



# DAG VAN DE BIOMEDICI



**Donderdag 3 april 2014**



## **Beste studenten en participanten,**

De opleidingsraad Biomedische Wetenschappen wil jullie graag welkom heten op de 'Dag van de Biomedici 2014'.

Met dit initiatief willen wij alle studenten Biomedische Wetenschappen de kans geven om kennis te maken met het onderzoek dat verricht wordt op de VUB campus Jette. Een breed spectrum van onderwerpen zal aan bod komen, waaronder diabetes, gentherapie, multiple myeloma en nog veel meer. Deze dag is een geschikt moment voor de jongere generatie om al eens na te denken over hun toekomstige stages.

Ook voor de Erasmus studenten uit de richting Biomedische Laboratorium Technologie, is het interessant om met de onderzoekslabo's van de VUB kennis te maken. Dit zijn de plaatsen waar vele FBT-laboranten te werk gesteld zijn, en waar je eventueel ook een stage kan lopen.

We beginnen deze dag met de presentaties van de 2<sup>de</sup> master studenten over het 'biomedisch' onderzoek dat ze verrichten ter voorbereiding van hun thesis. Tijdens de middag kunnen jullie genieten van een lunch in het Forum/Atrium en een drankje in de Tempus.

In de namiddag mag je sprekers verwachten uit een brede waaier van het beroepenveld. Daarnaast vertellen 2<sup>de</sup> Masterstudenten over hun ervaringen tijdens een stage in het buitenland.

Aan het einde van deze gevulde dag willen wij jullie graag uitnodigen om deel te nemen aan de labdrink in de Tempus. Tijdens de labdrink kan je als jonge student een praatje slaan met de master studenten.

Happy Meeting

## Programma: Deel I

<b>08.00 – 08.30</b>	<b>Registratie</b>
<b>08.30 – 08.45</b>	<b>Welkomstwoord</b> door prof. Karin Vanderkerken
<b>08.45 – 09.00</b>	<b>Baldan Jonathan:</b> Effect of thyroid hormone on human and murine pancreas
<b>09.00 – 09.15</b>	<b>Batota Luna:</b> Development of liver-specific transcriptional cis-regulatory modules (HS-CRM) and improved factor IX variant for hemophilia gene therapy
<b>09.15 – 09.30</b>	<b>Braye Aude:</b> In-vitro propagation of human spermatogonial stem cells
<b>09.30 – 09.45</b>	<b>De Ridder Ulrike:</b> Targeting Raf-1 in NSCLC
<b>09.45 – 10.00</b>	<b>Deneyer Lauren:</b> A mouse model of depression and anxiety can be induced by chronic corticosterone injections
<b>10.00 – 10.15</b>	<b>Giron Philippe:</b> The use of mRNA-lipoplexes for transfection of dendritic cells. The road to efficient tumor antigen loading and maturation of dendritic cells
<b>10.15 – 10.35</b>	<b>Frisdrank – Pauze</b>
<b>10.35 – 10.50</b>	<b>Krasniqi Ahmet:</b> In vivo administration of mRNA as a novel vaccination approach for the treatment of HIV-1
<b>10.50 – 11.05</b>	<b>Lambrecht Joeri:</b> The role of microRNAs in hepatic stellate cell activation and liver fibrosis
<b>11.05 – 11.20</b>	<b>Maes Anke:</b> The anaphase promoting complex as a possible new target in multiple myeloma
<b>11.20 – 11.35</b>	<b>Schwarze Julia Katharina:</b> Myeloid cells in colorectal cancer: a possible link between immunosuppression and radioprotection through arginase-1 overexpression
<b>11.35 – 11.50</b>	<b>Van Audenaerde Jonas:</b> Isolation and characterization of vital HIV from semen
<b>11.50 – 12.05</b>	<b>Vanheuverzwyn Raya:</b> The role of the insulin-like growth factor (IGF)-I receptor in IGF-I and estradiol mediated neuroprotection using a rat model for focal cerebral ischemia

## Programma: Deel II

<b>12.05 – 14.00</b>	<b>Lunch:</b> Forum/Atrium
<b>14.00 – 14.20</b>	<b>Kevin Ariën (Alumnus BMW - Instituut voor Tropische Geneeskunde):</b> Hoe bouwen aan een academische loopbaan? Enkele persoonlijke ervaringen.
<b>14.20 – 14.40</b>	<b>Nathalie De Clercq (Aspirant hoofdinspecteur - Politie Brussel):</b> Het leven na de dood, de job van forensisch onderzoeker.
<b>14.40 – 15.00</b>	<b>Dries Renmans (PhD student - VUB Laboratory of Molecular and Cellular Therapy):</b> Money money money: Doctoraatsbeurzen.
<b>15.00 – 15.20</b>	<b>Frisdrank – Pauze</b>
<b>15.20 – 15.40</b>	<b>Sofie Verachttert (Alumnus BMW - Lerarenopleiding):</b> Those who can, Teach!
<b>15.40 – 16.00</b>	<b>Jonathan Baldan en Joeri Lambrecht (2<sup>de</sup> Masterstudenten BMW):</b> Studying abroad.
<b>16.00 – 16.20</b>	<b>Katrien Van Beneden (Alumnus BMW - VUB/EhB):</b> Biomedische Wetenschappen; Een doctoraat; En wat daarna ?
<b>16.20 – 16.30</b>	<b>Slotwoord</b>
<b>16.30 – ...</b>	<b>Labdrink (Tempus)</b>



