corresponding non-toxic ligands and the fit was compared with the fit of the natural ligand in the wildtype LBD. Currently, the most promising design of ER-beta for a non-toxic ligand is being tested for activity in a NR-reporter assay in yeast. In the future, Molecular Dynamics simulations and binding energy calculations will be performed for further validation of the designs.

17. Habibe Jayron

A novel role for FHL2 in aging-related Type 2 Diabetes

Jayron Habibe[1,2], Maria Clemente[1], Mariska Vos[1], Max Nieuwdorp[3], Torsten Scheithauer[3], Daniel van Raalte[3], Etto Eringa[2], Carlie J. de Vries[1]

[1] Amsterdam UMC, AMC, Medical Biochemistry, Amsterdam, The Netherlands; [2] Amsterdam UMC, Vumc, Physiology, Amsterdam, The Netherlands; [3] Amsterdam UMC, AMC, Experimental Vascular Medicine, Amsterdam, The Netherlands.

Introduction
Type 2 diabetes (T2D) is a metabolic disease that is associated with aging. Methylation of the Four and a Half LIM domain 2 (FHL2) gene increases with aging, resulting in increased FHL2 expression in human pancreatic islets. To assess the role of FHL2 in modulation of β-cell function, glucose metabolism was studied in WT and FHL2-/- mice and human association of FHL2 expression and glucose/insulin metabolism gene expression profiles was determined.

Methods and results
The metabolic phenotype of WT and FHL2-/- mice was determined by glucose tolerance tests (GTT) revealing that FHL2-/- mice (22 wks old) have improved glucose clearance, both in oral GTT and intraperitoneal GTT and secrete more insulin. In addition, glucose-stimulated insulin secretion (GSIS) assays with isolated islets showed increased insulin secretion by FHL2-/- islets compared to WT islets. mRNA expression of the transcription factor MAFA (RT-qPCR) upstream of insulin is higher in FHL2-/- islets. Applying R2 software, shows in human islet RNA-seq databases a negative association of FHL2 expression with expression of the key regulatory transcription factors of insulin function MAFA, PDX1 and NEUROD1.

Conclusion
FHL2-/- mice display a favorable glucose metabolism compared to WT mice and in humans a strong association of FHL2 expression and insulin secretion and glucose metabolism pathways is observed.

We propose that FHL2 may negatively regulates insulin secretion in both mice and human islets and in doing so contributes the development of T2D.

18. Handle Florian

Drivers and therapeutic vulnerabilities in AR indifferent anti-androgen resistant prostate cancer cells

Florian Handle[1*], Stefan Prekovic[1,2*], Christine Helsen[1], Thomas Van den Broeck[1,3], Elien Smeets[1], Lisa Moris[1,3], Roy Eerlings[1], Sarah El Kharraz[1], Alfonso Urbanucci[4], Ian G. Mills[5,6,7], Steven Joniau[3], Gerhardt Attard[8], and Frank Claessens[1]

[1] Molecular Endocrinology Laboratory, Department of Cellular and Molecular Medicine, KU Leuven, Leuven, Belgium; [2] Division of Oncogenomics, Oncode Institute, Netherlands Cancer Institute, Amsterdam, The Netherlands; [3] Department of Urology, University Hospitals Leuven, Leuven, Belgium; [4] Department of Tumor Biology, Institute for Cancer Research, Oslo University Hospital, Oslo, Norway; [5] Centre for Molecular Medicine Norway, Nordic European Molecular Biology Laboratory Partnership, Forskningsparken, University of Oslo, Oslo, Norway; [6] Centre for Cancer Research and Cell Biology, Prostate Cancer UK/Movember Centre of Excellence for Prostate Cancer Research, Queen's University, Belfast, UK; [7] Nuffield Department of Surgical Sciences, University of Oxford, John Radcliffe Hospital, Oxford, UK; [8] UCL Cancer Institute, University College London, 72 Huntley Street, London, UK.
[*] These authors contributed equally to this work

Inhibition of the androgen receptor (AR) by second-generation anti-androgens is a standard treatment for castration resistant prostate cancer (CRPC), but it inevitably leads to the development of resistance. Importantly, AR independent non-neuroendocrine resistance mechanisms are on the rise since the development of efficient AR signaling inhibitors and comprise approximately 20% of CRPC patients in the clinic nowadays. However, this clinical setting remains poorly understood to date due to a lack of accessible and well-characterized models.

Two anti-androgen resistant cell lines were generated by continuous treatment of LNCaP cells with enzalutamide (ResA) or RD-162 (ResB) and they were fully resistant to enzalutamide in xenograft experiments. In addition, they were cross-resistant against apalutamide, darolutamide, abiraterone acetate, androgen deprivation, and docetaxel (partial) in vitro. Both cell lines are AR positive and androgen responsive but the activity of the AR is very low in presence of anti-androgens and not required for their anti-androgen resistant growth (AR indifferent). We performed GSEA transcriptome analysis to identify the drivers of anti-androgen resistance in these cells and found significant enrichment of the cell-cycle related E2F targets. Importantly, metastatic CRPC samples with low AR activity also had significantly increased E2F activity compared to those with high AR activity (N=41 and 108 respectively, $p<0.001$). Interestingly, ResB cells were strongly repressed by supra-physiological androgen therapy despite their AR indifferent phenotype.

The ResA and ResB cells are highly resistant against most clinically established therapies but remain sensitive to several therapeutic options that are currently evaluated in clinical trials. The model systems developed in this project may lead to a better understanding and the identification of therapeutic vulnerabilities of non-neuroendocrine, AR indifferent CRPC.

19. Kim Nari

Sexual dimorphism in bone size is mediated by neuronal ER alpha (ERα) signaling in females during late puberty

Nari Kim[1], Ferran Jardí[1], Vanessa Dubois[1], Leen Antonio[1], Rougin Khalil[1], Dieter Schollaert[1], Ludo Deboel[1], Brigitte Decallonne[1], Geert Carmeliet[1], Claes Ohlsson[2], Marie K. Lagerquist[2], Frank Claessens[3] and Dirk Vanderschueren[1]

[1] Clinical and Experimental Endocrinology, Department of Chronic Diseases, Metabolism, and Ageing (CHROMETA), KU Leuven, Leuven, Belgium; [2] Centre for Bone and Arthritis Research, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; [3] Molecular Endocrinology Laboratory, Department of Cellular and Molecular Medicine, KU Leuven, Leuven, Belgium.

