Belgian association for cancer research

ANNUAL MEETING

FRIDAY 7TH OF FEBRUARI 2020

Cancer metastases: from bedside to bench

ABSTRACT BOOK

Program Annual Meeting
Friday 7th of February

"Cancer metastases: from bedside to bench"

Venue: Vrije Universiteit Brussel, Medical Campus
Auditorium Brouwer, Forum & Atrium
Laarbeeklaan 103, 1090 Brussel

9.00-9.30 Registration & coffee - Auditorium Brouwer

9.30-12.00 MORNING SESSION - AUDITORIUM BROUWERS

CHAIRS: PROF. M. DE RIDDER & DR. I. DUFAIT

9.30 Introduction: management of oligometastases.
Prof. dr. Mark De Ridder, Radiotherapy department, UZ Brussel

9.45 **KEY LECTURE**: Combination of radiotherapy and immunotherapy
Prof. dr. Eric Deutsch, Gustave Roussy, Paris, France

10.15 Cancer cells take away food for their metastatic journey
Prof. dr. Olivier Feron, IREC, UCLouvain

10.45 Coffee break & poster viewing - Forum/Atrium

11.15 Young investigators: oral presentations of 4 selected abstracts

Specific targeting of type I interferon to the tumor microenvironment or to dendritic cells as a novel, generic, safe cancer immunotherapy
Anje Cauwels (UGent)

AXL: A potential therapeutic target to improve chemosensitivity in Multiple Myeloma
Niels Vandewalle (VUB)

CDK4 phosphorylation status and rational use of CDK4/6 inhibitors in advanced thyroid cancers
Jaime Pita (ULB)

Repurposing of the drug Auranofin for the treatment of p53 mutant non-small cell lung cancer: many ways to die
Laurie Freire Boullosa (UA)

12.00 BACR: general assembly (members only)

12.00-14.00 Lunch & poster session - Forum/Atrium

BELGIAN ASSOCIATION FOR CANCER RESEARCH

Program Annual Meeting
Friday 7th of February

"Cancer metastases: from bedside to bench"

14.00-16.30 AFTERNOON SESSION - AUDITORIUM BROUWER

CHAIR: PROF. A. BELLAHCÈNE

14.00 Young investigators: oral presentations of 4 selected abstracts

A phase I clinical trial on intratumoral administration of autologous CD1c (BDCA-1)+ myeloid dendritic cells plus talimogene laherparepvec (T-VEC) in patients with advanced melanoma
Julia Katharina Schwarze (VUB)

Palmitate: a driver of metastasis formation
Patricia Altea Manzano (KUL)

Zeb2 drives invasive and microbiota-dependent colon carcinoma
Ioanna Petta (UGent)

To induce tumor metabolic addiction and to harness it for synthetic lethality
Catherine Vander Linden (UCL)

15.00 Coffee break & poster viewing - Forum/Atrium

15.30 Unlocking the power of the methylome: a novel generation of biomarkers in cancer?
Dr. Ken Op de Beeck, Oncology department, Universiteit Antwerpen

16.00 Paracrine interactions in the tumor environment: consequences for metastasis and therapy response.
Prof. dr. Olivier De Wever, CRIG, University Ghent

16.30 Concluding remarks & award ceremony

WIFI

Network: BACR2020
PW: BACR2020@VUBJette
Twitter: #bacr2020

Abstract book online!

VRIJE UNIVERSITEIT BRUSSEL

Universitair Ziekenhuis Brussel

EACR European Association for Cancer Research

Program Annual Meeting
Friday 7th of February

"Cancer metastases: from bedside to bench"

Venue

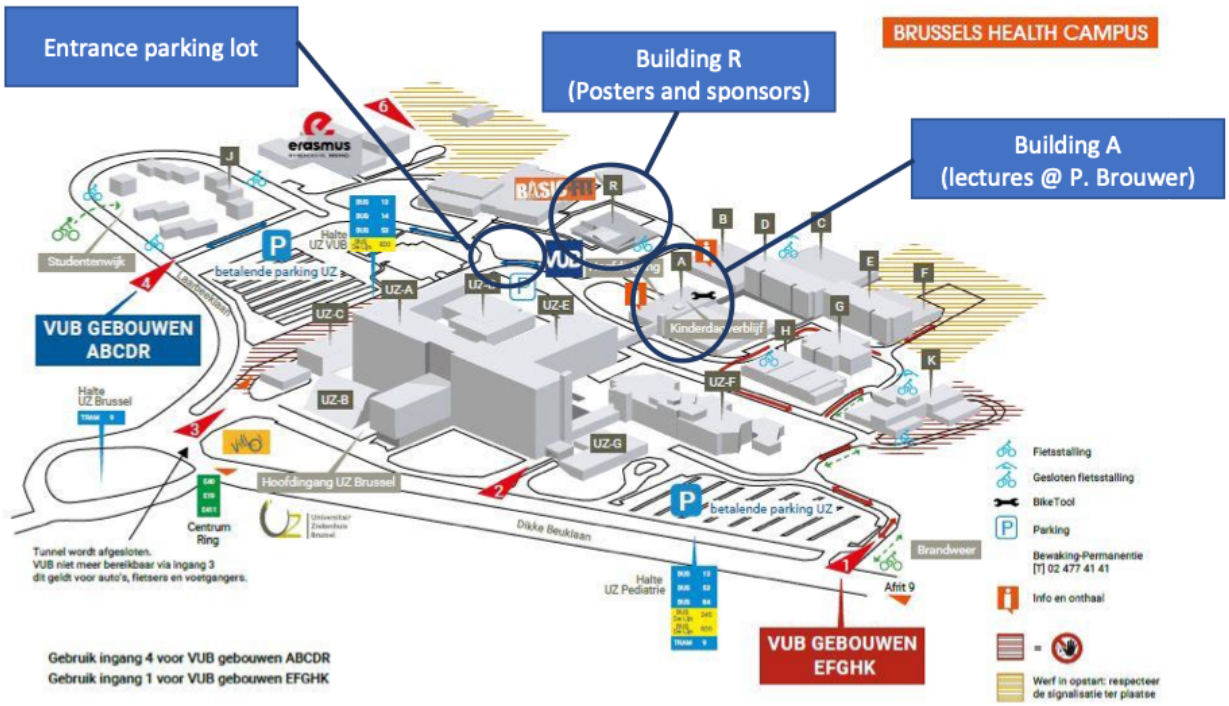
Location: Vrije Universiteit Brussel, Medical Campus

Lectures: Auditorium P. Brouwer (building A)
Breaks/lunch, poster walk and sponsor stands: Forum & Atrium (building R)

Adress: Laarbeeklaan 103, 1090 Brussel

Parking: Parking will be available at the Vrije Universiteit Brussel, Campus Jette Signs will guide you to the conference building.

Ground plan Medical Campus VUB



Organizing committee

The yearly BACR Annual Meeting is organized by the BACR board members on a rotational schedule. The 2020 edition of the BACR Annual Meeting is organized by the team of Prof. De Ridder of the Vrije Universiteit Brussel.

Members:

Prof. Mark De Ridder

Dr. Inès Dufait

Dr. Kathleen Leemans

Sven de Mey

Christelle Vandenhaute

Scientifique committee

The scientifique committee consists of all BACR board members:

Prof. Dr. Akeila Bellahcène

Prof. Dr. Tom Boterberg

Prof. Dr. Dirk Van Gestel

Prof. Dr. Geert Berx

Prof. Dr. Olivier Feron

Prof. Dr. Diether Lambrechts

Prof. Dr. Sandra Nuyts

Prof. Dr. Benjamin Beck

Prof. Dr. Filip Lardon

Prof. Dr. Pierre Lovinfosse

Prof. Dr. Philippe Martinive

Prof. Dr. Jan Vermorken

Prof. Dr. Jo Van Ginderachter

Prof. Dr. Mark De Ridder

Invited speakers

Mark De Ridder

Mark De Ridder graduated at the medical school of the Vrije Universiteit Brussel with maxima cum lauda in 1998. He combined his training in Radiotherapy and Oncology with a doctoral fellowship and defended his PhD "Hypoxic tumor cell radiosensitization through nitric oxide synthase: role of the NF-kB signalling pathway" in 2005. He was nominated professor of Oncology and Cancer Biology at the VUB in 2008 and head of the Radiotherapy department of the UZ Brussel in 2010. Mark De Ridder is the coordinator of the Spearhead Strategic Research Program "Societal Benefit of Markerless Stereotactic Body Radiotherapy: a Statistical Support based on Quantitative Imaging".

Eric Deutsch

Eric Deutsch, MD, PhD, full-Professor in Radiation Oncology at South-Paris University, head of the Inserm Unit 1030 « Molecular Radiology Laboratory » and Head of the Radiation Oncology Department in Villejuif, France. He is teaching radiobiology and medical physics at the Medical School of South-Paris University. Eric Deutsch's research has been dedicated to combination of novel anticancer drugs either used alone or in combination to radiotherapy. His interest focuses on cell death mechanisms, HPV related tumours and the tumourstroma interplay, on the clinical side, he is versed into the field of translational research and early clinical trials. He has investigated several first in human novel drugs radiotherapy combinations such as mTOR inhibitors, antiviral agents, immune modifiers and nanoparticles.

Olivier Feron

Olivier Feron is Professor of Translational Medicine at UCLouvain and hon. Research Director of the National Fund for Scientific Research (FNRS). He received his PhD in Molecular Pharmacology at the University of Louvain in 1995 and then trained from 1996 to 1998 as a post-doctoral fellow and Instructor in Medicine at Harvard Medical School (Brigham and Women's Hospital, Boston, USA). Back in Belgium, he developed his own group, progressively shifting his focus from the cardiovascular area to oncology. Today, he is the head of the Cancer Translational Research Laboratory within the Pole of Pharmacology and Therapeutics at the Institut de Recherche Expérimentale et Clinique (IREC, UCLouvain).

Ken Op de Beeck

Ken Op de Beeck is a post Doctoral Researcher at Center for Oncological Research at the University of Antwerp. He received his PhD "Elucidation of the role of the DFNA5 gene in the pathophysiology of hering impairment and cancer" in Biomedical Sciences at the University of Antwerp in 2011.

Olivier De Wever

Olivier De Wever is currently a Senior Researcher and an Associate Professor at the Laboratory of Experimental Cancer Research at the University Hospital of Ghent. He graduated in Pharmacy (great distinction) by the University of Ghent in 1999 and developed his PhD research in the Laboratory of Experimental Cancer Research defining "A road map for cancer invasion: implication of myofibroblasts and other factors", under the supervision of Marc Mareel and Marc Bracke (2004). He performed his Post Doctoral Research in the framework of the European project METABRE and supported by Ghent University Research Council and by Flanders Fund for Scientific Research. His work is being dedicated to understand the role of the tumour microenvironment, namely fibroblasts/myofibroblasts and adipocytes on cancer cell invasion and metastasis, dissecting the associated-molecular mechanisms. Olivier De Wever's team is also expert on the role of extracellular vesicles on the cancer cells-microenvironment crosstalk.