## Abstracts 2de Master BMW voor het MOSA Conference 2014 - International Students Research Conference on Health

Sinds 2003 nemen de studenten 2<sup>de</sup> master Biomedische Wetenschappen deel aan een internationaal studentencongres in Maastricht. Op dit congres krijgen (bio)medische studenten de mogelijkheid om hun eigen wetenschappelijk onderzoek voor te stellen. De auteurs met de beste ingezonden abstracts krijgen de mogelijkheid tot een orale presentatie en de andere auteurs stellen hun onderzoek voor met een poster presentatie. Zowel de orale als de poster presentatie wordt beoordeeld door een internationale jury.

De afgelopen jaren behaalden verschillende van onze studenten prijzen op het congres:

2013 – **Mathias van Bulck** behaalde de First Poster Prize met de abstract 'ENU mutagenesis as a discovery tool of potential driver genes in pancreatic tumourigenesis' (The Garvan Institute of Medical Research, Australia). **Inès Dufait** behaalde de First Oral Prize met 'Myeloid-derived suppressor cells as a biomarker of tumor growth and radiosensitivity: Role of hypoxia-inducible arginase-1' (VUB, Translational Radiation Oncology and Physics).



2011 – **Bart Legein** kreeg de publieksprijs voor de beste posterpresentatie met de abstract 'Tamoxifen Inhibits beta cell proliferation and biases beta cell neogenesis mechanisms in mouse pancreas'.

2010 – **Lien Thoen**: prijs van beste orale presentatie met 'The role of lipolysis in hepatic stellate cell activation'.

**Liesbeth Bieghs**: publieksprijs voor de beste poster presentatie met 'Forodesine reduces proliferation and induces apoptosis in multiple myeloma'.



2009 – **Violette Coppens**: prijs van beste orale presentatie met 'Human endothelial cells improve the outcome of the experimental islet transplantation'. **Sander Van Lint**: publieksprijs voor de beste poster presentatie met 'Development of a multiplex PCR for occurrence of uniparental disomy in all chromosomes of human embryonic stem cell lines'.

2007 – **Inge Neels**: prijs voor de beste poster presentatie. **Miguel Lemaire**: publieksprijs voor de beste poster.

2005 – **Christelle Orlando**: prijs voor de beste mondelinge presentatie. **Mieke Geens**: publieksprijs voor beste poster. **Tessa Dieltjens**: tweede plaats voor beste poster.

2003 – **An Verloes**: prijs van beste mondelinge presentatie. **Pieter Goossens** behaalde de eerste prijs voor de beste poster presentatie.

## Effect of thyroid hormone on human and murine pancreas

Authors: Jonathan Baldan, Isabelle Houbracken, Luc Bouwens

Cell Differentiation Lab, Vrije Universiteit Brussel, Belgium

**Introduction:** Transdifferentiation is a process whereby a specific mature cell-type converts to another mature cell type. As pancreatic acinar cells show high plasticity and account for the majority of pancreatic cells, transdifferentiation towards insulin producing cells using environmental factors is investigated as beta cell source for diabetic patients. EGF and LIF/CNTF showed to induce STAT3-dependent acinar to beta cell transdifferentiation in vivo and in vitro in rodent, but not on human cells in vitro. Furuya et al. showed transdifferentiation of TRalpha1 transduced murine acinar cells towards beta like cells when stimulated with thyroid hormone T3. We hypothesise that the STAT3 and PI3K/Akt pathway activation by T3 could have a similar effect on human exocrine cells.

**Material & methods:** Human exocrine pancreatic cells, AR42J cell line, C57Bl/6 mice, Ela-YFP, RIP-Cre-LacZ transgenic mouse strains. Western Blot (WB), qRT-PCR, immunohistochemistry, adenoviral production (pAdEasy). Thyroid hormone (T3).

**Results:** CMV- and amylase-driven FLAG-TRalpha1 constructs were cloned in pShuttle vector. Functionality was validated by transfection (AR42J). Adenoviral production is ongoing.

We are testing the effect of T3 in vivo in mice (7 and 35 days after alloxan-injection) and in vitro on human cells. We observed a difference in glycaemia (↓), pancreas (↑) and body weight (↑) between treated (4mg/kg T3) and non-treated normoglycaemic mice but did not observe a difference in alloxan-induced diabetic mice (7 days alloxan). Analysis of proliferation is ongoing but suggests an increased proliferation of acinar cells.