Gender dimorphism in bone architecture emerges largely during puberty. Estrogen receptor alpha (ERα) is indispensable to mediate the stimulatory effects of estrogens (E2) on peak bone mass acquisition during puberty in boys and girls. At the end of puberty, elevated E2 levels exert inhibitory actions on growth plate closure thereby limiting longitudinal growth in girls. As a result, boys develop taller and wider bones than girls. Previous studies have mainly focused on the stimulatory E2 actions on bone. Therefore, the mechanisms of gender-specific inhibitory E2 effects underlying sexual bone dimorphism remain elusive. In this study, we aimed to determine whether neuronal ERα would mediate inhibitory E2 actions on skeletal dimorphism during puberty. We generated mice with a targeted deletion of ERα in extrahypothalamic neurons and determined their bone phenotype. A tamoxifen-induced knockout at 6 weeks of age reduced ERα mRNA levels in both cerebral cortex and brain stem by 50%, while ERα expression was unaffected in hypothalamus and non-neuronal tissues. Serum T and E2 remained normal in both sexes, indicating that hypothalamus-pituitary-gonadal function was not affected by ERα disruption. Inactivation of neuronal ERα did not alter body weight in males, but female N-ERαKO were 6.3% heavier ($p < 0.01$) and 2% longer ($p < 0.05$) compared to control littermates. In female N-ERαKO mice, femoral and L5 vertebral lengths increased by 2.4% ($p < 0.01$) and 4.8% ($p < 0.01$), respectively. Radial bone expansion at femoral midshaft also increased in female N-ERαKO concomitantly with higher serum levels of IGF-I as well as IGFBP-3, a marker of GH secretion. Furthermore, the three-point bending test revealed increased bone strength in female N-ERαKO compared to control littermates. In contrast, inactivation of neuronal ERα had no major effect on bone growth in males. In conclusion, neuronal ERα limits female bone size and strength.

20. Kojic Aleksandar

Tumor-specific ERalpha cistrome in endometrial cancer is enriched for somatic mutations

Aleksandar Kojic[1], Marlous Hoogstraat[1], Karianne Schuurman[1], Tracy OMara[2], Abishek Singh[1], Lodewyk Wessels[1], Dylan Glubb[2], Wilbert Zwart[1]

[1] Netherlands Cancer Institute, Amsterdam, Netherlands; [2] QIMR Berghofer Medical Research Institute, Brisbane, Australia.

Endometrial cancer (EC) is the second most-common gynecological cancer. More than 80% of all ECs express Estrogen Receptor alpha (ERα) and are considered hormone-driven. To date, biological implications of healthy- and tumor-specific ERα chromatin interactomes have largely been unexplored. As preliminary datasets, I integrated ERα and H3K27Ac ChIP-seq data in healthy and tumoral clinical samples and whole genome sequencing data of metastatic endometrial samples. This revealed almost 4,000 tumor-specific ERα enhancers, ~600 of which harboring somatic variants. Additionally, I performed H3K27Ac Hi-ChIP in healthy and cancer endometrial cell lines and identified potential target genes of tumor-specific enhancers. I hypothesize that essential ERα enhancers and alterations invoked by somatic mutations, impact 3D genome organization and drive tumorigenesis. Using custom enhancer-centered CRISPR-Cas9 and CRISPRi genetic screens, I aim to identify those tumor-specific ERα enhancers that drive estrogen-driven proliferation of endometrial cancer cell lines. Additionally, I will perform Hi-C in normal and tumoral endometrial tissue to prioritize individual enhancers and their target genes for downstream functional/mechanistic experiments in vitro. These studies are aimed to reveal which ERα sites -harboring somatic mutations in endometrial cancer specimens- drive cancer cell proliferation. To elucidate the functional impact of somatic mutations at essential ERα sites, we will perform STARR-seq to determine enhancer activity, both in the wildtype and mutant setting. Through multidimensional omics data integration, this project is specifically geared to elucidate the molecular mechanisms of endometrial cancer development and progression, and the functional impact of non-coding somatic mutations on ERα action in 3D genomic space.

21. Koorneef Lisa

Selective antagonist CORT125281 prevents glucocorticoid receptor activity in a tissue-specific manner and improves glucocorticoid-induced hyperinsulinemia

Lisa L. Koorneef[1,2], Jan Kroon[1,2], Eva M.G. Viho[1,2], Lucas F. Wahl[1,2], Kim M.L. Heckmans[1,2], Marloes M.A.R. van Dorst[1,2], Menno Hoekstra[3], René Houtman[4], Hazel Hunt[5], Onno C. Meijer[1,2]

[1] Department of Internal Medicine, Division of Endocrinology, Leiden University Medical Center, Leiden, The Netherlands; [2] Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, Leiden, The Netherlands; [3] Leiden Academic Centre for Drug Research, Division of BioTherapeutics, Leiden University, The Netherlands; [4] Pamgene International, Den Bosch, The Netherlands; [5] Corcept Therapeutics, Menlo Park, California, USA.

Glucocorticoids mediate numerous essential processes in the human body via binding to the glucocorticoid receptor (GR). Excessive activation of GR signaling can cause disease, and GR antagonists can be used to treat many symptoms of glucocorticoid-induced pathology. The purpose of this study was to characterize the tissue-specific properties of the selective GR antagonist CORT125281. We evaluated the antagonistic effects of CORT125281 upon acute and chronic corticosterone exposure in mice. In the acute corticosterone setting, hypothalamus-pituitary-adrenal-axis activity was investigated by measurement of basal- and stress-induced corticosterone levels, adrenocorticotropic hormone levels and pituitary proopiomelanocortin expression. GR signaling was evaluated by RT-PCR analysis of GR-responsive transcripts in liver, muscle, brown adipose tissue (BAT), white adipose tissue (WAT) and hippocampus. Pretreatment with a high dose CORT125281 antagonized GR activity in a tissue-dependent manner. We observed complete inhibition of GR-induced target gene expression in the liver, partial blockade in muscle and BAT, and no antagonism in WAT and hippocampus. Tissue distribution only partially explained the lack of effective antagonism. Chronic CORT125281 treatment did not affect basal- and stress-induced activity of the hypothalamus-pituitary-adrenal neuroendocrine axis. In the chronic corticosterone setting, insulin, glucose and lipid concentrations were measured in plasma, and immune cell status was determined in whole blood. CORT125281 partially prevented corticosterone-induced hyperinsulinemia (-40%, p=0.02), but not hyperlipidemia and immune suppression. CORT125281 antagonizes GR transcriptional activity in a tissue-dependent manner and improves corticosterone-induced hyperinsulinemia. Tailored dosing of CORT125281 may allow tissue-specific inhibition of GR transcriptional activity.

22. Kroon Jan

Glucocorticoid receptor activity in the liver is dependent on androgen receptor signaling

Jan Kroon[1], Rob Buurstede[1], Karolien de Bosscher[2], Onno Meijer[1]

[1] Department of Medicine, Division of Endocrinology, Leiden University Medical Center, Leiden, The Netherlands; [2] Department of Biomolecular Medicine, University of Ghent, Ghent, Belgium.

Glucocorticoid signaling in the liver is sexually dimorphic, but the relative contribution of sex hormones to this phenomenon remains unclear. To address this, we investigated whether androgen receptor signaling influences glucocorticoid receptor (GR) activity in murine liver.

To activate the GR, male C57BL6/J mice were exposed to corticosterone by slow-release pellets. To determine whether AR signaling is essential for adequate GR signaling, mice were treated with a selective AR antagonist. As expected, corticosterone treatment increased hepatic expression of the classical GR-target genes (Fkbp5, 9-fold, $p<0.001$; Mt2a, 20-fold, $p=0.07$; Gilz, 2-fold; $p<0.01$) and lipid metabolism-associated genes (Mttp, 2-fold; Apob, 1.5-fold; both $p<0.05$). Strikingly, treatment with the AR antagonist diminished corticosterone-induced expression of GR-target genes, revealing that hepatic GR signaling is AR-dependent.