Abstracts Overview

N°	Presenting author	Type	N°	Presenting author	Type
1	Tess Van Meerhaeghe	Poster	38	Victor Delprat	Poster
2	Melissa Van De Gucht	Poster	39	Jaime Pita	Oral
3	Elyne Backx	Poster	40	Gitta Boons	Poster
4	Mohammad Rahimi Gorji	Poster	41	Kirsten De Ridder	Poster
5	Coline Wery	Poster	42	Marie Ebroin	Poster
6	Sven de Mey	Poster	43	Sandrina Martens	Poster
7	Sofie Meulewaeter	Poster	44	Eva Blondeel	Poster
8	Zohra Benyahia	Poster	45	Hasan Baysal	Poster
9	Luca Zampieri	Poster	46	Laurie Freire Boullosa	Oral
10	Tim Bomberna	Poster	47	Jinthe Van Loenhout	Poster
11	Charlotte Pilard	Poster	48	Tal Flieswasser	Poster
12	Marieke Bamps	Poster	49	Giel Tanghe	Poster
13	Kim Van de Loock	Poster	50	Kim Van der Eecken	Poster
14	Anje Cauwels	Oral	51	Yannick Verhoeven	Poster
15	Carmen Terren	Poster	52	Christophe Deben	Poster
16	Tatiana Revenco	Poster	53	Anthony Genna	Poster
17	Michiel De Coster	Poster	54	Julia Katharina Schwarze	Oral
18	Catharina Muylaert	Poster	55	Dionysia Dimitrakopoulou	Poster
19	Hannah Zaryouh	Poster	56	Ali Jalili	Poster
20	Léopold Thabault	Poster	57	Sam Donche	Poster
21	Panagiotis karras	Poster	58	Patricia Altea Manzano	Oral
22	Panagiotis karras	Poster	59	Hui Wang	Poster
23	Elien De Thaye	Poster	60	Laura Soumoy	Poster
24	Laura Vanderwaeren	Poster	61	Ioanna Petta	Oral
25	Nona Laura Lisa Merckx	Poster	62	Ahmad Najem	Poster
26	Jolien De Munck	Poster	63	Wout de Mey	Poster
27	Marine Blackman	Poster	64	Malak Sabbah	Poster
28	Niels Vandewalle	Oral	65	Tiamiou Assia	Poster
29	Lien Lippens	Poster	66	Gilles Rademaker	Poster
30	Laetitia Gheysen	Poster	67	Tom Wissocq	Poster
31	Mary Glorieux	Poster	68	Sandy Anania	Poster
32	Ines De Pauw	Poster	69	Esther Hoste	Poster
33	Hanne Verswyvel	Poster	70	Thomas Ertveldt	Poster
34	Sonia Furgiuele	Poster	71	Catherine Vander Linden	Oral
35	Abraham Lin	Poster	72	Emeline Dierge	Poster
36	Tânia Capelôa	Poster	73	Eva Reijmen	Poster
37	Lindsay A Broadfield	Poster	74	Philip Vlummens	Poster

Oral Abstracts

Abstract n° 14

Specific targeting of type I interferon to the tumor microenvironment or to dendritic cells as a novel, generic, safe cancer immunotherapy

A. Cauwels (1), S. Van Lint (1), A. Van Parys (1), F. Paul (2), G. Garcin (2), B. Vandekerckhove (3), N. Kley (4), G. Uzé (2) and J. Tavernier (1;4)

(1) VIB Medical Biotechnology Center & Gent University, Belgium
(2) CNRS UMR5235, University Montpellier, France
(3) Department of Clinical Chemistry, Microbiology and Immunology, UZ Gent, Belgium
(4) Orionis Biosciences, Gent, Belgium

Presenting author: Anje Cauwels – VIB-UGent Center for Medical Biotechnology, A. Baertsoenkaai 3, Gent - anje.cauwels@vib-ugent.be

Recently, immunotherapy has been set forth as the fourth pillar for cancer treatment, next to surgery, chemotherapy and radiotherapy. Immunotherapeutic therapies include checkpoint inhibition targeting antibodies, T lymphocyte and Dendritic Cell (DC) based cellular therapies, and cytokines. Type I IFN has been approved for treating various solid and blood tumors. However, best results are obtained with high doses and the numerous systemic toxic side effects of IFN are severely dose-limiting. To curtail cytokine toxicity, we developed AcTakines, Activated-by-Targeting Cytokines, mutated immunocytokines (with reduced receptor affinity) fused to cell-specific targeting moieties such as single domain antibodies, peptides or ligands.
As mouse AcTaferon (AFN = type I IFN based AcTakine), we use hIFN·2-Q124R, which breaches the cross-species barrier and is weakly active on mouse cells. In vivo treatment of CD20+ A20 lymphoma or B16-CD20+ melanoma tumors with CD20-targeted AFN reduced tumor growth similar to high dose WT mIFN immunocytokine. In sharp contrast to the latter, however, tumor-targeted AFN did not cause systemic toxicity (body weight, temperature, blood cell counts). The AFN antitumor effect depended on host cell IFNAR1, was lost in Batf3ko or CD8-depleted animals, and in mice lacking IFNAR1 on CD11c+ cells, indicating the critical involvement of conventional DC (cDC)(1). Furthermore, selective targeting of AFN to Clec9A+ XCR1+ cDC1 was sufficient to cause tumor stasis, without toxicity, in mouse melanoma and breast carcinoma, and using a human AcTaferon in a human lymphoma model in humanized mice(2). When CD20-AFN or Clec9A-AFN were combined with immunogenic chemotherapy, low-dose TNF, or immune checkpoint blockade such as anti-PDL1 and anti-CTLA4, complete tumor regression and long-lasting tumor immunity (memory) could be observed, still without adverse effects(1,2).
Collectively, our findings indicate that tumor- or DC-targeted AFNs provide novel, highly efficient and safe cancer immunotherapies, synergizing with existing therapies to completely eradicate tumors, and capable of converting immunologically "cold" into "hot" tumors. In case of DC-targeting, there is no need for tumor-specific markers or ex vivo cell manipulations, in contrast to other cellular therapies, and generic application is possible in a broad range of malignancies.

(1) Cauwels et al., OncoImmunology 2017
(2) Cauwels et al., Cancer Res 2018

Abstract n° 28

AXL: A potential therapeutic target to improve chemosensitivity in Multiple Myeloma

Niels Vandewalle (1), Philip Vlummens (1,2), Nathan De Beule (1), Sylvia Faict (1), Inge Oudaert (1), Ken Maes (1), Elke De Bruyne (1), Eline Menu (1), Karin Vanderkerken (1), Kim De Veirman (1)

(1) Department of Hematology and Immunology-Myeloma Center Brussels, Vrije Universiteit Brussel, Brussels, Belgium
(2) Department of Clinical Hematology, Ghent University Hospital, Gent, Belgium

Presenting author: Niels Vandewalle – Vrije Universiteit Brussel, Hematology and Immunology, Laarbeeklaan 103, 1090 Jette - Niels.vandewalle@vub.be

Introduction
The AXL receptor tyrosine kinase (AXL) has emerged as a promising therapeutic target for cancer therapy. Recent studies revealed a crucial role of AXL signaling in proliferation, survival and therapy resistance in different hematological cancers. In this study, we aimed to investigate the role of AXL in Multiple Myeloma (MM), focusing on cellular dormancy and chemoresistance.

Material & methods
The investigation of cellular dormancy was performed as described by Lawson, et. al (Nature Communication, 2015). The effects of R428 (BGB324|Bemcentinib, Sigma-Aldrich), a highly potent and AXL-specific small molecular inhibitor, on viability and induced apoptosis of MM cells was determined by Cell Titer Glo and AnnexinV/7AAD staining respectively. AXL expression in human myeloma cell lines (JJN3, U266 and LP-1) and murine 5TGM1 cells was analyzed by qRT-PCR. Patient cohorts (TT2/TT3/MMRF cohort) were used to correlate AXL expression and overall survival in newly diagnosed and relapsed MM patients.

Results
Using the in vivo 5TGM1 dormancy model, we demonstrated an increased expression of AXL (4x higher) in dormant MM cells compared to proliferating MM cells (n=3, $p<0,05$). Myeloma cell lines (JJN3, U266, LP1, 5TGM1) had a very low Axl expression, however, treatment with melphalan induced a significant upregulation of Axl in all cell lines, while bortezomib had no effect (n=3, $p<0.05$). The combination of melphalan and R428 significantly increased apoptosis of JJN3 (>10%), U266 (>20%) and LP-1 (>10%) cells compared to single agent therapy (n=6) ($p<0.01$). Using patient cohorts, we observed that AXL expression correlated with a good overall survival, indicating it's role in myeloma cell dormancy ($p=0.006$). Interestingly, AXL expression was increased in relapsed patients compared to newly diagnosed patients and correlation with overall survival disappeared.

Conclusion
We observed that AXL is highly expressed in dormant MM cells. Despite its association with a good prognosis in newly diagnosed MM patients, AXL serves as an interesting target to eradicate dormant myeloma cells as AXL inhibitors affect viability and induce apoptosis of myeloma cells, especially in combination with melphalan. Therefore, AXL can be considered as a new therapeutic strategy, to target residual cancer cells in MM patients.

Abstract n° 39

CDK4 phosphorylation status and rational use of CDK4/6 inhibitors in advanced thyroid cancers

Jaime Pita (1,2), Katia Coulonval (1,2), Sabine Paternot (1,2), Giuseppe Costante (3), Myriam Decaussin-Petrucci (4), John Copland (5), Jacques Dumont (1), Pierre Roger (1,2), Eric Raspé (1,2)

(1) Institut de Recherche Interdisciplinaire en Biologie Humaine et Moléculaire (IRIBHM), Université Libre de Bruxelles (ULB);
(2) ULB-Cancer Research Center (U-CRC);
(3) Institut Jules Bordet, ULB;
(4) Service d'anatomie-pathologique, Hospices Civils de Lyon; (5) Department of Cancer Biology, Mayo Clinic, Florida

Presenting author: Jaime Pita – Université Libre de Bruxelles, IRIBHM, 808 Route de Lennik, 1070 Anderlecht - jaimpita@ulb.ac.be

The cyclin-dependent kinases CDK4/6 are key regulators of the cell cycle entry, by phosphorylating the onco-suppressor retinoblastoma protein (pRb). Thus, CDK4/6 inhibitors (CDK4i) emerge as new drugs to treat various pRb-proficient chemotherapy-resistant cancers. Our group showed that presence of activating T172-phosphorylation of CDK4 in breast tumors correlates with their sensitivity to Palbociclib [1]. Thyroid follicular cell-derived tumors are subdivided into well-differentiated (WDTC), poorly differentiated (PDTC) and anaplastic thyroid carcinomas (ATC). Although rare (1 to 2%), ATC contribute up to 50% of the deaths attributable to thyroid cancer and most cases are resistant to targeted therapies. Molecular characterization of PDTC and ATC suggests that CDK4i could be considered for treating dedifferentiated thyroid cancers.
CDK4 posttranslational modifications were investigated using 2D-gel electrophoresis. Consistent with the quiescent state of the thyroid tissue, phosphorylated CDK4 could not be detected in non-malignant tissues (n=13). CDK4 phosphorylation was detected in all WDTC (n=15) and lymph node metastases (n=5), in PDTC (10/12) and in ATC (5/11). Sensitivity to three CDK4i was assessed (by BrDU and viability assays) in 12 ATC- and 9 WDTC-derived cell lines. All except 3 cell lines were sensitive to CDK4i with either full or partial inhibition of DNA synthesis. As drug responses were lower using viability assays, these compounds are probably mainly cytostatic. As in the breast cancer cell lines [1], detection of CDK4 T172-phosphorylation in thyroid cancer cell lines predicted their sensitivity to CDK4i. At the protein level, resistant cell lines were characterized by barely detectable pRb phosphorylation and highly expressed p16. In all sensitive cell lines, phosphorylated pRb was detected. Palbociclib combination with MEK and BRAF inhibitors was shown to be highly effective, being able to completely arrest clonogenic ability and prevent known resistance mechanisms, even in cells with partial response to Palbociclib.
The presence of the phosphorylated CDK4 (the actual CDK4i target) and the growth inhibition of all ATC cancer cell lines support CDK4i as a very promising option to treat or control, at least, some ATC, which presently are incurable and lead to patients death within few months.