Human exocrine pancreatic cells were cultured for 5 and 10 days in different conditions: 0, 30, 100 nM T3 in suspension (S) and monolayer (ML). ML: No effect on proliferative activity. Observation of larger number of insulin-immunoreactive cells proved to be uptake from medium (no co-staining for C-peptide). S: Upregulation of TRbeta but not TRalpha isoform is suggested (WB). Differential gene expression will be performed for the two culture methods.

**Discussion & conclusions:** TH seems to have little effect on differentiation and proliferation of human acinar cells in vitro (ML), whereas in vivo in mice it stimulated acinar cell proliferation. We are further evaluating the effect of T3 on acinar cells and want to overexpress TRa1 receptor by transduction.

## Development of liver-specific transcriptional cis-regulatory modules (HS-CRM) and improved factor IX variant for hemophilia gene therapy

Authors: Luna Batota, Nisha Nair, Hanneke Evens, Shilpita Sarcar, Sumitava Dastidar, Thierry Vandendriessche, Marinee K. Chuah

Department of Gene Therapy and Regenerative Medicine (GTRM), Vrije Universiteit Brussel, Belgium

**Introduction:** Hemophilia A and B are X-linked genetic disorders caused by a monogenic mutation in the factor VIII (FVIII) and factor IX (FIX) gene, respectively. The disease is characterized by spontaneous intra-articular and soft tissue bleedings, which can result in crippling arthritis. Classic treatment for hemophilia is administering clotting factor concentrates that have disadvantages, namely repetitive administration and inhibitor development. Due to which, gene therapy is a promising alternative. Moreover, there are advantages like availability of many target organs, study models and that an increase of protein expression above 1% leads to therapeutic effect. In this study, we have improved the constructs for hemophilia gene therapy and we have achieved therapeutic effect in vivo.

**Material & methods:** Various cloning techniques were used to generate different variants of FIX and FVIII constructs. These constructs were then tested in vivo by hydrodynamic administration of the plasmid in C57/BL6 and FIX KO mice. The functionality of the constructs was assessed by testing mice plasma for expression of clotting factors by ELISA and FIX activity assay. The specificity of the construct was checked with an AAV vector system by bio-distribution analysis using RT-PCR. Statistical analysis was done using student t-test.

**Results:** We used two different approaches to improve the therapeutic construct. First, we developed a liver-specific cis-regulatory module (HS-CRM8); secondly we modified the clotting factor transgene itself.

The HS-CRM8 gave a 10-15 fold increase in FIX expression and 2-4 fold increase in FVIII expression. The liver-specificity of the HS-CRM8 was confirmed by using an AAV construct with GFP reporter gene. We initially codon-optimized the FIX gene followed by a substitution mutation of arginine at position 338 to leucine (R338L) to obtain a hyperfunctional FIX. Combination of codon optimization and R338L led to an overall 15 fold augmentation of FIX expression (3 fold by codon-optimization and 5 fold by R338L mutation).

**Discussion & conclusions:** The HS-CRM8 developed by us was liver-specific and improved the FIX and FVIII expression significantly. Furthermore, clotting factor expression and activity was augmented significantly due to the mutations introduced in the transgene. The in vivo study needs to be further validated using AAV vector system.



## In-vitro propagation of human spermatogonial stem cells

Authors: Aude Braye, Yoni Baert, Herman Tournaye, Ellen Goossens

Department of Embryology and Genetics - Biology of the Testis, Vrije Universiteit Brussel, Belgium

**Introduction:** Pre-pubertal boys undergoing chemo- or radiotherapy as part of a cancer treatment or diagnosed with Klinefelter syndrome are at risk of losing their reproductive stem cells, i.e. the spermatogonial stem cells (SSCs). Consequently, they are often confronted with fertility problems once they reach adulthood. Since sperm cryopreservation is impossible before puberty, an alternative fertility preservation strategy includes SSC cryopreservation followed by autotransplantation or in-vitro maturation with in-vitro proliferation of SSC as an important however missing link. Rodent SSCs have been shown to grow as clusters in-vitro. Even though long-term propagation of human SSCs has also been claimed, recent reports on the existence of other testicular multipotent cell types that share certain markers with SSCs indicate otherwise. In addition, a marmoset study showed the tendency of testicular somatic cells to overgrow the culture, which adversely affects SSC maintenance in-vitro. The aim of this study is to re-evaluate whether long-term in-vitro propagation of human SSCs is feasible.

**Material & methods:** Normal adult testicular tissue was obtained from vasectomy reversal patients and prostate cancer patients undergoing an orchidectomy or pulpectomy. Testicular cells were isolated and cultured as described by *Sadri-Ardekani et al.* Cellular composition of the culture will be monitored at passage 0, 1 month and 2 months in culture with qRT-PCR and immunofluorescence. To unambiguously determine SSC presence and proliferation during culture, co-expression of VASA (spermatogenic marker) and UCHL1 (spermatogonial marker) will be studied by immunofluorescence after culturing FACS-sorted SSCs on mitotically inactivated feeder cells.