We previously showed that tissue levels of corticosterone were unaffected after AR antagonist treatment. This rules out that AR influences hepatic glucocorticoid levels by altering metabolism or tissue uptake of corticosterone. To explore the mechanism that underlies GR-AR crosstalk in the liver, we interrogated GR DNA-binding using ChIP-qPCR. Corticosterone treatment resulted in enrichment of GR to the genomic loci of multiple GR-regulated regions, including the Fkbp5 GRE. Surprisingly, AR antagonist treatment actually enhanced GR recruitment to the Fkbp5 GRE (2.5-fold).

In conclusion, AR inhibition diminishes GR transcriptional activity in the liver, despite a robust increase in GR DNA-binding. We conclude that GR transcriptional activity in the liver is AR-dependent, but not mediated via glucocorticoid regeneration, GR protein expression and GR-DNA binding. We hypothesize that AR-driven co-factors are required for adequate GR signaling in the liver, or alternatively, AR is involved in the GR transcriptional complex in the liver.

23. Lee Xiao Yin

Computational design of androgen receptor LBD dimerization inhibitors

Xiao Yin Lee[1,2], Tien Nguyen[1], Frank Claessens[2], Christine Helsen[2], Arnout RD Voet[1]

[1] Biochemistry Department, Celestijnenlaan 200G, KU Leuven, Heverlee 3001, Belgium; [2] Laboratory of Molecular Endocrinology, Department of Cellular and Molecular Medicine, KU Leuven, Leuven 3000, Belgium.

Androgen receptor antagonists are used in the metastatic stage of prostate cancer therapy but also as neo-adjuvant treatment. Currently, all clinically used AR antagonists target the ligand binding pocket of the androgen receptor ligand binding domain (AR LBD). Although resistance against these antagonists will occur within months of start of treatment, the AR itself remains a survival factor for most cases. Therefore, targeting the dimerization of the androgen receptor ligand binding domain might be an alternative therapy, which could overcome those resistance mechanisms. Here we delivered insight into the dynamics of the androgen receptor ligand binding domain homodimer through comparison of the crystal structures available for the AR LBD dimer and GR LBD dimer. We explored the druggability of detected binding sites at the surface of the AR LBD where binding of small-molecules could inhibit dimerization. Hit compounds from commercial databases were discovered by combining receptor-based pharmacophore modelling and docking methods. Preferred compounds were tested for biological activity on a stable androgen-responsive luciferase reporter cell line. Improved derivatives were identified using a SAR by catalogue approach. Crystallography studies with AR LBD are used to validate the binding site of the dimerization inhibitors.

24. Lefere Sander

Differential therapeutic effects of single and pan-PPAR agonists on experimental steatohepatitis and hepatic macrophage biology

Sander Lefere[1,2], Tobias Puengel[2], Jana Hundertmark[2,3], Oliver Krenkel[2], Christian Penners[2], Anna Katharina Frank[2], Vanessa Adarbes[4], Céline Estivalet[4], Anja Geerts[1], Lindsey Devisscher[5], Guillaume Wettstein[4], Frank Tacke[2,3]

[1] Department of Gastroenterology and Hepatology, Hepatology Research Unit, Ghent University, Ghent, Belgium; [2] Department of Medicine III, University Hospital Aachen, Aachen, Germany; [3] Department of Hepatology/Gastroenterology, Charité University Medical Center, Berlin, Germany; [4] Inventiva, Daix, France; [5] Department of Basic and Applied Medical Sciences, Gut-Liver Immunopharmacology Unit, Ghent University, Ghent, Belgium.

Peroxisome proliferator-activated receptors (PPARs) are essential regulators of whole-body metabolism. We compared selective PPAR agonists with the pan-PPAR agonist lanifibranor as therapeutic agents in NAFLD, and determined their effects on macrophage biology.

Lanifibranor or selective PPARα (fenofibrate), PPARγ (pioglitazone) and PPARδ (GW501516) agonists were administered in mice with choline-deficient, amino acid-defined high-fat diet-induced steatohepatitis. Acute liver injury was induced by carbon tetrachloride (CCl4). Murine bone marrow-derived macrophages (BMDM) were stimulated in vitro with palmitic acid and treated with PPAR agonists. Inflammatory activation markers were investigated in classical CD14+ CD16- monocytes isolated from patients with NAFLD.

Lanifibranor improved all histological features of steatohepatitis in mice, including liver fibrosis, thereby combing and exceeding specific effects of the single PPAR agonists. Its potent anti-steatotic efficacy was confirmed in a 3D liver-biochip model with primary cells. Infiltrating hepatic monocyte-derived macrophages (MoMF) were reduced following PPAR agonist administration, especially with lanifibranor, even after short-term treatment. Reduced MoMF paralleled improved steatosis and hepatitis. In the CCl4 model of acute injury in mice, neither single nor pan-PPAR agonists directly affected monocyte recruitment. Lanifibranor attenuated palmitic acid-induced inflammatory activation of primary macrophages in vitro in a PPARδ-dependent fashion. In line, classical monocytes from patients with fibrotic NAFLD phenocopied the expression profile of palmitic acid-treated macrophages.

Conclusion: Pan-PPAR agonists combine the beneficial effects of selective PPAR agonists and may counteract inflammation and disease progression more potently. PPARδ agonism and lanifibranor directly modulate macrophage activation, but not infiltration, thereby synergizing with beneficial metabolic effects o

25. Leijten - van de Gevel Iris

Elucidating the prerequisites of forming the allosteric pocket in RORγt

Iris A. Leijten – van de Gevel[1], L. Brunsveld[1]

[1] Laboratory of Chemical Biology, Department of Biomedical Engineering, Eindhoven University of Technology, the Netherlands.

The retinoic acid receptor-related orphan receptor-γt (RORγt) is one of 48 members of the human nuclear receptor (NR) superfamily. Within this family, there is a high level of homology and all members have a similar domain structure. The ligand binding domain (LBD), which upon compound binding can induce an altered stability and resulting from that cofactor recruitment, is especially highly conserved. This presents the challenge of specifically targeting one NR.

In 2015, an allosteric binding pocket in RORγt was discovered (Scheepstra M *et al.* 2015 Nat Commun). Despite current efforts in engineering compounds binding RORγt allosterically, the mechanism underlying the formation of this pocket is poorly understood. To elucidate if the pocket is a unique asset of RORγt or could also be formed in other NRs we aim to identify the prerequisites for the allosteric pocket.

Key-differences between RORγt and other NRs in the allosteric region were identified. A unique feature within the ROR family is the presence of a helix 11 prime (H11') connecting H11 and H12, which, upon allosteric compound binding, unfolds and spans over the pocket. H11' in RORγt was shortened in order to determine if NRs with a shorter linker could be able to form the allosteric pocket. Further, residues in direct contact with the ligand or connecting distant helices in the allosteric fold were pinpointed. Wildtype RORγt LBD was mutated at these locations and the mutants were tested in biochemical assays.

Based on our findings we can conclude it would be very challenging, if not impossible, to form a similar pocket in another NR, since the combination of crucial residues and linker length is unique for RORγt. This confirms the selectivity of targeting this pocket and gives opportunities for treating autoimmune diseases.

26. Meijer Femke

Ligand-based design of allosteric RORγt inverse agonists

Femke A. Meijer[1], Richard G. Doveston[1,2], Rens M.J.M. de Vries[1], Gael M. Vos[1], Luc Brunsveld[1]

[1] Laboratory of Chemical Biology, Dept. of Biomedical Engineering and Institute for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven, The Netherlands; [2] Leicester Institute of Structural and Chemical Biology and Dept. of Chemistry, University of Leicester, Leicester, UK.