1.Raspé, E. et al. (2017) EMBO Mol Med. 9,1052-1066.

Abstract n°46

Repurposing of the drug Auranofin for the treatment of p53 mutant non-small cell lung cancer: many ways to die.

Laurie Freire Boullosa, Jinthe Van Loenhout, Tal Flieswasser, Filip Lardon, Evelien Smits, Christophe Deben

Center for Oncological Research, University of Antwerp, Belgium

Presenting author: Laurie Freire Boullosa – Center for Oncological Research, Medicine and Health Sciences, universiteitsplein 1, 2610 Antwerp - laurie.freireboullosa@uantwerpen.be

Auranofin (AF) is an orally available, organogold compound that is FDA-approved for its use in rheumatoid arthritis. AF gained interest as an anti-cancer agent since it acts as a thioredoxin reductase (TrxR) inhibitor. The Cancer Genome Atlas shows that high expression of TXNRD1 in lung adenocarcinoma patients negatively affects outcome, making TrxR1 an attractable target in non-small cell lung cancer (NSCLC). The goal of this study was to determine if mutant p53 expression can be used as a predictive biomarker for AF treatment in NSCLC. In addition, we aimed to obtain a profound understanding of the molecular mechanisms that govern the sensitizing effect of mutant p53 towards AF-treated NSCLC cells by investigating the type of (immunogenic) cell death.

A strong inverse correlation was observed between mutant p53 protein expression and AF IC50-values in a panel of NSCLC and pancreatic cells, indicating that mutant p53 can sensitize cancer cells for AF. This sensitizing effect was confirmed in the NSCLC cell line NCI-H1299 (p53 deletion, null) and its two isogenic derivates (mutant p53 R175H or R273H knock-in). Transcriptome analysis of the isogenic NSCLC cells after exposure to a medium dose of AF revealed the up- and downregulation of genes involved in ferroptosis. Using the IncuCyte ZOOM, we observed that a cytotoxic concentration of AF induced cell death through various mechanisms, including apoptosis and ferroptosis, which were dependent on p53 expression levels rather than the type of mutation. Moreover, all types of AF-induced cell death were immunogenic since the release of damage-associated molecular patterns (ecto-calreticulin, ATP and HMGB1) and dendritic cell maturation (CD86 and MHC-II) occurred in all three cell lines independently of the type of cell death. In vivo validation using the gold-standard vaccination assay is ongoing.

In conclusion, mutant p53 protein expression renders NSCLC more susceptible to AF. In an isogenic setting, medium levels of mutant p53 sensitize NSCLC cells for apoptosis while cells with higher levels are effectively killed through ferroptosis. Both AF-induced cell death mechanisms were immunogenic. Altogether, AF presents a potential novel therapeutic strategy to efficiently kill p53 mutated NSCLC cells through the induction of immune stimulatory effects.

Abstract n°54

A phase I clinical trial on intratumoral administration of autologous CD1c (BDCA-1)+ myeloid dendritic cells plus talimogene laherparepvec (T-VEC) in patients with advanced melanoma

Julia Katharina Schwarze (1), Gil Awada (1), Louise Cras (3), Ramses Forsyth (3), Inès Dufait (1), Ivan Van Riet (2), Bart Neyns (1)

(1) Medical Oncology, Universitair Ziekenhuis Brussel, Laarbeeklaan 101, 1090 Brussel;
(2) Stem cell laboratory, Department of Hematology, Universitair Ziekenhuis Brussel, Laarbeeklaan 101, 1090 Brussel;
(3) Department of Anatomopathology, Universitair Ziekenhuis Brussel, Laarbeeklaan 101, 1090 Brussel

Presenting author: Julia Katharina Schwarze – UZ Brussel, Medical oncology, Laarbeeklaan 1010, 1090 Brussel - julia.katharina.schwarze@vub.be

Background: Intratumoral (IT) myeloid dendritic cells (myDC) play a pivotal role in initiating antitumor immune responses and re-licensing of antitumor cytotoxic T-lymphocytes within the tumor microenvironment. IT injection of the oncolytic virus T-VEC leads to the release of maturation signals and tumor antigens that can be captured and processed by IT co-administered CD1c (BDCA-1)+ myDC, reinvigorating the cancer immunity cycle.

Methods: Patients (pts) with advanced melanoma who failed standard therapy were eligible for IT injections of ≥1 non-visceral metastasis with T-VEC (10^6 PFU/mL; max total volume of 4 mL) on day 1 followed by IT injection of autologous CD1c (BDCA-1)+ myDC on day 2. Injection of T-VEC (10^8 PFU/mL; max total volume of 4 mL) was repeated on day 21 and every 14 days thereafter. Pts were treated with 0.5x10^6, 1x10^6, or 10x10^6 CD1c (BDCA-1)+ myDC in cohort-1/-2/-3, respectively. Primary objectives were safety and feasibility. Repetitive biopsies of treated lesions were performed.

Results: In this ongoing trial, 2 pts were treated in cohort-1, 2 pts in cohort-2, and 3 pts in cohort-3. Pts received a median of 6 (range 3-10) injections of T-VEC. All pts are evaluable for response. The best overall tumor response (iRECIST) was a CR (pathologic CR) and one PR (confirmation pending; pCR of treated lesions). Both pts were treated in cohort-3 and had previously progressed on anti-PD-1-, and one patient also on anti-CTLA-4 therapy. Adverse events include G1 fever in 4 pts, G1-2 flu-like symptoms in 5 pts, transient G1-2 local pain and redness at the injection-site in 3 pts, and G1 gastrointestinal symptoms in 4 pts. The patient with CR developed an asymptomatic G3 eosinophilia during treatment; the patient with PR developed a transient purpuric rash at the site of skin metastases after the first treatment. Multiplexed immune-profiling (Ultivue) of baseline and on-treatment tumor biopsies is ongoing.
Conclusions: IT co-injection of autologous CD1c (BDCA-1)+ myDC with T-VEC is feasible and tolerable and resulted in encouraging early signs of anti-tumor activity in patients with immune checkpoint inhibitor refractory melanoma.

Abstract n°58

Palmitate: a driver of metastasis formation

Patricia Altea-Manzano (1,2), Alex Cuadros (1,2), Joke Van Elsen (1,2) and Sarah-Maria Fendt (1,2)

(1) Laboratory of Cellular Metabolism and Metabolic Regulation, VIB Center for Cancer Biology, VIB, Herestraat 49, 3000 Leuven, Belgium.
(2) Laboratory of Cellular Metabolism and Metabolic Regulation, Department of Oncology, KU Leuven and Leuven Cancer Institute (LKI), Herestraat 49, 3000 Leuven, Belgium.

Presenting author: Patricia Altea Manzano – KUL-VIB Center for Cancer Biology, Laboratory of Cellular Metabolism and Metabolic Regulation, Herestraat 49, 3000 Leuven - patricia.alteamanzano@kuleven.vib.be

Metastasis is the leading cause of breast cancer-related death worldwide (1). Despite the advances in effective primary tumor treatments, metastatic tumors (mostly in lung, liver and brain) remain elusive for current therapies. This is mainly due to the fact that disseminated breast cancer cells acquire new features (not observed in the primary tumor) to overcome the selective pressure of the circulation and the new conditions in the distant site (2). Recent evidences point out that metastatic cells rewire their metabolism to support their transition through the metastatic cascade (3) and adapt to the different nutrients availability in the new organ (4). Understanding the rewired metabolic dependencies in the new environment will allow targeting the capacity of metastatic cells to colonize distant sites and form secondary tumors. Measurements of the nutrient levels in mouse interstitial fluids showed palmitate as one of the most abundant fatty acids in the lungs and liver. Higher levels of palmitate were found in metastatic lung tissue compared to breast primary tumor. In an in vitro setting, palmitate supplementation was able to drive a significant advantage in the anchorage-independent growth (3D colonies). We demonstrated that this palmitate is being mainly metabolized through the fatty acid oxidation pathway (FAO) in colonizing tumor cells, generating high levels of mitochondrial acetyl-coA. This increase in intracellular acetyl-coA levels (palmitate-dependent) has a strong impact on the acetylation of histones marks related to gene expression. Accordingly, increased histone acetylation levels were found in metastatic lung tissues compared to primary breast tumor in vivo. Interestingly, when the rate-limiting FAO enzyme CPT1a is inhibited, both in vitro and in vivo metastatic potential are significantly reduced. Taking together our data suggest a novel role of palmitate metabolism regulating gene expression to promote metastasis formation, a role that might in fact offer new therapeutic opportunities to prevent and treat metastatic breast cancer.

Abstract n°61

Zeb2 drives invasive and microbiota-dependent colon carcinoma

Karolina Slowicka (1,2,3), Ioanna Petta (1,3,4), Esther Hoste (1,2), Emilie Dumas (1,3,4), Mozes Sze (1,2), Hanna Vikkula (1,2), Konstantina Zafeiropoulou (1,3,4), Enrico Radaelli (5,6), Jody J. Haigh (7,8), Sven Jonckheere (2,9), Joachim Taminau (2,9), Niels Vandamme (1,2,9), Pieter Van Vlierberghe (9,10), Gert De Hertogh (11), Pamela Baldin (12), Emre Etlioglu (13), Pratyaksha Wirapati (14), Sabine Tejpar (13), Steven Goossens (2,9,10), Lars Vereecke (1,3,4), Geert van Loo (1,2,3), Geert Berx (2,9)

(1) VIB Center for Inflammation Research, 9052 Ghent, Belgium
(2) Department of Biomedical Molecular Biology, Ghent University, 9052 Ghent, Belgium
(3) Ghent Gut Inflammation Group (GGIG), Ghent University , 9000 Ghent , Belgium.
(4) Department of Rheumatology, Ghent University, 9000 Ghent, Belgium
(5) KU Leuven Center for Human Genetics, 3000 Leuven, Belgium
(6) VIB Center for the Biology of Disease, 3000 Leuven, Belgium
(7) Research Institute in Oncology and Hematology (RIOH), CancerCare Manitoba, Winnipeg, Manitoba, Canada
(8) Department of Pharmacology and Therapeutics, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada
(9) Cancer Research Institute Ghent (CRIG), 9000 Ghent, Belgium
(10) Department of Biomolecular Medicine, Ghent University, 9000 Ghent, Belgium
(11) Department of Pathology, University Hospital Leuven, KU Leuven, 3000 Leuven, Belgium
(12) Department of Pathology, Cliniques Universitaires Saint Luc, Université Catholique de Louvain (UCL), 1200 Brussels, Belgium
(13) Department of Oncology, University Hospital Leuven, KU Leuven, 3000 Leuven, Belgium
(14) Swiss Institute of Bioinformatics, 1015 Lausanne, Switzerland.