**Results:** Cell cultures were established of fresh (n=3) and cryopreserved (n=3) testicular fragments of which material is currently being collected for analyses. After 7 and 14 days of culturing sorted SSCs on non-proliferating feeders, only sporadic single VASA/UCHL1-positive cells were found. Remarkably, cluster formation did occur but were devoid of these cells.

**Discussion & conclusions:** First results contradict the maintenance of SSCs by the method of *Sadri-Ardekani et al.* but further confirmation is required. In addition, the composition of cell types that do occur will be studied.

## Targeting Raf-1 in NSCLC

Authors: Ulrike De Ridder, Amir Noeparast, Jacques De Grève, Erik Teugels

Laboratory for Medical and Molecular Oncology (LMMO), Vrije Universiteit Brussel, Belgium

**Introduction:** Non small cell lung cancer (NSCLC) is the most common type of lung cancer. Development of new targeted therapies to improve clinical outcome and long time survival rate of NSCLC is highly required. Thanks to genomic analysis of biopsy specimen, new found genetic alterations lead to the discovery of new targeted therapeutic strategies. For controlling cell proliferation and differentiation the mitogen activated protein kinase (MAPK) or RAF/MEK/ERK pathway is significant. Aberrant regulation due to over expression or activating mutations of receptor tyrosine kinase (RTK) or mutations of the Ras and Raf proteins kinase are associated to cancer. MAPK signaling in normal cells, can be triggered by ligand binding (for example EGFR) to the extracellular domain of RTK. Consequently Ras will catalyze its exchange into the active Ras-GTP form. B-Raf is one of the extensively studied effectors of Ras. It is a member of the mammalian Raf proteins (or MAP3K proteins), these serine/threonine kinases can be distinguished into 3 types; A-Raf, B-Raf and Raf-1 (or C-Raf) .

In a study of Karreth et al. it has been shown that depletion of C-Raf, in KRAS mutated NSCLC, significantly reduced tumor formation in mice. Another article by Blasco et al. is consistent with these results and proves that transformed cells, expressing oncogenic KRAS, signal through C-Raf exclusively; independent of B-Raf. Their data also demonstrate that concomitant depletion of C-Raf together with downstream signalling effectors MEK or ERK leads to tumor reduction. These findings aimed to target the MAPK signaling pathway and to investigate the role of C-Raf inhibitors on KRAS-mutated cell lines in vitro.

**Material & methods:** To address whether the selective inhibition of C-Raf kinase activity in KRAS-mutated lung cancer can lead to growth inhibition, we used a competitive C-Raf 1 kinase inhibitor and a novel selective type II inhibitor of C-Raf on three different KRAS-mutated cell lines derived from human lung, namely A427, A549 and H358. The C-Raf inhibitors are tested alone and in combination with Trametinib, a MEK-inhibitor, already in phase II clinical trial. To determine the effect of the drug in cell culture we performed a cell proliferation assay using the CyQUANT kit (Invitrogen). Protein expressing is assessed by western blot analysis.

**Results:** The first results show us that the type II inhibitor on its own already has an effect on the A427 cell line.

**Discussion & conclusions:** If the following data support this trend we should ask ourselves the question why oncogenic KRAS prefers to signal through C-Raf. Although C-Raf might be a promising target for novel therapeutic approaches there is still much to learn about the MAPK signaling pathway.

## A mouse model of depression and anxiety can be induced by chronic corticosterone injections

Authors: Lauren Deneyer, Thomas Demuyser, An De Prins, Giulia Albertini, Eduard Bentea, Joeri Van Liefferinge, Ellen Merckx, Ann Massie, Ilse Smolders

Center for Neurosciences, Vrije Universiteit Brussel, Belgium

**Introduction:** Depression and anxiety are among the most common psychiatric disorders, leading to diminished everyday functioning and inferior quality of life. Stress is a causative factor in the pathogenesis of this debilitating illness, since it is involved in the overactivation of the hypothalamic-pituitary-adrenal (HPA) axis, with consequent elevated levels of plasma cortisol. In this study, we will determine whether administration of chronic exogenous corticosterone (CORT) may lead to anxiety- and depressive-like behavior in mice. Via administration of CORT either through chronic subcutaneous (s.c.) injections or slow-release pellets, we will create an animal model for depression and anxiety. This animal model will then be used in further pharmacological research towards novel future antidepressant treatments.

**Material & methods:** Male C57/BL6 mice were randomly assigned to 2 treatment groups and their respective control groups. The first group was injected s.c. with CORT (20 mg/kg) or vehicle for 21 days between 9 and 11 a.m. The second group was briefly anaesthetized prior to the implantation of slow-release CORT pellets (18 mg/kg/day) or placebo pellets. To verify the anxiety- and depressive-like state, both experimental groups were subjected to a battery of behavioral tests. Anxious behavior was measured using the light dark paradigm, elevated plus maze and open field test. The depressive-like state was analyzed by measuring the duration of immobility in the mouse-tail suspension and forced swim test. The novelty suppressed feeding test allowed us to determine both anxiety- and depressive-like behavior. Finally, the saccharine intake test revealed the level of anhedonia. Furthermore, blood was collected after the 21-day treatment period and after the behavioral tests in week 5. An ELISA-assay was performed on all blood samples to determine the CORT levels.