RORγt is a key regulator in the development of Th17 cells. The inhibition of this protein could be beneficial in therapies for autoimmune diseases. Recently, an allosteric binding site for RORγt has been identified and this alternative type of inhibition shows many advantages over orthosteric targeting.

Currently, the examples of allosteric RORγt ligands are limited to one series of closely related indazoles. In order to better exploit the strategy of allosteric modulation for therapeutic purposes and improve the PK profile, the aim was to identify novel chemotypes that effectively target the allosteric site of RORγt.

In silico pharmacophore screening and docking studies were used to discover a novel class of allosteric ligands. Isoxazole compound FM26 was found as a lead compound, which clearly inhibits coactivator recruitment, significantly reduces IL-17a mRNA expression levels, has a promising ADME profile and shows a clear allosteric binding mode in the co-crystal structure.

27. Özgün Fatma

Characterization of androgen receptor variant 7 dimerization in prostate cancer

Fatma Özgün[1], Martin E. van Royen[2], Adriaan B. Houtsmuller[2], Nathan A. Lack[1,3]

[1] Koç University, School of Medicine, Rumelifeneri Yolu, Sarıyer, 34450 İstanbul, Turkey; [2] Erasmus Optical Imaging Centre, Department of Pathology, Erasmus MC, 3015 GE.

Prostate cancer is an extremely common disease that affects an estimated one out of seven men in their lifetime. While the cancer can often be treated through surgery or radiotherapy, those patients with late-stage, recurrent or metastatic forms of prostate cancer are often given androgen deprivation therapy. While this treatment is initially effective the cancer almost always develops resistance. Recent studies have proposed that androgen receptor variants can cause AR signaling in the absence of androgen and may drive CRPC.

While AR variants correlate with CRPC, it is unclear how they initiate transcription as they are missing a key domain involved in activation and dimerization. Therefore, to better understand the mechanism of variant dimerization we developed an acceptor photobleaching FRET based methodology to test if the most commonly observed variant, ARv7, can form dimers. We demonstrated that ARv7 formed heterodimers with flAR at N/C orientation, with no ARv7/ARv7 N/C homodimerization being detected. To quantify the relative affinity of the ARv7 with flAR, we conducted FRET with a “tunable” system that can vary the flAR and ARv7 expression. We demonstrated that ARv7 interacts with flAR with a same affinity as flAR. To better understand the bound DNA occupancy of the ARv7, we performed fluorescence recovery after photobleaching (FRAP) experiments. We found that the ARv7 residence time on DNA was markedly shorter than flAR. These results suggest that ARv7 itself does not form long-term interactions with DNA.

In conclusion, ARv7 interacts with flAR to form heterodimers. ARv7 does not form stably bound fractions on DNA and has much shorter occupancy. Further FRET studies in combination with FRAP is in progress to better understand the function of AR variant heterodimers in CRPC as a potential therapeutic target.

28. Prekovic Stefan

Multimodal influence of chromatin-interacting factors on glucocorticoid receptor transcriptional programs

Stefan Prekovic[1], Karianne Schuurman[1], Selcuk Yavuz[1], Isabel Mayayo-Peralta[1], and Wilbert Zwart[1]

[1] *Division of Oncogenomics, Oncode Institute, Netherlands Cancer Institute, Amsterdam, The Netherlands.*

Unravelling mechanisms by which glucocorticoid receptor (GR) directly regulates gene expression is quintessential to an understanding of its role in maintaining various aspects of human homeostasis. Upon ligand binding, the GR translocates to the nucleus, where it will interact with chromatin to activate or repress target genes.

To improve our understanding of how GR regulates gene expression, we performed immunoprecipitation experiments followed by mass spectrometry to identify its interacting partners in various cell lines. Our comprehensive snap-shot of the GR protein-interactome in cancer cells identified proteins either directly related to RNA Pol II complex recruitment, or to the establishment of a chromatin remodelling machinery.

These findings led us to further investigate involvement of chromatin-related factors and how these influence GR transcriptional output. We show that various chromatin-related factors colocalize with GR on the genome, and could potentially be related to chromatin looping, and glucocorticoid-driven changes in chromatin accessibility and acetylation status of various histone proteins. In addition, glucocorticoid-induced alteration in phosphorylation status of various chromatin-remodelling proteins was observed using mass spectrometry.

To address the functional properties of these interactions we generated knockout models of cohesin subunits (SA1/SA2), cohesin release factor (WAPL), and chromatin remodeling complex members (SMARCA2, SMARCB1, and ARID1A). The functional impact of each knockout on GR function was assessed by RNA and GR-ChIP sequencing.

Our studies reveal a gene-specific role of cohesion SA2 in context of GR signalling, and connect loss of chromatin remodelling capabilities to alterations in the GR signalling axis. Our data sheds light on an important understudied aspect of GR biology and repositions chromatin remodelling as an important and context-dependent modulator of GR transcriptional output.

29. Preuss Jonathan Michel

Augmented acute lung injury and dysregulated immune response of GRdim mice under intensive care management in endotoxemia

J.M. Preuss[1], M. Wepler[2,3], T. Merz[2], C. Hartmann[2,3], U. Wachter[2], O. McCook[2], J. Vogt[2], S. Kress[2], M. Groeger[2], M. Fink[2], A. Scheuerle[4], P. Moeller[4], S. Hafner[3], M. Georgieff[3], T. Datzmann[2,3], E. Calzia[2], M. Kalbitz[5], U. Burret[1], R. Engelhardt[2], B. Stahl[2], M. Huber-Lang[6], P. Radermacher[2], J.P. Tuckermann[1], S. Vettorazzi[1]

[1] Institute of Comparative Molecular Endocrinology, Ulm University, Ulm; [2] Institute for Anaesthesiological Pathophysiology and Process Engineering, University Hospital, Ulm; [3] Department of Anesthesiology, University Hospital, Ulm; [4] Institute of Pathology, University Hospital, Ulm; [5] Department of Traumatology, Hand-, Plastic-, and Reconstructive Surgery, Center of Surgery, Ulm University, Ulm; [6] Institute of Clinical and Experimental Trauma-Immunology, University Hospital, Ulm.

Bacterial induced endotoxin shock is one important cause for intensive care unit (ICU) cases with a high mortality rate. Endotoxemia is often accompanied by acute lung injury (ALI) or the acute respiratory distress syndrome (ARDS) in patients and characterized by a reduced lung compliance. Endogenous glucocorticoids (GCs) play a decisive immune modulatory role and have anti-inflammatory effects during sepsis. Mice with attenuated GC receptor (GR) dimerization (GRdim) show an increased lethality and more pronounced hypothermia in LPS-induced endotoxemia than WT animals. To resemble the septic patient under intensive care management we analyzed GRdim mice under intensive care conditions (lung-protective ventilation, catecholamine administration and fluid resuscitation) during LPS induced endotoxic shock. GRdim mice showed a higher lethality after endotoxic shock in the ICU and had an increased catecholamine need to keep hemodynamic stability. Additionally GRdim mice showed elevated Il-10 plasma level and increased Tgfb1 expression in the lung. Furthermore, estrogen dependent immune suppression might be enhanced in GRdim mice reflected by sex dependent dysregulation in plasma cytokine level (like Mip-1b and Il-6) in response to LPS. Intriguingly GRdim mice showed a significant reduced lung compliance in the ICU compared to WT in the LPS and the PBS treated control group too. Expression of pulmonary function related genes like Spd and Scnn1b were impaired in GRdim animals in response to LPS indicating surfactant dysregulation in the alveoli. Male GRdim mice showed increased and females reduced expression of Mpo in the lung. Osteopontin (Spp1), a crucial mediator during human and rodent lung inflammation, was increased in the lungs of GRdim mice. This was substantiated by an increased Spp1 expression of GRdim derived macrophages in response to LPS in vitro. Therefore Opn might be a target for the reduced lung compliance and survival rate of GRdim mice in endotoxic shock.