Presenting author: Ionna Petta – VIB-Ghent Universtity, Department of Rheumatology, Corneel Heymanslaan 10, 9000 Ghent - ioannap@irc.vib-UGent.be

Colorectal cancer (CRC) is one of the most prevalent cancers in Western society, and increasing evidence indicates a strong contribution of environmental factors and the intestinal microbiota to CRC development. A specific subset of CRC is characterized by a transcriptional profile linked to 'epithelial-to-mesenchymal transition' (EMT), a cellular process crucial during development but often deregulated in cancer. We have identified a synergistic tumor-promoting mechanism through which both EMT and the intestinal microbiota are required to induce spontaneous invasive CRC development.
Intestinal epithelial cell (IEC)-specific transgenic expression of the EMT regulator Zeb2 in mice (Zeb2IEC-Tg/+ mice) leads to increased intestinal permeability and spontaneous invasive colon carcinoma development. Already at young age, Zeb2IEC-Tg/+ mice all develop a dysplastic colonic, but normal small intestinal, epithelium which progresses to severely inflamed neoplastic lesions over time. Tumor-bearing Zeb2IEC-Tg/+ mice are characterized by marked intestinal dysbiosis. Remarkably, broad spectrum antibiotic treatment or germfree rederivation completely prevents cancer development in Zeb2IEC-Tg/+ mice, indicating a crucial involvement of the intestinal microbiota during tumor development in this model. In human CRC patients, expression of ZEB2 and other EMT markers is associated to a specific CRC subtype with worse outcome and is linked to inflammatory gene expression profiles similar to those observed in Zeb2IEC-Tg/+ mice. Together, our study describes a strong synergistic mechanism through which the resident intestinal microbiota can boost invasive CRC in an EMT-prone environment. Zeb2IEC-Tg/+ mice represent the first model of spontaneous microbiota-induced invasive CRC, which will help to unravel host-microbiome interactions driving CRC development in humans.

Abstract n°71

To induce tumor metabolic addiction and to harness it for synthetic lethality.

Catherine Vander Linden, Cyril Corbet, Estelle Bastien, Céline Guilbaud, Laurenne Petit and Olivier Feron

Pole of Pharmacology and Therapeutics (FATH), Institut de Recherche Expérimentale et Clinique (IREC), UCLouvain, Brussels

Presenting author: Catherine Vander Linden – IREC, Pharmacology and Therapeutics, Avenue Hippocrate 57, 1200 Brussels - c.vanderlinden@uclouvain.be

Not accepted for publication.

Poster Abstracts

Abstract n°1

Dilemma of immune checkpoint inhibitors in renal transplant patients with cutaneous squamous cell carcinoma.

Tess Van Meerhaeghe (1), Alain Le Moine (1) and Sandrine Aspeslagh (2)

(1) Department of Nephrology, Erasmus Hospital-ULB, Brussels, Belgium
(2) Department of Medical Oncology, Erasmus Hospital-ULB, Brussels, Belgium

Presenting author: Tess Van Meerhaeghe – Hostpital Erasme, Nephrology, Route De Lennik 808, 1070 Brussels - vmtess@hotmail.com

Introduction
Only a few case reports and case series were published on the use of ICI in organ transplant patients. Here we report two patients with prior renal transplant and advanced squamous cell carcinoma (cSCC) of the skin who have been treated with anti-PD1. We examined possible prognostic factors for graft rejection and tumor response.

Case descriptions
The first patient described is a 50 year old man, who received a third deceased donor kidney transplantation in 2013 for a primary focal and segmental glomerulosclerosis. His post-transplant period was complicated by recurrent episodes of cSCC. Despite treatment with reduction of immunosuppression, surgery, radio – and chemotherapy the disease progressed. Treatment with Cemiplimab (humanized anti PD-1 monoclonal antibody) was initiated with partial tumor response. His kidney function remained stable during the whole treatment course. The second case is a 74 year old man who received a renal transplant from a heart-beating donor in 2012 for nephrangiosclerosis. He suffered from recurrent presternal cSCC, treated surgically with concomitant radiotherapy and reduction in immunosuppression. The patient was started on Cemiplimab for progressive disease. Patient had complete tumor response, but developed acute T-cell cell mediated graft rejection early in the treatment course.

Conclusion
In the present case reports, we see a favorable response to anti-PD-1 in patients with previous renal transplant. The risk of graft rejection is not negligible. Potential risk factors identified for graft rejection were total years of transplantation and previous cumulative dose of radiation. In order to identify markers predictive of cancer response and graft rejection additional multi-center studies are eagerly awaited. Only then, we will be able to stratify renal graft patients into those that could benefit from ICI treatment at high and low risk of graft rejection.

Abstract n°2

Inhibition of PHGDH in improving radioresponse in cancer

Melissa Van De Gucht (1), Hui Wang (1), Sven de Mey (1), Inès Dufait (1), Ka lun Law (1), Thierry Gevaert (1), and Mark De Ridder (1)

(1) Department of Radiotherapy, Universitair Ziekenhuis Brussels, Vrije universiteit Brussel, Brussels, Belgium

Presenting author: Melissa Van De Gucht – Vrije Universiteit Brussel, TROP, Department of Radiotherapy, Laarbeeklaan 103, 1090 Brussels - melissa.van.de.gucht@vub.be

Background: Metabolism of cancer is reprogrammed and characterized by increased glycolysis. As a bypass of glycolysis, serine pathway is aberrantly active in varying cancer types, proving essential precursor for DNA and antioxidants of which disrupting either could lead to radiosensitization. We, therefore, explored whether inhibition of the rate-limiting enzyme phosphoglycerate dehydrogenase (PHGDH) of serine synthesis could enhance radioresponse.

Material and methods: Colorectal cancer cells HCT116 and breast cancer cells MDA231 were exposed to PHGDH inhibitor NCT503 for 16h at indicated concentrations. Expression and activity of PHGDH were assessed respectively by RT-PCR and Western blot and commercial kit. Cell viability was measured by MTT and apoptosis. Radiosensitivity of tumor cells was evaluated by colony formation assay under aerobic and hypoxic conditions. To unravel underlying mechanisms, reactive oxygen species (ROS), apoptosis, and DNA damage were measured by flow cytometry. Production of glutathione and NADPH were assessed by commercial kits.

Results: NCT503 inhibited the activity of PHGDH in a dose-dependent manner in all cancer cell lines. Consistently, cell viability was reduced after exposure to NCT503. At non-toxic doses, NCT503 enhanced hypoxic radioresponse. Regarding the radioresponse in aerobic condition, NCT503 did not exhibit any impact. In mechanical studies, we uncovered that NCT503 triggered overproduction of ROS, along with decreased levels of glutathione and NADPH. In line, ROS scavenger NAC counteracted the radiosensitization induced by NCT503, confirming that overproduction of ROS is the main mechanism. In addition, increased apoptosis and DNA damages were observed after exposure to NCT 503, most likely caused by ROS.

Conclusion: Our results demonstrate that inhibition of PHGDH in serine pathway overcome hypoxic radioresistance in cancer cells through overproduction of ROS, which will be validated in vivo models.

Abstract n°3

Discovery analysis of a cancer-relevant transcription factor uniquely expressed in dedifferentiated human acinar cells and not in duct cells

Elyne Backx (1), Elke Wauters (2), Mathias Van Bulck (1), Jonathan Baldan (2), Ellis Michiels (1), Zhidong Ling (2), Luc Bouwens (2), Patrick Jacquemin (3), Isabelle Houbracken (2), Ilse Rooman (1)

(1) Oncology Research Centre, Vrije Universiteit Brussel
(2) Diabetes Research Centre, Vrije Universiteit Brussel
(3) Cell Differentiation Lab, De Duve Institute, Université Catholique de Louvain

Presenting author: Elyne Backx – Vrije Universiteit Brussel, LMMO, Laarbeeklaan 103, 1090 Brussels - ebackx@vub.be

Pancreatic acinar cells can dedifferentiate and acquire ductal characteristics, termed acinar-to-ductal metaplasia (ADM), which is critical in tumor development. Nevertheless, duct cells themselves are less prone for development of pancreatic cancer (PC) than dedifferentiated acini. We aimed to clarify which genes are unique for dedifferentiated acini. For this, mixed exocrine preparations of acinar and duct cells were obtained from human pancreatic donor organs and cultured to induce dedifferentiation. We FACS-purified the dedifferentiated acinar cells and duct cells and compared their expression signature. RNAseq analysis detected 1219 genes unique for dedifferentiated acinar cells (Adj $P<0.01$, log fold changes of ≤ -2). The most differentially expressed transcription factor (log fold change=-6.09; adj. P=1,04-123) encodes for a known oncogene. We confirmed that it is highly expressed in embryonic acinar cells and in chronic pancreatitis where acinar cells dedifferentiate but not in duct cells nor differentiated acinar cells. Additionally, during human acinar cell dedifferentiation as well as in PDAC, there is a moderate correlation between our gene of interest (GOI) and SOX9, an important transcription factor regulating ADM and indispensable for tumour formation in the pancreas. In vitro and in vivo depletion of GOI in dedifferentiated acinar cells gives rise to a distinct phenotype with more prominent cell death and prolonged immune infiltration.
In conclusion, we report here the purification and transcriptional profiling of the two human pancreatic exocrine cell types. We uncovered a transcription factor, important in cancer, that is characteristic of dedifferentiated acinar cells.

Abstract n°4

A numerical simulation of pressurized intraperitoneal aerosol chemotherapy (PIPAC) validation part.

Mohammad Rahimi-Gorji (1,2,3), Charlotte Debbaut (2,3), Wouter Willaert(1,3), Patrick Segers (2,3), Ghader Ghorbaniasl (4), Wim Ceelen (1,3)

(1) Laboratory for Experimental Surgery, Department of Human Structure and Repair, Ghent University, Ghent, Belgium
(2) IBiTech – bioMMeda, Ghent University, Ghent, Belgium
(3) CRIG – Cancer Research Institute Ghent, Belgium
(4) Department of Mechanical Engineering, Vrije Universiteit Brussel, Brussels, Belgium.

Presenting author: Mohammad Rahimi Gorji – Ghent University, Department of Human Structure and Repair, Conreel Heymanslaan 10, 9000 Ghent - mohammad.rahimigorji@ugent.be

1. INTRODUCTION

Pressurized IntraPeritoneal Aerosol Chemotherapy (PIPAC) is a new and innovative drug delivery method developed to treat peritoneal metastasis using laparoscopy. The aerosol distribution by PIPAC, however, is not homogeneous and therapy may need optimization for the individual patient, given the variability in PM. A homogenous aerosol distribution in the area of interest is therefore a goal in PIPAC. We therefore started modeling PIPAC with a computational fluid dynamics (CFD) method to understand and optimize particle deposition. In this study, we aim to validate the CFD model by comparing the calculated results with in-vitro PIPAC experiments in a box model.