**Results:** The results of our behavioral tests show that repeated CORT injections significantly increase anxiety. A strong trend towards increased depressive-like behavior was observed as well. In contrast, slow-release pellets were not able to provide the same results as no clear difference was observed between the control and treated group. Moreover, repeated CORT injections significantly increased basal serum CORT levels after 21 days in contrast to the control group and pellet-treated animals.

**Discussion & conclusions:** Our results demonstrate that exogenous administration of CORT produces a reliable mouse model for anxiety and depression, only when administered through injections and not through pellets. It also highlights the importance of chronic high levels of CORT and its contribution to the etiology of depression.

## The use of mRNA-lipoplexes for transfection of dendritic cells. The road to efficient tumor antigen loading and maturation of dendritic cells

Authors: Philippe Giron<sup>a,b,c</sup>, Heleen Dewitte<sup>b</sup>, Karine Breckpot<sup>c</sup>, Ine Lentacker<sup>b</sup>

<sup>a</sup> Master student Biomedical sciences, Faculty of Medical and Pharmaceutical Sciences, Vrije Universiteit Brussel, Belgium

<sup>b</sup> Laboratory of General Biochemistry and Physical Pharmacy, Ghent Research Group on Nanomedicine, Faculty of Pharmaceutical Sciences, Ghent University, Belgium

<sup>c</sup> Laboratory of Molecular and Cellular Therapy, Department of Physiology-Immunology, Faculty of Medical and Pharmaceutical Sciences, Vrije Universiteit Brussel, Belgium

**Introduction:** Dendritic Cell (DC) vaccines are a potent and emerging immunological approach for the treatment of cancer. They aim to induce an active cellular immune response targeted against specific tumor antigens. For a DC vaccine to be effective, 2 major requirements should be met: (i) DCs must be loaded with antigens, and (ii) antigens must be presented by fully mature DCs (mDCs).

The vaccine should induce presentation of tumor antigens on the surface of mDCs. An effective way of loading DCs with antigens is through transfection with mRNA encoding tumor antigens. To meet the second requirement, maturation signals are delivered to these DCs. The maturation signals induce the transition of immature DCs (iDC) to mDCs. These mDCs have the capacity of propagating cellular immune responses, whereas iDCs lack this capacity.

**Material & methods:** DC vaccines are classically generated *ex vivo*. These vaccines are patient-specific, costly, time consuming, and limit the up scaling of this immunotherapy. *In vivo* DC vaccines can overcome these major limitations. To address the need for *in vivo* applicable DC vaccines, we aim to develop mRNA-lipoplexes that can (i) transfect DCs with tumor antigen mRNA and (ii) simultaneously deliver maturation stimuli. mRNA-lipoplexes are complexes of negatively charged mRNA to cationic liposomes, resulting in the formation of stable nanoparticles. They are formed spontaneously when mRNA is mixed with cationic liposomes. Liposomes were modified to induce maturation by incorporating the adjuvant lipid 'monophosphoryl lipid A' (MPLA). The capacity of the mRNA-lipoplexes to transfect and/or to mature DCs was evaluated on primary murine iDC cultures. To evaluate the (i) antigen loading capacity, transfection efficiencies were evaluated based on transfection efficiency of Green Fluorescent Protein (GFP). (ii) The maturation capacity of the lipoplexes was determined based on the expression of maturation surface markers (e.g. CD40).

**Results:** Basic GFP-mRNA-lipoplexes show transfection efficiencies up to 50% and induce a shift in mDCs from 7% to 17%. By including MPLA into the lipoplexes, similar transfection efficiencies are obtained, however they induce a strong shift towards mDCs, up to 88%.

**Discussion & conclusions:** In conclusion, we demonstrate a superior maturation capacity of MPLA modified liposomes over standard liposomes.

## **In vivo administration of mRNA as a novel vaccination approach for the treatment of HIV-1**

Authors: Ahmet Krasniqi, Patrick Tjok Joe, Ioanna Christopoulou, Alex Olvera van der Stoep, Beatriz Mothe, Christian Brander, Kris Thielemans, Xavier Saelens, Joeri L. Aerts

Laboratory for Molecular and Cellular Therapy (LMCT), Vrije Universiteit Brussel, Belgium

**Introduction:** Therapeutic vaccination with ex vivo modified autologous dendritic cells (DCs) has been proposed as an alternative for antiretroviral therapy, since it enhances HIV-1-specific immune responses, resulting in the temporary control of the viral load without cART. In vivo modification of DCs via the direct administration of messenger RNA (mRNA) encoding for target antigens has been shown to retain the potency of a DC vaccine while bypassing the complexity and cost of ex vivo modification. Therefore, in this project we evaluated the efficacy of an intranodally (IN) delivered therapeutic HIV-1 vaccine comprised of mRNA encoding rationally HIV-1 antigens (HIVACAT) and immunostimulatory molecules (TriMix).