30. Ramos Pittol Jose Miguel

RXR inhibits FXR isoform-selective gene activation via a switch in binding motif preference

Jose M. Ramos Pittol[1], Jelmer J. Dijkstra[2], Ellen C.L. Willemsen[1], Suzanne W. van der Veen[1], Michiel Vermeulen[2], Saskia W.C. van Mil[1]

[1] Center for Molecular Medicine, University Medical Center Utrecht, University Utrecht, Utrecht, The Netherlands; [2] Department of Molecular Biology, Faculty of Science, Radboud Institute for Molecular Life Sciences, Oncode Institute, Radboud University Nijmegen, Nijmegen, The Netherlands.

The Farnesoid-X-receptor (FXR) is a nuclear receptor regulating metabolic processes. FXR is expressed as four isoforms (α1-4), which bind to a DNA motif (IR-1) and subsequently activate transcription. FXRα2/4 additionally bind to a selective DNA motif (ER-2), with target genes of therapeutic interest. However, FXR activation from ER-2sites has seldom been reported. Here we show that the Retinoid-X-receptor (RXR) plays a pivotal role in FXR target gene selectivity. RXR is the classical heterodimerization partner of FXR but is not required for FXR binding to ER-2 sites. Instead, RXR inhibits FXR transactivation from ER-2 sites in reporter constructs and cells. Concomitantly, abrogation of the FXR-RXR interaction selectively negates activation from IR-1 sites while retaining ER-2 activation capacities. Thus, our results reveal a novel mechanism behind FXRα2/4 DNA binding selectivity, and demonstrate the FXR-RXR interaction as a target to promote transcription from ER-2 sites upon FXR agonism.

31. Salem Fatouma

PPARg deficiency in RORgt positive cells aggravates experimental colitis

Fatouma Salem, Maroua Ferhat, Mahdia Taieb, Jean-Yves Jouzeau, Gerard Eberl, David Moulin

Introduction
Peroxisome proliferator activated receptor gamma (PPARγ) is a ligand-dependent transcription factor, and a member of the nuclear receptor superfamily that acts as a “molecular sensor”, able to control many physiological functions including glucose and lipid homeostasis, cell growth and differentiation. More recently, PPARγ has emerged as an important player of immune response through its ability to mitigate the expression of inflammatory cytokines. Of interest, PPARγ has been shown to be a master regulator of IL-17-expressing cells through inhibition of the transcription factor RORγt (RAR-related orphan receptor gamma t) and influence the local balance between Th17 and Treg during intestinal inflammation.

Objective
To investigating the interplay between metabolic sensing through PPARγ and IL-17-expressing cells during intestinal inflammation.

Methods
To study the functional role of PPARγ in IL-17-producing cells, we conditionally disrupted the PPARγ gene in cells expressing RORγt using Cre/loxP–mediated DNA recombination. The generated PPARγ fl/fl RORγt Cre+ (deficient mice) and PPARγ fl/fl RORγt Cre- (control mice) were treated with agonist anti-CD40 antibody or given 3% DSS (Dextran Sodium Sulfate) in drinking water for 3, 5 or 7 days, to induce colitis. Animals were monitored regularly, by a daily record of weight loss rate, faeces consistency and blood presence in stools, to follow the colitis severity during the experience.

Results
Daily clinical scores of weight loss, faeces consistency and bloodiness demonstrate an elevated inflammation stated in the deficient group compared to WT. Variation of frequency of RORgt+ cells (Th17 and their innate counterpart ILC3) were observed between the two studied genotypes. These findings support a key role for metabolic sensing through PPARγ in modulating immunity.

32. Sermikli Benan Pelin

Role of myeloid-expressed the RAR-related Orphan Receptor Alpha (RORα) in metabolic diseases

Sermikli BP*, L'homme L*, Molendi-Coste O*, Fleury S, Quemener S, Joseph ML, Pineau L, Gross B, Vallez E, Muhr-Tailleux A, Staels B, Dombrowicz D

**These authors contributed equally to this work*
Univ. Lille, INSERM, CHU Lille, Institute Pasteur de Lille, U1011, EGID, France

Retinoic acid receptor-related orphan receptor alpha (RORα) is a nuclear receptor involved in the pathophysiology of metabolic diseases and a critical transcription factor for various immune cell types. Taking into account the importance of RORα in metabolic homeostasis and immunoregulation, and the role of myeloid cells in the pathogenesis of metabolic diseases, we investigated the role of myeloid-expressed RORα in the context of obesity and obesity-associated diseases. Thus, we generated a myeloid-cell specific RORα-deficient mouse model by crossing mice harboring a floxed Rora allele with transgenic mice expressing Cre-recombinase under the control of Lysozyme M promoter. Diet-induced obesity model achieved by feeding RORα+/+ (WT) and their littermates RORαLysM/LysM (MKO) mice with high fat (HFD) or a chow diet (CD) for 12 weeks. We didn't observe genotype effect on body weight nor peripheral glycemic control suggesting that myeloid-expressed RORα doesn’t play a role in obesity development and obesity-induced insulin resistance. Liver steatosis was also comparable. To further investigate the role of myeloid-expressed RORα in liver diseases, we fed mice with a NASH-inducing diet (choline deficient, methionine restricted, 2% cholesterol and high in sucrose) for 8 weeks and then classical NASH parameters investigated. Our findings suggest that RORα doesn't play a role in NASH development and its complications.

33. Severson Tesa

Defining active enhancers that drive prostate cancer development, metastasis and progression

Tesa M. Severson[1], Yanyun Zhu[1], Guido Jenster[2], Geert van Leenders[3], Henk van der Poel[4], Lodewyk Wessels[5], Andries M. Bergman[6], Wilbert Zwart[1,7]

[1] Division of Oncogenomics, Oncode Institute, Netherlands Cancer Institute, 1066 CX, Amsterdam, The Netherlands; [2] Department of Urology, Erasmus MC, PO Box 2040, 3000 CA, Rotterdam, The Netherlands; [3] Department of Pathology, Erasmus MC, PO Box 2040, 3000 CA Rotterdam, The Netherlands; [4] Department of Urology, Antoni van Leeuwenhoek Ziekenhuis, 1066 CX, Amsterdam, The Netherlands; [5] Division of Molecular Carcinogenesis, Oncode Institute, Netherlands Cancer Institute, 1066 CX, Amsterdam, The Netherlands; [6] Division of Medical Oncology, The Netherlands Cancer Institute, Amsterdam, The Netherlands; [7] Laboratory of Chemical Biology and Institute for Complex Molecular Systems, Department of Biomedical Engineering, Eindhoven University of Technology, PO Box 513, 5600 MB, Eindhoven, The Netherlands.