2. MATERIALS AND METHODS

Experiments were performed in the Experimental Surgery Lab using a Plexiglas box model. The box size was 185 x 135 x 152 (mm) and it was infused by CO2 gas maintained at a pressure of 12 mmHg. Black ink was used in the experiment, injected through a nebulizer and high-pressure injector, where the experiment was repeated for six times. Tissue was placed on four plates inside the box (A, B, C and D) to allow us to visualize and quantify the distribution of ink. The CFD geometry model was generated by COMSOL Multiphysics. The same procedures as the experiment were considered: insufflating CO2 into the box to reach 12 mmHg pressure. CFD Module was applied under initial and boundary conditions (inlet flow with 12 mmHg and no-slip conditions for the walls). Then injecting a volume of 20 mL of black ink (density: 1071.9 kg/m3, viscosity: 4.875*10-3 Pa.s) with a flow rate of 0.5 mL/s at a fixed injector position in the top surface was modeled.

3. RESULTS AND DISCUSSION

The results show the tissues after the experiment and comparison between the experimental and simulated distribution of ink over the 4 plates. An overall good agreement was observed between the calculated and the experimental results. Not surprisingly, most aerosol was deposited on the bottom surface due to the gravity. To overcome this problem, an electrostatic field can be imposed, yielding ePIPAC. This will be the topic of our future experimental and computational work.

Abstract n°5

Role of Lipid droplets in cancer progression and resistance to anti-cancer drugs.

Coline Wéry (1), Adrien Godfroid (1), Geraldine Luis (1), Donovan Jonlet (1), Agnès Noel (1), Olivier Feron (2), Nor Eddine Sounni (1)

(1) Laboratory of Tumor & Development Biology (LBTD), GIGA-Cancer, ULiège, Belgium ;
(2) Pole of Pharmacology and Therapeutics (FATH), Institut de Recherche Expérimentale et Clinique (IREC), UCLouvain, Belgium

Presenting author: Coline Wery – Uliège, GIGA-Cancer, Avenue Hippocrate 13, 4000 Liège - coline.wery@uliege.be

A growing number of evidences report that hypoxia in tumor is a key driver of cancer malignancy. Cancer cell adaptation to hypoxia increases their survival potential but also resistance to the anti-cancer treatments. Hypoxia occurring naturally in tumors or induced by anti-cancer drugs is also described to induce metabolic alterations, acquisition of fast-growing properties as well as promoting metastatic dissemination. Here we explored tumor hypoxic areas detected by pimonidazol staining in MDA-MB231 xenografts grown in RAG1-/- mice and in the syngeneic Lewis Lung Carcinoma tumors, grown in C57bl/6 mice, and find an increased accumulation of lipid droplets (LDs), revealed by adipophilin (perilipin-II) staining. Also, an increase in the fatty acid binding protein-4 (FABP4), the long chain fatty acids transport was found specifically expressed in the vasculatures adjacent to tumor hypoxic areas. Incubation of cancer cells in hypoxia resulted in an increased LDs when compared to cells incubated in normoxia that was reduced by FABP4 inhibitors in vitro. We are investigating the underlying molecular mechanisms of cancer cells adaption to hypoxia and the role of lipid metabolism and LDs structures in cancer cells. We have generated cancer cells expressing shRNA against adipophilin, the key protein of the LDs dependent hypoxia. These cells will be characterized for their proliferation, migration and response to anticancer drugs in vitro, and for tumor growth and response to anti-angiogenic drugs in vivo. The lipid composition of LDs associated with hypoxia will be analyzed by lipidomic analysis after their isolation. Our study will shed light on the role of LDs in cancer cell responses to hypoxia, acidosis and to variety of therapeutics. The objective is to better understand of the link between cancer cell adaptation to the microenvironmental stresses, lipid metabolism and disease progression.

Abstract n°6

Dichloroacetate radiosensitizes hypoxic tumor cell through enhanced production of reactive oxygen species.

Sven de Mey (1), Melissa Van De Gucht (1), Hui Wang (1), Heng Jiang (1), Inès Dufait (1), Ka Lun Law (1), Cyril Corbet (2), Thierry Gevaert (1), Olivier Feron (2), Mark De Ridder (1)

(1) Department of Radiotherapy, Universitair Ziekenhuis Brussel, Vrije Universiteit Brussel, Brussels, Belgium
(2) Pole of Pharmacology and Therapeutics (FATH), Insitut de Recherche Expérimentale et Clinique (IREC), Université Catholique de Louvain, Brussels, Belgium

Presenting author: Sven de Mey – Vrije Universiteit Brussel, TROP, Department of Radiotherapy, Laarbeeklaan 103, 1090 Brussels – sven.de.mey@vub.be

Background and Purpose: Dichloroacetate (DCA) is a well-tolerated, oral drug used in the treatment of metabolic diseases. DCA has been found to have anti-tumorigenic activity in various cancer models. However, the radiomodulatory effect of DCA is largely unknown. DCA Inhibits pyruvate dehydrogenase kinase (PDK) which activates pyruvate dehydrogenase (PDH) and can shift tumor cell metabolism from anaerobic glycolysis to glucose oxidation. Promotion of the oxidative metabolism leads to enhanced ROS production. ROS are the primary effector molecules of radiation, and increase of ROS production by pharmacological modulation is known to enhance radioresponse. We therefore investigated the radiosentizing effect of DCA in breast cancer cells.

Material and Methods: The radiosensitizing effect of DCA on cancer cells was examined *in vitro* in two different breast cancer cell lines (4T1 and EMT6) by clonogenic assay in both aerobic and hypoxic conditions. These results were validated in a 3D spheroid model. PDH phosphorylation was measured by western blot. Lactate production and extracellular acidification rate (ECAR) were measured by a commercial kit and the Seahorse analyzer. ROS production was measured by FACS.

Results: DCA increased PDH activity, while decreasing ECAR and lactate. Next, ROS was significantly increased under hypoxic conditions, but not under aerobic conditions. Consistently, DCA radiosensitized hypoxic tumor cells, while leaving the intrinsic radiosensitivity unchanged.

Conclusion: Our results suggest that DCA can increase hypoxic radioresponse by overproducing ROS in the tumor cells, and providing a rationale for exploring the efficacy of DCA with radiotherapy.

Abstract n°7

Nanobody-mediated imaging of PD-L1 provides a rationale to combine Galsome vaccination with immune checkpoint blockade

Thomas Ertveldt (1,*), Sofie Meulewaeter (1,2,3,*), Ine Lentacker (2,3), Rein Verbeke (2,3), Stefaan De Smedt (2,3), Oscar Olarté (4), Kurt Barbé (4), Marleen Keyaerts (5, 6), Heleen Dewitte (2,3), Karine Breckpot (1)

(1) Laboratory for Molecular and Cellular Therapy, Department of Biomedical Sciences, Vrije Universiteit Brussel, Brussels, Belgium
(2) Ghent Research Group on Nanomedicines, Faculty of Pharmacy, Ghent University, Ghent, Belgium
(3) Cancer Research Institute Ghent (CRIG), Ghent University Hospital, Ghent University, Ghent, Belgium
(4) Department of Biostatistics and Medical Informatics, Vrije Universiteit Brussel, Brussels, Belgium
(5) In Vivo Cellular and Molecular Imaging Laboratory, Vrije Universiteit Brussel, Brussels, Belgium
(6) Nuclear Medicine Department, UZ Brussel, Brussels, Belgium
(*) shared first author.

Presenting author: Sofie Meulewaeter – Vrije Universiteit Brussel, LMCT, Ghent University, Research group on nanomedicines, CRIG, 9000 Ghent - sofie.meulewaeter@ugent.be

Designing therapeutic vaccines has been a major focus in cancer immunotherapy. We have developed Galsomes, a cancer vaccine consisting of a lipid nanoparticle that incorporates tumor antigen mRNA and the glycolipid α-galactosylceramide. Consequently, vaccination with Galsomes activates two types of adaptive and innate immune killer cells: cytotoxic T lymphocytes (CTLs), natural killer T (NKT) cells, respectively. To fully capitalize on these cells' ability to eradicate cancer cells, it is critical to understand which barriers within the tumor-bearing host might form obstacles for their activity. Programmed death-ligand 1 (PD-L1) is such a barrier that can act on CTLs and NKT cells both at the time of activation as at the time of their effector function. There are indications that Galsome vaccination triggers expression of PD-L1, but up to now, no detailed information on the timing and location of this checkpoint expression is available. We previously developed a nanobody-mediated strategy to noninvasively image PD-L1. In this study, we used this nanobody-mediated SPECT/CT imaging strategy to determine the spatio-temporal expression of PD-L1 upon Galsome vaccination in the B16-OVA mouse melanoma tumor model. Moreover, ex vivo analysis was performed to support the SPECT/CT images. We also studied how Galsome vaccination combined with PD-L1 blockade impacts on the therapy outcome. Noninvasive, nanobody-mediated imaging of PD-L1 and ex vivo analysis was performed at several time points after Galsome vaccination in B16-OVA bearing mice, showing upregulation of PD-L1 expression as soon as one day after vaccination in organs targeted by the vaccine (lung, spleen, lymph node), while PD-L1 upregulation in the tumor environment occurred at later time points. Corroborating these findings, we showed that Galsome vaccination combined with anti-PD-L1 monoclonal antibody therapy significantly improved therapy outcome. In conclusion, Galsome vaccination in combination with PD-L1 blockade represents a promising, more effective treatment regimen for melanoma, a treatment regimen that is supported by noninvasive imaging of PD-L1 upon Galsome vaccination.

Abstract n°8

Characterization of the molecular and functional consequences of MCT1 inhibition in cancer treatment

Zohra Benyahia, Loïc Hamelin, Thibaut Vazeille, Pierre Sonveaux

Pole of Pharmacology & Therapeutics, Institut de Recherche Expérimentale et Clinique (IREC), University of Louvain (UCLouvain), Brussels, Belgium.

Presenting author: Zohra Benyahia, Univeristy of Louvain, IREC, FATH, Avenue Hippocrate 57, 1200 Brussels - zohra.benyahia@uclouvain.be

Introduction: Monocarboxylate transporters (MCTs) convey lactate exchanges in many tumors. In solid tumors, MCT4 primarily promotes lactate export from glycolytic cancer cells. Comparatively, because it simultaneously control lactate-dependent metabolic symbiosis in cancer,1 lactate-dependent angiogenesis2 and metastasis,3 MCT1 has been suggested as a new anticancer target. Hence, AZD3965 is the first MCT1 inhibitor to enter clinical trials for cancer patients (ClinicalTrials.gov NCT01791595). However, the physiological roles of MCT1 are not well known. They are the focus of our in vitro study.
Experimental: Responses of MCF7 human breast cancer cells to AZD3965 were compared to that of human T47D and MCF10A normal breast cells and human BJ fibroblasts. Cell viability was tested using crystal violet; the expression of MCT1, MCT4 by RT-qPCR and WB; glucose consumption and lactate and pyruvate release enzymatically on a CMA600 analyzer; and oxygen consumption on a Seahorse bioenergetic analyzer.

Results: In the absence of exogenous lactate and in the presence of glucose, doses from 1 nM to 10 µM of AZD3965 did not affect cell viability. However, it increased MCT4 expression in the four cell lines. From a metabolic standpoint, treated normal and cancer cells all had enhanced glucose consumption, decreased lactate and pyruvate release and increased oxygen consumption linked to mitochondrial ATP production.