**Material & methods:** To determine the immunogenicity of the vaccine, C57BL/6 mice received three IN immunizations with mRNA encoding for TriMix and HIVACAT. Two weeks after the last immunization, splenocytes were isolated for ELISPOT. We also performed an in vivo cytotoxicity assay to determine the lytic capacity of the induced cytotoxic T cells. Thereafter, we evaluated whether IN mRNA immunization can control a natural influenza infection in mice. For this purpose, BALB/C mice received three IN immunizations with mRNA encoding for TriMix and influenza nuclear protein 1 (Flu-NP1). Following the challenge with influenza, mice were either followed up for weight evolution or used for further immune analysis.

**Results:** Immune responses against the HIVACAT immunogen were detected in all immunized mice, with an increase in vivo cytotoxicity after the third immunization. IN mRNA immunization was shown to control the influenza infection.

**Discussion & conclusions:** In the future, we will also evaluate IN mRNA immunization both in a prophylactic and in a therapeutic setting in a chronic model of Lymphocytic Choriomeningitis Virus (LCMV), as the immune responses against chronic LCMV closely resemble those occurring during an HIV-1 infection. Finally, we will test our vaccination approach in a humanized mouse model, where the human T cells are selected on a human thymus.

However, our results demonstrate the feasibility of an mRNA-based vaccine to enhance HIV-1-specific immune responses. Furthermore, we also showed that our vaccination strategy is capable of controlling an influenza infection thereby establishing a proof of concept for our HIV-1 vaccine.

## The role of microRNAs in hepatic stellate cell activation and liver fibrosis

Authors: Joeri Lambrecht<sup>1</sup>, Inge Mannaerts<sup>1</sup>, Mar Coll<sup>2</sup>, Adil El Taghdouini<sup>1</sup>, Pau Sancho-Bru<sup>2</sup>, Leo. A. van Grunsven<sup>1</sup>

<sup>1</sup> Laboratory of Liver Cell Biology, Vrije Universiteit Brussel, Belgium - <sup>2</sup> Liver Unit, Hospital Clínic, Institut d'Investigacions Biomèdiques August Pi i Sunyer, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas, Barcelona, Spain

**Introduction:** Liver fibrosis is a pathology, resulting from sustained scar formation in response to chronic injury. The main player in this disease is found to be the Hepatic Stellate Cells (HSCs), which undergo a process called HSC activation in the presence of hepatocyte damage and inflammation. HSC activation results in a myofibroblastic phenotype, characterized by alpha-SMA and collagen expression, excessive deposition of fibrillar matrix and cell proliferation. Recent studies showed that microRNAs (miRNAs) are involved in HSC activation. MiRNAs can regulate the expression of complementary mRNA sequences by their degradation or by inhibition of their translation into protein.

In this study we aim to identify miRNAs that could be key regulators of HSC activation and could be used as targets for anti-fibrotic therapy or that can serve as HSC markers in the diagnosis of liver injury.

**Material & methods:** We previously integrated HSC gene- and miRNA profiling of human HSCs: quiescent HSCs from 4 human donor livers were isolated by FACS and compared to culture-activated human HSCs using Affymetrix HG-U219 and miRNA-Taqman® Array. MiRNA and gene expression profiles were integrated based on miRNA-mRNA interaction. In this study, the expression of differentially regulated miRNAs was confirmed in in vitro and in vivo activated primary mouse HSCs. For the induction of liver fibrosis and in vivo HSC activation we used two BalbC mice models, carbon tetrachloride intoxication and bile duct ligation. Gain and loss of function studies for the miRNAs were followed by an EdU proliferation assay and qPCR for HSC activation markers.

**Results:** Two miRNAs that came out of this analysis are miR-21 and miR-192. MiR-21 is up-regulated upon HSC activation (in vitro and in vivo) and inhibition of its expression using a miR-21 inhibitor sequence reduced the expression of alpha-SMA and col1a1. In addition, the expression of miR-21 target genes Btg2, PTEN, and Spry1 was enhanced by miR-21 inhibition. MiR-192 is down-regulated during HSC activation and overexpression of this miRNA, although not affecting the mRNA levels of alpha-SMA and col1a1, strongly reduced HSC proliferation. MiR-21 nor miR-192 were clearly HSC specific.

**Discussion & conclusions:** In conclusion, both in vitro and in vivo activated mHSCs display a significant change in the expression patterns of miR-21 and miR-192, confirming our observations in human. Functional analysis shows that both microRNAs affect different aspects of HSC activation supporting their potential as therapeutic targets.