Background
Prostate cancer is the most common non-cutaneous cancer in men and is dictated by activity of the Androgen Receptor (AR). The epigenetic landscape has been shown to be important in prostate tumorigenesis from normal epithelium. Currently, little is known about the epigenetic interactions and gene regulation of the natural course of the disease from oncogenesis to metastatic outgrowth without the selective pressure of treatment and how that compares to primary and castration resistant metastatic prostate cancer (mCRPC).

Aim
Elucidation of the underlying mechanisms of transformation from normal tissue to primary and metastatic prostate cancer. We aim to identify state-specific enhancer activity, dictating genetic programs and transcription factor action that drive tumor development and progression.

Methods
To identify genomic binding sites for enhancers, we use chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq) of fresh-frozen samples for the epigenetic mark H3K27ac. These data are coupled with ChIP-seq data for AR, its pioneer factor FOXA1 and chromatin insulator CTCF, along with gene expression data for all samples.

Results
We have generated high quality H3K27ac, AR, FOXA1 and CTCF ChIP-seq data for normal tissue (n=27), primary tumor (n=29), treatment naïve metastases (n=19) and metastatic CRPC tissue (n=6). Remarkably, we observed the greatest differences in binding sites between healthy/primary and both metastases types in CTCF binding. These differential sites were H3K27acetylated and intra-TAD, promoter/enhancer selective indicating control at the 3D genomic level.

Conclusions
We find the most prominent difference is enhancer-facilitating CTCF binding in the acquisition of metastatic features. These data suggest that major changes in promoter-enhancer loops in the natural course of prostate cancer happen exclusively in the transition point from primary tumor to metastasis and importantly, before CRPC development.

34. Vanden Berghe Wim

Identification of CALD1 DNA Methylation as a saliva specific biomarker for early life psychosocial deprivation stress associated with institutional care

Stacy Drury[1,4], Ken Declerck[2], Kyle Esteves[4], Chris Jones[1], Sabine Langie[3,7] Charles Zeanah[4], Nathan Fox[5], Charles Nelson[6], Wim Vanden Berghe[2], Patrick De Boever[3,7]

[1] Tulane Brain Institute, Tulane University, New Orleans, LA, USA; [2] Laboratory of Protein Chemistry, Proteomics and Epigenetic Signaling, Department of Biomedical Sciences, University of Antwerp, Wilrijk, Belgium; [3] Center for Environmental Sciences, Hasselt University, Diepenbeek, Belgium; [4] Department of Psychiatry, Tulane University; [5] Department of Human Development, University of Maryland; [6] Department of Pediatrics and Neuroscience Harvard University; [7] Health Unit, Flemish Institute for Technological Research (VITO), Mol, Belgium.

Epigenetic DNA methylation changes are hypothesized to be one mechanism through which early life stress experiences shape adverse neurodevelopment across social, emotional, behavioral, physiological and neurobiological health outcomes associated with psychosocial deprivation during institutional care. In this study we utilized an innovative work flow to identify epigenetic mechanistic changes that are likely contributors to the lasting impact of early life stress on the hypothalamic pituitary adrenal axis (HPA). Leveraging an existing prospective randomized controlled trial of foster care compared to institutional caregiving we took a step wise analytic approach to relating methylation, gene expression and cortisol within salivary samples from the same individuals. Upon comparison of genomewide DNA methylation and gene expression profiles associated with the different care groups, IPA network analyses identified gene enrichment involved in nervous system development, cognition, behavior, and psychological disorders. Further comparison with gene expression profiles before and after the Trier social stress test (TSTT) identified a nuclear receptor coordinated stress dependent gene network, involving the glucocorticoid receptor. One of the genes with the highest correlation between TSST specific changes in cortisol levels, DNA methylation and gene expression was CALD1, a gene with established links to glucocorticoid stress responsiveness, neuronal migration and also the impact of cortisol stress hormones on neuronal morphology, dendritic spines and migration. Furthermore, chronic stress-induced shrinkage of dendritic spine subtypes has been shown to impair feedback mechanisms of the HPA-axis, cause detoriation of sensory functions and attrition of neuroplasticity. These findings revealed an important mechanistic link for CALD1 in the lasting neurodevelopmental impacts of early life stress.

35. Vanderhaeghen Tineke

Zinc and glucocorticoids control microbe-induced interferon signatures in intestinal epithelium of mice

Vanderhaeghen Tineke, Souffriau Jolien, Van Looveren Kelly, Wallaeys Charlotte, Timmermans Steven, Eggermont Melanie, Ballegeer Marlies, De Bosscher Karolien, Beyaert Rudi, Libert Claude

Center for Inflammation Research, VIB, Ghent, Belgium. Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium.

The signal transduction and mechanism of induction of inflammation by tumor necrosis factor (TNF) is well-known in vivo. Based on phenotypes of glucocorticoid receptor (GR) mutant mice, we uncovered new insights in the biology of TNF. Since GRdim mice, with reduced GR dimerizing function, and GRVillKO mice are severely sensitive for TNF-induced lethal systemic inflammatory response syndrome (SIRS), and TNF has strong effects on intestinal epithelium cells (IECs), the data suggested that TNF-induced SIRS is blocked at the level of IECs by GR. However, unchallenged GR mutant mice express high levels of interferon-stimulated genes (ISGs) at the IECs, encoding necroptosis inducing genes e.g. Ripk3, Mlkl and Zbp1. This suggested that this ISG signature is induced by gut microbes and that GR mutant mice, hence, lost the control over this ISG expression in IECs. This gives TNF a wild card to induce necroptosis, mainly in crypts of these mice. Interestingly, an anti-inflammatory treatment, used e.g. to protect piglets against intestinal infection and diarrhea, is zinc (Zn). Zn pretreatment protects mice against TNF-induced SIRS, but not in GRdim, GRVillKO or adrenalectomized mice. In line with our new hypothesis, by RNA sequencing in IECs, Zn leads to strong reduction of ISG genes in GRWT mice, also in GR mutant mice. Zn has similar effects in GRdim mice, but as these mice have basically strongly induced ISGs, Zn reduces these genes only partially. In GRWT animals, Zn reduces TNF-induced cell death in crypts and the outflux of gut microbes into the tissues, such as spleen and mesenteric lymph nodes. The mechanism by which Zn reduces ISG expression is now under investigation, but preliminary data suggest a direct effect on the composition of the gut microbiome in the ileum. As Zn and GR both control the microbe-induced ISG signature, it is hence interesting to observe that Zn and glucocorticoids (dexamethasone) have additive protective effects against TNF-induced SIRS.

36. Vandewalle Jolien

Glucocorticoid resistance in liver as a driver of sepsis pathology

Vandewalle Jolien, Timmermans Steven, Van Wyngene Lise, Van Looveren Kelly, Vanderhaeghen Tineke, Wallaeys Charlotte, Eggermont Melanie, Libert Claude

Center for Inflammation Research, VIB, Ghent, Belgium. Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium.