Conclusions: In the presence of glucose, MCT1 inhibition by AZD3965 did not kill cancer and normal cells, but induced a switch to a more oxidative metabolism in both types of cells. The switch could be related to the functional replacement of MCT1 by MCT4, which is less efficient than MCT1 to export lactate.

Abstract n°9

Mitochondrial alterations associated to cisplatin resistance in ovarian cancer

Luca X Zampieri (1), Debora Grasso (1), Tânia Capelôa (1), Pierre Sonveaux (1)

(1) Pole of Pharmacology and Therapeutics, Institute of Experimental and Clinical Research (IREC), University of Louvain (UCLouvain), Brussels, Belgium

Presenting author: Luca Zamieri – Univerisity of Louvain (UCLouvain), Pole of Pharmacology and Therapeutics (FATH), Rue du Campanile 12/205, 1200 Brussels - luca.zampieri@uclouvain.be

Ovarian cancer is the 5th cause of cancer-related deaths among women. Platinum-based chemotherapy often leads to increasingly resistant recurrences. Here, we hypothesized that metabolic alterations could contribute to resistance to cisplatin, and we metabolically compared isogenic sensitive and resistant human ovarian carcinoma cell lines to identify new therapeutic approaches.
Our models were SKOV-3 and COV-362 human ovarian cancer cells and their resistant variants that were selected in vitro following exposure to increasing doses of cisplatin. Enzymatic assays on a CMA600 analyzer were used to evaluate glucose consumption and lactate production, Seahorse analysis for mitochondrial respiration, and immunofluorescence for mitochondrial morphology (TOM20) and mitophagy (p62).
Cisplatin-resistant cancer cells had a decreased glycolytic rate, whereas mitochondrial respiration was increased. Compared to sensitive cells they also presented a bigger mitochondrial surface. To test the hypothesis of a higher mitochondrial turnover, resistant cells were treated with bafilomycin A1. It caused an accumulation of p62 co-localized with mitochondria (mitophagy inhibition) and, when used in combination with cisplatin, prevented the increase in mitochondrial surface.
Fitter mitochondria, probably resulting from a higher mitochondrial turnover, induce a switch from a glycolytic to an oxidative metabolism in cisplatin-resistant ovarian cancer cells. Targeting mitophagy could thus be an effective therapeutic strategy to prevent or overcome cisplatin resistance.

Abstract n°10

Targeted drug delivery for liver cancer: modelling the impact of cancer burden on the particle distribution

Tim Bomberna (1), Ghazal Adeli Koudehi (1), Patrick Segers (1), Charlotte Debbaut (1)

(1) Ghent University, IBiTech-bioMMeda, Belgium

Presenting author: Tim Borberna – Ghent University, IBiTech-bioMMeda, Corneel Heymanslaan 10, 9000 Ghent - Tim.Bomberna@UGent.be

Hepatocellular carcinoma (HCC) is the most common liver malignancy in the world. HCC patients for whom resection is not possible can be treated by transarterial chemo-embolization (TACE). In TACE, particles are injected in the feeding arteries of the tumour which permanently damage the tumour tissue. Since the goal of TACE is to steer particles towards the tumour tissue and limit the toxicity for healthy tissue, target-specificity is a key parameter. The target-specificity of the proposed therapy can be estimated by simulating the particle distribution in the patient-specific geometry using computational fluid dynamics. Using these simulations, the set of injection parameters (injection location, injection velocity, etc.) which maximize particle deposition at the target site can be identified for each patient. The goal is to tailor the therapy to each patient specifically.

In this study, the impact of cancer burden on the blood flow and the particle distribution throughout the arterial tree was investigated. A detailed dataset of a patient-specific cirrhotic liver vasculature was obtained by combining vascular corrosion casting and micro-CT imaging. The arterial network of the liver was segmented and the resulting 3D geometry was meshed. Simulations were run for eleven different cancer scenarios, each varying in total cancer burden and tumour nodule locations. Cancer tissue was modelled as having over a four-fold increase in arterial perfusion as compared to healthy tissue [1].

The results show that cancer burden has a substantial impact on the blood flow and particle distribution in patient-specific geometries. For the non-cancerous case, 16.58% of particles reached the left lobe of the liver. For cases in which tumour nodules were modelled in the left lobe, this fraction increased from low cancer burden (32.41%) to high cancer burden (69.77%). For cases in which tumour nodules were constricted to the right lobe, this fraction decreased from low cancer burden (13.61%) to high cancer burden (6.46%). It is clear that cancer burden is an important parameter to consider in simulating particle distribution for TACE.

[1] J. Aramburu et al, "Liver cancer arterial perfusion modelling and CFD boundary conditions methodology", 2016.

Abtract n°11

Combining PD-1/PD-L1 blockade and RANKL inhibitors to treat breast cancers unresponsive to standard therapy

Charlotte Pilard (1), Patrick Roncarati (1), Elodie Hendrick (1), Alizée Lebeau (1), Diane Bruyère (1), Thomas Lerho (1), Marie Ancion (1), Célia Reynders (1), Philippe Delvenne (1), Michael Herfs (1), Pascale Hubert (1)

(1) Laboratory of Experimental Pathology, GIGA-Cancer, University of Liege, Liege, Belgium

Presenting author: Charlotte Pilard, ULiège, Laboratory of Experimental Pathology, Avenue de l'Hôpital 1, 4000 Liège - cpilard@uliege.be

Immunotherapy using immune checkpoint inhibitors (like PD-1/PD-L1) has been demonstrated as a promising strategy for the treatment of cancers that don't respond to classical chemoradiotherapy. Given that cancer cells have the potential to express many immunosuppressive molecules, the combination of immune checkpoint inhibitors with drugs thwarting tumor immunosuppressive microenvironment could represent a promising strategy. Among these immunosuppressive molecules, RANKL, a member of the TNF superfamily, which affects the immune system and bone remodeling, has been shown to be a key factor promoting the progression of breast cancer. In addition, RANKL induces the formation of tolerogenic dendritic cells and Treg cells, which promotes immunotolerance to the tumor.
The aim of this research project is to study the impact of several RANKL inhibitors on triple negative breast cancer and to analyze the efficiency of their association with anti-PD-1/PD-L1 agents.
RANKL/PD-L1 expression profile on specimens from each breast cancer subtypes showed that both immunosuppressive molecules are expressed by all breast cancers with a significantly more intense immunoreactivity for triple negative breast cancers.
We next studied RANKL and PD-L1 expression in several murine and human breast cancer cell lines by immunohistochemistry. Most of these latter expressed both proteins. Moreover, the secretion of RANKL was analyzed by ELISA. We found that RANKL is secreted in their extracellular environment. Several RANKL inhibitors were then tested in vitro: anti-RANKL antibody, RANK-Fc, Isoliquiritigenin and Gallocatechin gallate. The efficacy of these inhibitors was indirectly evaluated with the murine macrophage RAW264.7 cell line which undergoes, in the presence of RANKL, an osteoclast differentiation (TRAP and CTSK expression). The efficacy of RANKL inhibitors was then evaluated, in this model, by RT-qPCR. Apoptosis and proliferation of the cancer cell lines in the presence of the inhibitors were also analyzed.
The two most efficient inhibitors will be selected and tested in vivo. Several murine triple negative breast cancer cell lines will be sub-cutaneously injected mice and the efficacy of both RANKL and PD-L1 inhibitors will be evaluated (separately or in combination). The infiltration of tumor microenvironment by different immune cell populations, the presence of metastasis and the tumor growth will be analyzed.

Abstract n°12

HPV-positive and HPV-negative Head and neck squamous cell carcinoma cell lines have different radioresistant mechanisms involving the DNA damage response

Marieke Bamps (1), Rüveyda Dok (1), Sandra Nuyts (1,2)

(1) Laboratory of Experimental Radiotherapy, KU Leuven, Department of Oncology, University of Leuven, Leuven, Belgium
(2) Leuven Cancer Institute, Department of Radiation Oncology, University Hospital of Leuven, Leuven, Belgium

Presenting author: Marieke Bamps - KU Leuven, Oncology, Herestraat 49, 3000 Leuven - marieke.bamps@kuleuven.be

Background: Up to 50% of patients with locally advanced head and neck squamous cell carcinoma (HNSCC) treated with (chemo)radiotherapy (RT) develop locoregional recurrences. These local recurrences are often related to intrinsic or acquired radioresistance. Although several oncological processes, such as the DNA damage response (DDR) are linked with radioresistance, the exact underlying mechanisms are not fully understood. Therefore, we focus on the involvement of the DDR in radioresistance.

Methods: We generated and characterized acquired radioresistant cells from Human papillomavirus (HPV)-positive and HPV-negative HNSCC cell lines by exposing parental cells to a fractionated RT schedule with fractions of 2 Gray (Gy) upto a total dose of 60 Gy. The influence of DDR on RT response in parental and radioresistant cells was assessed via a loss of function CRISPR-Cas9 screen targeting several DDR genes. The findings of the loss of function CRISPR-Cas9 screen were validated with commercially available inhibitors.

Results: We were able to generate different clones of HPV-positive cell line SCC154 and HPV-negative cell line SCC61 with a stable radioresistant phenotype of at least 3 months. No differences in doubling time were observed between HPV-positive radioresistant cells and parental cells. In contrast, HPV-negative radioresistant cells showed a faster doubling time compared to parental cells. Less G2/M arrest was observed after RT in HPV-positive radioresistant cells accompanied by an increased DNA repair capacity compared to parental cells. The loss of function CRISPR-Cas9 screen showed the importance of cell cycle, non-homologous end joining and homologous recombination in the RT response of HPV-positive radioresistant cells. HPV-negative radioresistant cells differed in cell cycle kinetics after RT, but no differences in DNA repair kinetics compared to parental cells. In concordance with differences in cell cycle kinetics, the loss of function CRISPR-Cas9 screen showed that general DDR and cell cycle genes were important for RT response in HPV-negative radioresistant cells. Inhibition of cell cycle proteins CHK1 and CHK2 with CHK1/2 inhibitor AZD7762 reverts the radioresistant phenotype of HPV-negative radioresistant cells. Further experiments are necessary to unravel the underlying mechanisms of HPV-positive and HPV-negative radioresistant cells and eventually improve RT treatment.