## The anaphase promoting complex as a possible new target in multiple myeloma

Authors: Anke Maes, Susanne Lub, Ken Maes , Eline Menu , Elke De Bruyne, Karin Vanderkerken, Els Van Valckenborgh

Department of Hematology and Immunology, Myeloma Centre Brussels, Vrije Universiteit Brussel, Belgium

**Introduction:** Multiple myeloma (MM) is a plasma cell disorder, characterized by an accumulation of malignant plasma cells in the bone marrow. Despite the discovery of novel drugs, MM is still an incurable disease. The anaphase-promoting complex (APC) is an E3 ubiquitin ligase and contributes to the cell cycle by ubiquitylation of cell cycle proteins such as securin and cyclin B and initiating anaphase. Genetic changes affecting the APC and its regulator, the spindle assembly checkpoint are described in MM patients and are associated with chromosomal instability and aneuploidy. The purpose of this study is to examine the ubiquitin ligase APC as a possible new target in MM.

**Material & methods:** The APC inhibitor proTAME (pT) was tested on different cell types such as human MM cell lines (HMCL) LP-1 and RPMI, MM patient cells, stromal cells and endothelial cells. Cells in metaphase were morphologically counted on May-Giemsa Grunwald stained cytopspins using a light microscope. Viability and apoptosis was determined by respectively the CellTiterGlo assay (Promega) and Annexin V/7AAD flow cytometry staining. Expression of apoptotic and cell cycle proteins was determined by western blot. Statistical analysis was performed by the Mann-Whitney t-test.

**Results:** HMCL were treated with pT and the effect on mitosis was analysed by morphology. Treatment of HMCL with pT induces a clear metaphase arrest and this was accompanied with an accumulation of cyclin B which is consistent with inhibition of the APC. A prolonged metaphase can lead to cell death; therefore we measured viability and apoptosis. We observed a significant dose-dependent decrease in viability and increase in apoptosis when the HMCL were treated with pT. Similar effects were observed on MM cells purified from patient samples. In contrast, no effect on viability could be observed on other cell types from the bone marrow micro-environment such as stromal cells and endothelial cells. We further analysed the apoptosis mechanisms by western blot and could clearly detect an increased cleavage of caspase 3, 8, 9 and PARP in MM cells treated with pT.

**Discussion & conclusions:** From these results we can conclude that pT induces a prolonged metaphase in HMCL which results in a reduced viability and an induction of apoptosis. These findings suggest that the APC complex may be a promising target in MM.



## **Myeloid cells in colorectal cancer: a possible link between immunosuppression and radioprotection through arginase-1 overexpression**

Authors: Julia Katharina Schwarze, Wim Leonard, Inès Dufait, Heng Jiang, Ka Lun Law, Mark De Ridder

Department of Radiotherapy , UZ-Brussel - Vrije Universiteit Brussel, Belgium

**Introduction:** Myeloid cells can polarise towards a pro-tumoral or an anti-tumoral phenotype, marked by the alternative activation of Arginase-1 (Arg-1) and inducible Nitric oxide synthase (iNOS), respectively. This study explored the ARG signature of myeloid cells in colorectal cancer (CRC) patients and in a mouse colon tumor and its possible role in radioprotection of hypoxic tumor cells.

**Material & methods:** (a) Blood of CRC patients and healthy donors was analysed by flow cytometry (FCM). Clinical data available in the EMD for patients participating in phase II trials was analyzed. (b) MDSC from spleen or tumor from CT26 tumor-bearing mice were examined for their suppressive activity, Arg-1 expression, and their ability to deplete L-arginine (L-arg). 0,04mM L-arg culture medium was depleted at different cell densities of MDSC and was used to culture RAW-macrophages for 48hrs. Induction of iNOS production by RAW was achieved by IFN-gamma/LPS administration and NO production was determined.

**Results:** (a) In CRC patients the levels of Arg-1 over-expressing neutrophils and Lin-HLA-DR-CD15<sup>+</sup>CD16<sup>-</sup> MDSC were significantly increased (3- to 5-fold). An increased NLR (>7.0) predicts a poor prognosis, which suggests a N2 polarisation of neutrophils. (b) The growth of CT26 tumor was linked to a progressive accumulation of MDSC that displayed clear immunosuppressive effects on T cells. Within CT26 tumors, 44% of myeloid cells were ARG<sup>+</sup>, contrasting to only 2% in the spleen. However, the latter myeloid subset could acquire the ARG<sup>+</sup> phenotype following hypoxic conditioning, and thereby deplete L-arg. On the other hand, splenocytes from tumor-free mice did not show ARG activation or L-arg depletion. In the TMCS, M1 macrophages caused drastic (up to 2.2-fold) radiosensitisation of CRC cells through NO-induced oxygen sparing, while M2 macrophages contributed to hypoxic radioprotection. Thus, the shift in L-arg turnover from the iNOS towards ARG pathway abrogates the radiosensitising potential of M1 macrophages and sustains hypoxic radioresistance.

**Discussion & conclusions:** CRC in both the clinical and preclinical settings is associated with the progressive expansion of ARG<sup>+</sup> myeloid cells including MDSC. This subset indicates a protumor polarisation of the immune system, and may provide a mechanistic link between immunosuppression and radioprotection through L-arg depletion.