Sepsis is a life-threatening condition resulting from a dysregulated response to infection, with a global burden of at least 19 million cases annually. Recently the World Health Organization (WHO) has acknowledged sepsis as a global health priority issue. Here we demonstrate that induction of a genome-wide glucocorticoid resistance (GCR) in the liver of cecal ligation and puncture (CLP) mice abrogates glucose production via liver gluconeogenesis. This in turn could contribute to lethal hypoglycemia and hyperlactatemia through defects in the cori-cycle. A second consequence of this GCR is that no therapeutic, anti-inflammatory effects can be expected from GCs. To unravel the underlying mechanism of GCR in sepsis, we studied GR levels, nuclear translocation and DNA binding of GR after DEX stimulation in liver. We found that the GR was unable to bind DNA in septic CLP livers. GCR could not be reverted in ADX mice or TNFR1KO mice. In conclusion, we show that in sepsis, there is a genome-wide GCR contributing. The consequences of this GCR are two-fold. On the one hand no therapeutic effects can be seen with GCs in sepsis and on the other hand physiological functions of endogenously produced GCs are failing. It is our ultimate goal to revert GCR in sepsis to preserve the functions of the GR, but this needs further investigation.

37. Van Looveren Kelly

Development and study of new dimerization mouse tools

Kelly Van Looveren[1,2], Tino Hochepied[1,2], Steven Timmermans[1,2], Melanie Eggermont[1,2], Robbert J Rottier[3,4], Karolien De Bosscher[5,6], Claude Libert[1,2]

[1]VIB Center for Inflammation Research, Ghent, Belgium; [2]Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium; [3]Department of Pediatric Surgery, Erasmus Medical Center, Sophia Children's Hospital, Rotterdam, The Netherlands; [4]Department of Cell Biology, Erasmus Medical Center, Rotterdam, The Netherlands; [5]Department of Biochemistry, Ghent University, Ghent, Belgium; [6]Receptor Research Laboratories, Nuclear Receptor Lab, Medical Biotechnology Center, VIB, Ghent, Belgium.

The glucocorticoid receptor (GR) regulates genes implicated in control and regulation of metabolism, inflammation, development and other key processes. GR regulates gene expression through various mechanisms, e.g. as a homodimeric transcription factor that binds to glucocorticoid response elements (GREs) in promoter regions of GC-inducible genes or e.g. as a monomer which reduces transcription of inflammatory genes by transrepression. GR homodimers are essential to mediate the anti-inflammatory properties of GCs in acute inflammatory settings, such as Systemic Inflammatory Response Syndrome (SIRS). Most of these data are based on GRdim (GRA465T) mice. These mice express a mutant version of GR, carrying a missense mutation in the DNA binding domain, which leads to a reduced GR dimerization and DNA binding, yet maintains an intact monomer profile. However, the GRdim mutant protein has been shown to induce gene expression in a sequence and context-dependent manner. These results were explained by findings showing extra GR dimerization contacts in their ligand-binding domain (LBD). Using CRISPR/Cas9, we generated a GRI634A mouse, containing a point mutation in the dimer interface of the LBD and a GRA465T/I634A mouse, which contains both point mutations in DBD and LBD. Surprisingly, homozygous GRA465T/I634A mice appeared not to be viable. Therefore, we use two different approaches to characterize this new mutant mouse: (i) investigate the sensitivity of heterozygous mice for an inflammatory stimulus and (ii) generate mouse embryonic fibroblasts (MEFs) to characterize the double mutant cells in detail. Via RNAseq we compare the expression profile of GRA465T/I634A with GR+/+ cells, the GRA465T mutant and GR-/- MEF cells and investigate the nature of the dimers as well try to investigate the potential of the monomers. Finally, we study the pathology underlying the perinatal mortality of these double mutant mice.

38. Van Moortel Laura

The quest for improved glucocorticoid receptor ligands: a study of the plasticity of the glucorticoid receptor using selective modulators

Laura Van Moortel[1,2], Jonathan Thommis[1,2], Jarne Pauwels[2], Delphine De Sutter[2], Sven Eyckerman[2], Kris Gevaert[2], Karolien De Bosscher[1,2].

[1] Translational Nuclear Receptor Research lab, Albert Baertsoenkaai 3, 9000 Gent, Belgium; [2] VIB-UGent Center for Medical Biotechnology, Albert Baertsoenkaai 3, 9000 Gent, Belgium.

Exogenous glucocorticoids (GCs) are widely used for the treatment of inflammatory disorders and hematological cancers. Unfortunately, the use of GCs is associated with numerous side effects, limiting their long-term use. Despite the efforts of pharma, the search for so-called 'selective glucocorticoid receptor (GR) modulators' (SEGRMs) has not led to compounds with a real improved benefit-risk ratio so far. It is our belief that this is at least partially due to the neglect of GR's ability to adapt its behavior depending on various factors (e.g. ligand type, cofactor profile, cell type).

The goal of my project is to study whether and how different types of ligands alter the conformation of GR, and how this is linked to its cofactor recruitment and eventual signaling profile. We expect the conformation of GR will be a much better predictor for its signaling behavior than most of the current screening tools (usually ligand-binding assays and a minimal set of reporter genes). In line herewith, we expect to find cofactors of which the absence or presence in the GR signaling complex may be linked to either a favorable or unfavorable GR signaling profile.

In the first part of the project, different GR ligands from pharma (agonist, antagonist or SEGRM) are being characterized with our screening platform (a.o. reporter gene assays, qPCR and dimerization assays), with the aim to profile the downstream GR signaling behavior. The second part of the project consists of mapping conformational differences induced in GR by different ligand types via limited proteolysis followed by a mass spectrometry read-out. In the last part of the project, we will use proximity-dependent biotin identification (BioID) to characterize the conformation-dependent interactome of GR. To minimize the number of false-positive interactions due to overexpression, the miniTurbo tag will be introduced at the N-terminus of endogenous GR via CRISPR-Cas9 knock-in.

39. Viho Eva

Understanding the nature of CORT118335 selective receptor modulation in liver lipid metabolism

Eva M.G. Viho[1], Loïs J. Aitatus[1], Lisa L. Koorneef[1], René Houtman[2], Hazel Hunt[3], Jan Kroon[1], Onno C. Meijer[1]

[1] Department of Medicine, Division of Endocrinology, Leiden University Medical Center, Leiden, The Netherlands; [2] Precision Medicine Lab, Pivot Park, Oss, The Netherlands; [3] Corcept Therapeutics, Menlo Park, CA 94025, U.S.A.

CORT118335 is a selective Glucocorticoid Receptor (GR) modulator that was previously shown to prevent diet-induced liver steatosis in male mice. While GR coregulator interactions induced by CORT118335 in vitro are clearly distinct from cortisol-induced interactions, the mechanisms underlying the beneficial properties of CORT118335 in liver lipid metabolism are yet unknown.

In order to unravel how CORT118335 prevents liver steatosis, we previously performed RNA-sequencing on livers of mice fed a high fat diet supplemented with vehicle, corticosterone or CORT118335. We identified a shortlist of approximately 30 genes that were significantly upregulated by corticosterone but that were not upregulated or even downregulated by CORT118335 treatment; and are thus likely involved in the beneficial effects of CORT118335. Gene enrichment pathways analysis reveals that these genes are mainly involved in lipid metabolism. Here, we evaluated the expression of genes in this shortlist and GR classic target genes in the human hepatocyte cell line HepG2 after CORT118335 and cortisol treatment at different time points (3h-6h-12h-24h). Although HepG2 cells do not exactly recapitulate the full GR target gene pattern of the mouse transcriptome data, the results suggest that CORT118335 acts as a partial agonist on most GR target genes in HepG2 cells, but that the extent of partial agonism is gene- and time-specific.