Abstract n°13

The Belgian Virtual Tumourbank (BVT) Project: Availability of metastasis in the BVT catalogue

Kim Vande Loock (1), Eva Van der Stock(1), Annelies Debucquoy(1), Katia Emmerechts(1), Liesbet Van Eycken(1), Etienne Marbaix(2) on behalf of the Steering Committee of the Belgian Virtual Tumourbank

(1) Belgian Cancer Registry, Koningsstraat 215 bus 7, 1210 Brussels, Belgium
(2) Service d'Anatomie Pathologique, Université Catholique de Louvain, St-Luc University Hospital, 10 Ave Hippocrate, 1200 Brussels, Belgium

Presenting author: Kim Vande Loock – Belgian Cancer Registry, Biobank, Koningsstraat 215, 1210 Brussels - kim.vandeloock@kankerregister.org

Biobanks play a critical role in cancer research by providing high quality biological samples for research. However, the availability of tumour samples in single research institutions is often limited, especially for less frequent sample types. The Belgian Virtual Tumourbank (BVT) network encompasses the tumour biobanks from eleven Belgian university hospitals that collect and store residual human tumour samples locally. In order to facilitate the search for tumour samples scattered among different institutions, data collected at sample level is made available for researchers via the online BVT catalogue. High quality of the data is guaranteed by automatic and manual controls performed by the BVT project team at the Belgian Cancer Registry.
Currently, more than 99,000 registrations are available in the catalogue for researchers in the oncology field. The availability of metastasis samples in the BVT catalogue was investigated. The majority of the samples in the catalogue are primary tumour samples (85%, 84,760 registrations), including benign, borderline, in situ and primary malignant tumours. However, the catalogue also contains 11,926 registrations (12%) from metastasis samples. The most common sample localisations of the metastasis samples in the BVT catalogue are lymph nodes (26.1%) and liver (25.9%). Soft tissue completes the top three of sample localisations with 10.8%. These metastases most commonly originate from primary tumours of colon and rectum (18.1%), breast (7.7%) and lung (7,5%). For 70.4% of the metastasis registrations, only the residual metastasis tissue is stored. For some patients, also additional types of material are stored at the local biobanks and registered in the BVT catalogue. The most common type is corresponding normal tissue: 21.3% of the registrations. Blood (8.2%), plasma (7.5%) and serum (5.7%) are also available in some local biobanks. More than 73% of the metastasis samples (9,517) are stored at -80°C.
Our data illustrate the great value of the BVT catalogue for cancer research, in particular for research on less frequent tumour samples such as metastases. Researchers that request access to the BVT catalogue can perform queries, based on specific search criteria, to trace the samples of interest located at different local biobanks of the BVT network.

Abstract n°15

In vivo study of follicular activation after transplantation of cryopreserved murine ovaries

Carmen Terren (1), Ancion Marie (1), Michelle Nisolle (1,2), Carine Munaut (1)

(1) Laboratory of Tumor and Development Biology, GIGA-Cancer, University of Liège, Tour de Pathologie (B23), Sart Tilman, B-4000 Liège, Belgium
(2) Department of Obstetrics and Gynecology, Hôpital de la Citadelle, University of Liège, B-4000 Liège, Belgium

Presenting author: Carmen Terren, ULiège, GIGA-Cancer (LBTD), Avenue Hippocrate 13, 4000 Liège - carmen.terren@uliege.be

Cryopreservation of ovarian tissue followed by its autotransplantation is currently the only option to preserve the fertility of prepubertal patients or when oncological care is urgent. However, this technique has certain limitations, including massive follicular loss during this process, resulting from follicular hyperactivation.

Our aim was to adapt an original transplantation model to study the ovarian follicular activation process. This model terms "ear mice model", previously develop to study angiogenesis and lymphogenesis, involved tissue transplantation between the ear skin. This model is intended to be an alternative to the ovarian orthotopic transplantation site. One important advantage is that ears are easily accessible for subsequent local treatments. To test this model, cryopreserved murine ovaries were transplanted between the two mouse ear skin layers for either 3 days or 3 weeks and analyzed by immunohistochemistry (5 ovaries per group). Ovarian vascularization was highly increased after 3 weeks of transplantation (CD31 and FITC staining). Computer-assisted detection of fibrosis revealed a decrease of fibrosis after 3 weeks of transplantation as compared to 3 days. Nucleus density and apoptosis were similar after 3 days or 3 weeks of transplantation. Conversely, cell proliferation increased after 3 weeks of transplantation.

We next used this model to highlight the various molecular signaling pathways responsible for follicular activation following ovarian transplantation. An increased expression of the phosphorylated forms of Jnk and mTOR after transplantation of cryopreserved ovaries for 4 weeks as compared to fresh transplants was detected. In order to limit this follicular activation after cryopreservation and transplantation of murine ovaries, specific inhibitors of the Jnk and mTOR pathway, SP600125 and Rapamycin, respectively, were injected locally during 3 days and transplants were recovered after 2 and 4 weeks (6 ovaries per group). No difference in p-Jnk and p-mTOR labeling density between control and inhibitors treated groups were observed.

In conclusion, preliminary result indicates that the "ear mice model" could be suitable for ovarian transplantation in order to study follicular survival and activation. The Jnk and mTOR pathways are implicated in the hyperactivation process after cryopreservation and transplantation of murine ovarian tissue. Their inhibition needs however to be improved.

Abstract n°16

Context dependency of epithelial to mesenchymal transition for metastasis

Tatiana Revenco (1), Adeline Nicodème (1), Ievgenia Pastushenko (1), Magdalena K. Sznurkowska (1), Mathilde Latil (1), Panagiota A. Sotiropoulou (1), Christine Dubois (1), Virginie Moers (1), Sophie Lemaire (1), Viviane de Maertelaer (2) & Cédric Blanpain (1, 3)

(1) Laboratory of Stem Cells and Cancer, Université Libre de Bruxelles, Brussels B-1070, Belgium
(2) RIBHM, Université Libre de Bruxelles, Brussels B-1070, Belgium
(3) WELBIO, Université Libre de Bruxelles, Brussels B-1070, Belgium.

Presenting author: Tatiana Revenco – ULB, Laboratory of Stem Cells and Cancer, route de Lennik 8080, 1070 Brussels - trevenco@ulb.ac.be

Metastasis is the terminal disease of oncological patients. It can occur from weeks to years following tumour resection and is incurable. Metastatic dissemination is a very complex process and it includes that cancer cells leave the tumour, extravasate and survive in the blood stream, intravasate from the vessels, invade the distant organs and grow to form overt metastases. Although the basic steps of metastatic cascade are known based on clinical observations, the mechanisms of each step are still elusive.

Epithelial to mesenchymal transition (EMT) has been proposed to be important for metastatic dissemination. EMT is a physiological process that occurs during embryonic development and is characterized by the loss of adhesion molecules and increased expression of mesenchymal markers. This process was also observed in several types of tumours and is correlated with poor prognosis, resistance to anti-cancer therapy and metastasis. However, recent studies have challenged the requirement of EMT for metastasis leaving the next questions open: is EMT important for metastatic dissemination? Does EMT occur at the primary tumour or in the blood? What is the phenotype of circulating tumor cells (CTCs)? Do all CTCs have the same potential to colonize the distant organ? Is mesenchymal to epithelial transition (MET) important for the formation of overt metastases?

Here, we assessed in different models of primary skin squamous cell carcinoma (SCCs) whether EMT is associated with metastasis. The incidence of metastasis was much higher in SCCs presenting EMT compared to SCCs without EMT, supporting the notion that a certain degree of EMT is required to initiate the metastatic cascade in primary skin SCC. Most circulating tumor cells presented EMT, whereas most lung metastasis did not present EMT, showing that MET is important for metastatic colonization. In contrast, transplanted mice with SCCs, whether displaying EMT or not, presented metastasis. Altogether, our data demonstrate that the association of EMT and metastasis is model-dependent, and that metastasis of primary skin SCCs is associated with EMT.

Abstract n°17

Loss of the tumor suppressor RASSF4 is associated with aggressive disease and poor outcome in Diffuse Large B Cell Lymphoma

Michiel De Coster(1), Philip Vlummens(1,2), Ken Maes(1), Kim De Veirman(1), Eline Menu(1), Karin Vanderkerken(1) and Elke De Bruyne(1)

(1) Department of Hematology and Immunology-Myeloma Center Brussels, Vrije Universiteit Brussel, Brussels, Belgium;
(2) Hematology, Department of Internal Medicine, Ghent University Hospital, Belgium.

Presenting author: Michiel De Coster – Vrije Universiteit Brussel, Department of Hematology and Immunology-Myeloma Center Brussels, Laarbeeklaan 103, 1090 Brussels - michiel.de.coster@vub.be

Mantle cell lymphoma (MCL) and diffuse large B cell lymphoma (DLBCL) are two aggressive B cell non-Hodgkin lymphomas. Currently, treatment consists of rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP). Unfortunately, most MCL and ±30-40% of the DLBCL patients will eventually relapse and die of refractory disease. This calls for the identification of novel targets involved in MCL and DLBCL pathogenesis. We recently showed that epigenetic repression of the tumor suppressor RASSF4 promotes RAS-driven malignant progression in the incurable plasma cell malignancy multiple myeloma (MM). Treatment with epigenetic modulating agents restored RASSF4 expression and RASSF4 overexpression sensitized MM cells to the standard-of-care agent bortezomib and the MEK1/2 inhibitor trametinib. So far, however, little is known about the expression and role of RASSF4 in DLBCL and MCL. Here, we investigated the prognostic value of RASSF4 in DLBCL and MCL using publicly available gene expression profiling data of two independent cohorts of DLBCL patients and one cohort of MCL patients. We found that DLBCL patients with low RASSF4 mRNA levels have a significant worse survival outcome and this especially when exposed to rituximab. In contrast, no significant difference in clinical outcome was found in MCL patients. Multivariate analysis furthermore revealed that RASSF4 is an independent good prognostic factor for overall survival in R-CHOP treated DLBCL patients. Importantly, R-CHOP treated DLBCL patients with low RASSF4 mRNA levels have a more complex karyotype, with 88% of the patients having a complex karyotype in the low expressers vs 57% in the high expressers (p=0.019). Moreover, GSEA analysis showed significant enrichment of genes involved in KRAS-, EGFR-, HGF-, PDGF-, HOXA5- and NF-κB-signaling, hypoxic response and condensation of prophase chromosomes in the low expressers. Finally, RASSF4 mRNA levels were found significantly reduced in human cell lines compared to primary cells. Together, our data suggest that RASSF4 is also a tumor suppressor in DLBCL and loss of RASSF4 favors disease progression. Moreover, our data suggest that RASSF4 is a new predictive marker for response to R-CHOP treatment. These findings thus provide the rationale for future preclinical studies studying the epigenetic regulation of RASSF4 and its role in DLBCL pathogenesis.

Abstract n°18

The role of the de novo DNA methyltransferase DNMT3B in multiple myeloma cell survival and drug resistance

Catharina Muylaert(1), Philip Vlummens(1,2), Ken Maes(1), Kim De Veirman(1), Eline Menu(1), Karin Vanderkerken(1) and Elke De Bruyne(1)

(1) Department of Hematology and Immunology-Myeloma Center Brussels, Vrije Universiteit Brussel, Brussels, Belgium;
(2) Hematology, Department of Internal Medicine, Ghent University Hospital, Belgium.