## Isolation and characterization of vital HIV from semen

Authors: Jonas Van Audenaerde, Katleen Vereecken, Johan Michiels, Leo Heyndrickx, Guido Vanham, Kevin Ariën

Virology Unit, Institute of Tropical Medicine, Antwerp, Belgium

**Introduction:** Till today, HIV remains one of the most serious infectious diseases with over 34 million people infected around the world. Semen is the most important carrier for transmission of HIV, both in homosexual and heterosexual transmission. Semen contains several substances like amines, nutrients and enzymes that protect HIV and could even increase viral infectivity. Despite decades of research, HIV transmission mechanisms are not completely understood yet because there is no consensus of the source for transmission, i.e. cell free virus (CFV) or cell-associated virus (CAV). Therefore, we want to isolate infectious seminal HIV, determine its source in semen and characterize the virus from this compartment phenotypically.

**Material & methods:** Several cell lines (CEM-SS, Jurkat, Sup-T1, R5MaRBLE and Ghost) were tested for sensitivity for virus pickup with both CFV and CAV HIV. After selection, the chosen cell lines were investigated for their preference for R5-tropic versus X4-tropic viruses. Semen samples, received from untreated HIV patients consulting the AIDS Reference Center of the Institute of Tropical Medicine in Antwerp, were fractionized in seminal plasma (SP) which contains only CFV and non-sperm cells (NSC) for CAV by centrifugation over a Nycodenz gradient. SP HIV was further isolated using the  $\mu$ MACSTM VitalVirus HIV Isolation kit. Both separated fractions were co-cultured with Ghost and R5MaRBLE cells. Infection after 7 days incubation is assessed by p24 ELISA and Luciferase activity, respectively. Blood from the same patients was also collected to isolate HIV for comparison with the seminal viruses.

**Results:** R5MaRBLE and Ghost showed to be the most sensitive cell lines for HIV isolation, both for the SP and the NSC fraction. Infectious HIV was isolated from two out of 19 semen samples. This relative low success rate is related to the low VL in semen of the patients tested. The isolated viruses are being characterized for viral fitness in comparison with HIV from the blood of these patients.

**Discussion & conclusions:** We have developed a new method to isolate infectious HIV from semen. We are currently characterizing the two seminal viruses in terms of nucleotide sequence and replication capacity in PBMC and cervical explant tissue.

## **The role of the insulin-like growth factor (IGF)-I receptor in IGF-I and estradiol mediated neuroprotection using a rat model for focal cerebral ischemia**

Authors: Raya Vanheuverzwyn, Wendy Stoop, Julie Verdood, Ron Kooijman

Experimental Pharmacology, Center for Neurosciences, Vrije Universiteit Brussel, Belgium

**Introduction:** Cerebrovascular accidents (CVA) are the leading cause of chronic acquired disability in adults and the third leading cause of death, after coronary heart diseases and cancer. Each year approximately 650.000 individuals suffer from a CVA of which 87% are ischemic strokes. Nowadays, reperfusion with tissue plasminogen activator is the only FDA approved acute treatment for ischemia. Due to the limited therapeutic time window and the severe inclusion criteria, this treatment is only accessible for the minority of patients. Recent data from experimental studies highlighted that female sex hormones, in particular 17 $\beta$ -estradiol (E2), and IGF-I are potential neuroprotective agents in neurodegenerative disorders. These neuroprotective effects, in response of neural tissue damage, depend on the interaction between IGF-I receptors and E2 receptors suggesting a parallel- or co-activation of IGF-I receptors and E2 receptors. In this study, we examined the role of the IGF-I receptors in the central nervous system in IGF-I- and E2-mediated neuroprotection using a rat model for focal cerebral ischemia.

**Material & methods:** Induction of focal cerebral ischemia in male albino Wistar Kyoto rats (WKY) was performed by intracerebral administration of endothelin-1 (Et-1, 200 pmol) in the vicinity of the middle cerebral artery. Intracerebroventricular (ICV) administration of JB-1, an IGF-I receptor antagonist, or saline was started 2h prior to stroke induction. Thirty minutes after stroke induction, IGF-I/placebo control and E2/placebo control were applied subcutaneously. All animals were sacrificed 24h after the insult. Neurological evaluation was performed and scored 24h before and after stroke induction by the neurological deficit score. Assessment of the infarct size and the activated microglia was performed respectively using cresyl violet staining and immunohistochemistry.

**Results:** Optimization of the protocol where saline was administered ICV in an Et-1 rat model for focal brain ischemia showed significantly larger infarct volumes in the untreated placebo group when compared to the IGF-I treated group.

**Discussion & conclusions:** IGF-I reduces the infarct volume and appeared to be neuroprotective in an Et-1 rat model for focal brain ischemia when administered 30 minutes after stroke induction. Future experiments using JB-1 will address the role of the IGF-I receptor in IGF-I and E2 mediated neuroprotection.

## Notities

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