We next evaluated how CORT118335 may induce a different expression pattern as compared to corticosterone. We hypothesized that CORT118335-specific effects relied on an altered GR-interactome. Indeed, the MARCoNI assay revealed that 5 coregulators interacted with GR in the presence of cortisol but not CORT118335. Our present hypothesis is that differential interaction with coregulator UBE3A is responsible for the selective modulator characteristics of CORT118335.


PARTICIPANTS
NRRN meeting
Ghent, 21.11.19

Title	First name	Last name	Institute/Company
Mrs.	Nur	Al Amery	Universitair Medisch Centrum Utrecht
Mr.	Jorge Miguel	Amaya	LUMC
Mrs.	Rosalie	Baak	Academic Medical Center Utrecht (UMCU)
Mrs.	Anouska	Borgman	UMC Utrecht
Dr.	Roger	Bouillon	KU Leuven
Dr.	Luc	Brunsveld	TU Eindhoven
Dr.	Lisa	Butler	University of Adelaide
Mr.	Rob	Buurstede	LUMC
Dr.	Frank	Claessens	KU Leuven
Dr.	Dorien	Clarisse	VIB-UGent
Mrs.	Maria	Clemente	Amsterdam UMC
Dr.	Charlotte	Cornil	University of Liège
Mrs.	Giulia	D´Arrigo	Eindhoven University of Technology
Mr.	Karel	David	KU Leuven
Dr.	Karolien	De Bosscher	VIB-UGent
Dr.	Catherine	de Bournonville	University of Liège
Mr.	Pim	de Vink	Eindhoven University of Technology
Mr.	Rens	de Vries	Eindhoven University of Technology
Mrs.	Carlie	de Vries	University of Amsterdam
Mr.	Koos	de Wit	UMC Utrecht
Mr.	Nick	Deploey	VIB-UGent
Dr.	Sofie	Desmet	Ghent University
Mr.	Wout	Devlies	KU Leuven
Dr.	Maria	Donaldson Collier	NKI
Dr.	Vanessa	Dubois	KU Leuven
Mr.	Roy	Eerlings	KU Leuven
Mr.	Nils	Eickhoff	Netherlands Cancer Institute (NKI)
Mrs.	Sarah	El Kharraz	KU Leuven
Dr.	Mélanie	Faure	University of Liège
Dr.	Maroua	Ferhat	IMoPA-UMR 7365 CNRS
Mrs.	Anne	Gallez	University of Liège
Dr.	Anja	Geerts	University Hospital Ghent
Mr.	Max	Gentenaar	Leiden University Medical Centre (LUMC)
Mrs.	Marie	Gerard	INSERM U1011
Mrs.	Purvi	Gupta	KU Leuven
Mr.	Jayron	Habibe	Amsterdam UMC location AMC
Dr.	Florian	Handle	KU Leuven
Dr.	Christine	Helsen	KU Leuven
Mr.	Miguel	Hernández Quiles	UMC Utrecht
Mr.	Twan	Hopstaken	Eindhoven University of Technology
Dr.	Rene	Houtman	Precision Medicine Lab
Dr.	Adriaan	Houtsmuller	Erasmus MC Rotterdam
Dr.	Johan	Jonker	UMCG
Dr.	Jean-Yves	Jouzeau	IMoPA

Dr.	Eric	Kalkhoven	UMC Utrecht
Mrs.	Rougin	Khalil	KU Leuven
Mrs.	Nari	Kim	KU Leuven
Mr.	Jan	Klomp	Lead Pharma
Mr.	Aleksandar	Kojic	Netherlands Cancer Institute (NKI)
Mr.	Auke	Koops	Technical University Eindhoven
Mrs.	Lisa	Koorneef	LUMC
Dr.	Jan	Kroon	Leiden University Medical Center
Mrs.	Xiao YIn	Lee	KU Leuven
Dr.	Philippe	Lefebvre	Inserm
Dr.	Sander	Lefere	Ghent University
Mrs.	Iris	Leijten - van de Gevel	Eindhoven University of Technology
Mr.	Yunkun	Li	VIB-UGent
Mrs.	Wenjun	Liao	Erasmus MC
Mrs.	Sanne	Lith	AMC
Mr.	Bert	Luyckx	VIB-UGent
Mr.	Brecht	Maertens	VIB-UGent
Dr.	Taieb	Mahdia	University of Lorraine - UMR7365CNRS
Dr.	Alireza	Mashaghi	Leiden University
Dr.	Onno	Meijer	Leiden Universtity Medical Center
Mrs.	Femke	Meijer	Eindhoven University of Technology
Dr.	Marcel	Meima	Erasmus MC
Dr.	Marie	Miglianico	ChromaCure SA
Dr.	David	Moulin	IMoPA
Dr.	Fatma	Özgün	Koc University in collaboration with Erasmus MC
Mr.	Nuno	Padrao	Netherlands Cancer Institute (NKI)
Mrs.	Els	Pardon	VUB
Mr.	Frank	Peelman	VIB-UGent
Mrs.	Christel	Péqueux	University of Liège
Dr.	Stefan	Prekovic	Netherlands Cancer Institute
Mr.	Jonathan Michel	Preuss	Institute for Comparative Molecular Endocrinology
Dr.	Jose Miguel	Ramos Pittol	UMC Utrecht
Mrs.	Fatouma	Salem	IMoPA
Mrs.	Benan Pelin	Sermikli	Pasteur Institute of Lille
Dr.	Tesa	Severson	Netherlands Cancer Institute (NKI)
Mr.	Joseph	Siefert	NKI-AvL
Dr.	Maud	Sigoillot	ChromaCure
Mrs.	Hetty	Sips	LUMC
Mr.	Gido	Snaterse	Erasmus MC
Mrs.	Bente	Somsen	Eindhoven University of Technology
Mr.	Jonathan	Thommis	VIB-UGent
Mr.	Steven	Timmermans	Ghent University
Mrs.	Janny	van den Burg	AMC
Mrs.	Suzanne	van der Veen	UMC Utrecht

Mr.	Ferdy	van Geest	Erasmus MC Rotterdam
Mrs.	Maisa	van Genderen	NKI, Technical University Eindhoven
Mrs.	Kelly	Van Looveren	VIB
Mrs.	Laura	Van Moortel	VIB-UGent
Mrs.	Karlien	Van Wesemael	VIB-UGent
Mrs.	Marie	Vandaudenard	Chromacure
Dr.	Wim	Vanden Berghe	University Antwerp
Mrs.	Tineke	Vanderhaeghen	VIB-UGent
Mrs.	Jolien	Vandewalle	VIB-UGent
Mrs.	Vanessa	Vermeirssen	Ghent University
Mrs.	Eva	Viho	Leiden University Medical Center
Mrs.	Emira	Visser	Technical University Eindhoven
Dr.	Arnout	Voet	KU Leuven
Mrs.	Charlotte	Wallaeys	VIB-UGent
Dr.	Alexandre	Wohlkonig	VUB/VIB
Mr.	Selçuk	Yavuz	Erasmus MC