Presenting author: Catharina Muylaert – Vrije Universiteit Brussel, Department of Hematology and Immunology-Myeloma Center Brussels, Laarbeeklaan 103, 1090 Brussels - catharina.muylaert@vub.be

Multiple myeloma (MM) is an incurable hematological malignancy characterized by the accumulation of malignant plasma cells in the bone marrow (BM). Although new treatments developed over the last few years have significantly increased the median life expectancy, there is still a high chance of relapse due to the development of DR. In order to counter relapse, the mechanisms involved in DR should be investigated more thoroughly. It is well known that MM is a heterogenous disease, both encompassing genetic and epigenetic aberrations. These epigenetic defects contribute to genomic instability, disease progression and highrisk disease. However, for most of the enzymes mediating the epigenetic modifications (so-called epigenetic modifiers or epiplayers), their exact role in MM cell biology and DR has not yet been sufficiently explored. Using the publicly available RNA-seq data form matched newly diagnosed and relapsed samples from the MMRF CoMMpass study, we recently found that the epiplayer DNA methyltransferase 3B (DNMT3B) is upregulated in relapsed samples compared to newly diagnosed samples. This suggests a role for DNMT3B in DR in MM. Here, we examined the expression and role of DNMT3B in MM cell biology. Using publicly available gene expression profiling data, we found that high DNMT3B mRNA expression is correlated with a worse disease outcome in both newly diagnosed and relapsed patients. Moreover, DNMT3B mRNA levels were found significantly increased in human cell lines compared to primary MM cells. Specific DNMT3B targeting by the selective inhibitor Nanaomycin A in cell lines with high DNMT3B levels strongly reduced MM cell viability and induced apoptosis. In contrast, bone marrow stromal cells (BMSC) were only slightly affected by Nanaomycin A, indicating that Nanaomycin A is not as toxic for the BMSC as for the myeloma cells. Together, our findings indicate that DNMT3B could indeed play an important role in the survival of MM cells. In the future, the role of DNMT3B in MM cell biology and drug resistance and the therapeutic and predictive potential of DNMT3B will be evaluated in 3D spheroid models, the murine 5TMM models and primary human samples.

Abstract n°19

The PI3K/Akt pathway as a promising therapeutic target in acquired cetuximab resistant head and neck squamous cell carcinoma

H. Zaryouh (1), H. Baysal (1), P. Pauwels (1,2), M. Peeters (1,3), J.B. Vermorken (1,3), F. Lardon (1), A. Wouters (1, *), I. De Pauw (1, *)

(1) Center for Oncological Research (CORE), University of Antwerp, Belgium
(2) Department of Pathology, Antwerp University Hospital, Belgium
(3) Department of Medical Oncology, Antwerp University Hospital, Belgium
(*) These authors share senior authorship.

Presenting author: Hannah Zaryouh – University of Antwerp, Center of Oncological Research (CORE), Universiteitsplein 1, 2610 Antwerp - hannah.zaryouh@uantwerpen.be

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer type worldwide and a challenging malignancy to treat. Despite initially promising results, resistance to therapies targeting the epidermal growth factor receptor (EGFR), such as cetuximab, remains a major roadblock in the search for effective therapeutic strategies in HNSCC. Constitutive activation of compensatory oncogenic signalling pathways has been proposed as a potential mechanism underlying cetuximab resistance. In this regard, increasing evidence suggests that aberrant signalling of the PI3K/Akt pathway is involved in resistance to cetuximab treatment. Therefore, this study aims to investigate whether inhibition of the PI3K/Akt pathway might be a novel therapeutic strategy to overcome acquired cetuximab resistance in HNSCC.

Acquired cetuximab resistant HNSCC cell lines (SC263-R and SCC22b-R) were generated by chronically exposing initially sensitive cell lines to cetuximab. In parallel, control cell lines (SC263-PBS and SCC22b-PBS) were established by exposing these cells to the vehicle control (PBS). Cytotoxicity of the Akt-inhibitor MK2206 (72h, 0-10µM) and the PI3K inhibitor buparlisib (72h, 0-1µM) was assessed under normoxic and hypoxic (1% O2) conditions using the sulforhodamine B (SRB) assay. IC50 values were calculated using the WinNonlin Software.

The Akt inhibitor MK2206 showed a clear concentration-dependent cytotoxic effect in both cetuximab sensitive and acquired resistant HNSCC cell lines with IC50 values ranging between 1.36 +/- 0.23 and 2.57 +/- 0.57 µM. Acquired cetuximab resistance had no significant effect on the cytotoxicity of MK2206 ($p=0.831$). Interestingly, MK2206 monotherapy demonstrated an increased cytotoxic effect under hypoxia ($p=0.009$). Furthermore, preliminary results indicate that the PI3K inhibitor buparlisib also has a cytotoxic effect in both cetuximab sensitive and acquired resistant HNSCC cell lines in a concentration-dependent manner. IC50 values of the first experiment were ranging between 0.50 +/- 0.02 and 0.66 +/- 0.04 µM under normoxic conditions and between 0.35 +/- 0.02 and 0.59 +/- 0.03 µM under hypoxic conditions. This experiment is currently being repeated.

In conclusion, these data support the hypothesis that targeting the PI3K/Akt signalling pathway with either MK2206 or buparlisib might be a promising novel therapeutic strategy to treat HNSCC patients experiencing acquired cetuximab resistance.

Abstract n°20

Towards the validation of a new anticancer strategy: Designed peptides targeting Lactate Dehydrogenase tetramerization process

Léopold Thabault,(1,2) Lucie Brisson,(2,3) Chiara Brustenga,(1) Santiago A. Martinez Gache,[4,5,6] , Julien R. C. Prévost,(1) Arina Kozlova,(1) Quentin Spillier,(1,2) Zohra Benyahia,(2) Joris Messens,(4,5,6) Tamara Copetti,(2) Pierre Sonveaux,(2), Raphaël Frédérick,(1)

(1) Louvain Drug Research Institute (LDRI), Université catholique de Louvain (UCLouvain), Brussels, Belgium.
(2) Pole of Pharmacology and Therapeutics, Institut de Recherche Expérimentale et Clinique (IREC), Université catholique de Louvain (UCLouvain), Brussels, Belgium.
(3) INSERM UMR1069, Nutrition, Croissance et Cancer, Université François-Rabelais, Tours, France.
(4) VIB-VUB Center for Structural Biology, Brussels, Belgium.
(5) Brussels Center for Redox Biology, Brussels, Belgium.
(6) Structural Biology Brussels, Vrije Universiteit Brussel, Brussels, Belgium.

Presenting author: Léopold Thabault - UCLouvain, IREC/LDRI, Avenue Mounier 73, 1200 Brussels - leopold.thabault@uclouvain.be

Lactate Dehydrogenases (LDH) have been shown to be involved in several oncogenic pathways such as Autophagy1, Metabolic symbiosis2, Angiogenesis3 and Invasiveness4. LDH are therefore considered as promising targets for cancer therapy. Yet, their highly polar catalytic site makes them a challenging target for small molecules inhibition. However this issue could be addressed with the development of allosteric site inhibitors. As LDH tetrameric state is mandatory for its activity, the study herein focuses on the prospect of targeting LDH tetramerization process.
LDH N-terminal domain is known to play a key role in its tetrameric assembly. Corresponding to an extended 19 amino-acids arm interacting with a lipophilic tetramerization site, this tetramerization domain was used in this study as a starting point for the development of new LDH tetramerization site ligands. The N-terminal domain derived peptide LB19 was therefore exploited as a starting compound with the objective to design new tool compounds targeting LDH tetramerization.
Using Nuclear Magnetic Resonance (NMR) and MicroScale Thermophoresis (MST) experiments, the characterization of the interaction between LB19 and LDH tetramerization site was performed. This evaluation led to the sequence optimization of LB19, yielding an 8 amino-acids peptide bearing all the interacting residues (LB8).
Following the identification of the minimal interacting sequence, an extensive study of the structure activity relationship (SAR) between LB8 and LDH tetramerization site was performed. This evaluation highlighted LB8 key interacting residues. Such SAR was further validated by performing single point mutations on LDH tetramerization domain and studying the resulting impact on LDH tetrameric stability. Exploitation of this SAR enabled the design of more rigid and potent analogs of LB8, leading to the cyclic peptide 1, an in vitro micromolar inhibitor of LDH tetramerization process.

Abstract n°21

In vivo Identification and characterization of melanoma metastatic initiating cells

Panagiotis Karras(1,2), Alberto Paulo De Borba Neto(1,2), Michael Dewaele(1,2), Joanna Pozniak(1,2) Florian Rambow(1,2) and Jean-Cristophe Marine(1,2)

(1) Laboratory for Molecular Cancer Biology, VIB Center for Cancer Biology
(2) Department of Oncology, KU Leuven, Belgium

Presenting author: Panagiotis Karras – VIB-CCB, Oncology, Herestraat 49, 3000 Leuven-panagiotis.karras@kuleuven.vib.be

An incomplete view of the mechanisms that drive metastasis, the primary cause of melanoma-related death, has been a major barrier to development of effective therapeutics and prognostic diagnostics. Increasing evidence indicates that the interplay between microenvironment, genetic lesions and cellular plasticity drives the metastatic cascade. We performed a longitudinal and exhaustive analysis of the diversity and trajectories of melanoma cell states during metastatic dissemination by combining single-cell profiling techniques with lineage tracing in clinically-relevant mouse models of melanoma. Among others, we identified one specific melanoma cell state with EMT characteristics, which is present in both mouse and human primary tumors, which drives the metastatic process. We deciphered the gene regulatory network underlying this particular state and developed therapeutic modalities that prevent phenotype switching into this state. This study highlights how understanding the magnitude and dynamics of non-genetic reprogramming in space and time at single-cell resolution can be exploited to develop therapeutic strategies that capitalize on non-genetic tumor evolution.

Abstract n°22

Melanoma tumor growth is fueled according to the hierarchical model

Panagiotis Karras(1,2), Alberto Paulo De Borba Neto(1,2), Florian Rambow(1,2), Benjamin D. Simons(3) and Jean-Christophe Marine(1,2)

(1)Laboratory for Molecular Cancer Biology, VIB Center for Cancer Biology
(2)Department of Oncology, KU Leuven, Belgium
(3)Wellcome Trust Centre for Stem Cell Research, University of Cambridge, Cambridge, UK.

Presenting author: Panagiotis Karras – VIB-CCB, Oncology, Herestraat 49, 3000 Leuven-panagiotis.karras@kuleuven.vib.be

Melanoma is notorious for its high degree of heterogeneity and plasticity, which are key drivers of metastatic spreading and therapy resistance. Studies addressing the contribution of the heterogeneous subpopulations to melanoma growth have so far remained limited to functional in vitro clonogenic assays and xenotransplantations and have led to conflicting conclusions. A key pending question in the field is whether melanoma follows the stochastic model of growth, where virtually all cells are equipotent in their ability to sustain tumor growth, or the Cancer Stem Cell (CSC) model, where only a small fraction of cells fuel tumor growth. To capture the dynamics of individual tumor growth capacity within their natural microenvironment, we performed multicolour lineage tracing in a spontaneous (NRAS-driven) mouse model of melanoma. Quantitative clonal analyses, dual pulse labelling proliferation assays, 3D tumor reconstruction and mathematical modeling revealed that the majority of labelled melanoma cells exhibited limited proliferative potential, whereas a small fraction of cells displayed the capacity to persist long term, giving rise to progenies that occupy a significant part of the tumour. These unbiased analyses uncovered the presence of two distinct proliferative cell states organized in a hierarchical manner indicating that the more persistent state has stem cell-like features, whereas the other gives rise to terminally differentiated tumour cells. The stemness properties of these cells exhibit a niche dependency and are maintained theire characteristics in close proximity of vessels. Importantly, their transcriptional activity was assessed by single cell –OMICs approaches and it is distinct from other melanoma cells. This study highlights the existence of CSCs in primary melanoma lesions, an observation that have important therapeutic implications.

Abstract n°23

Targeting both Hsp90 and MEK proteins as a therapeutic strategy in KRAS-mutant LGSOC

Elien De Thaye (1,2), Koen Van de Vijver (2,3), Glenn Wagemans (2,4), Elly De Vlieghere (2,4), Johanna Mestach (2,4), Emiel De Jaeghere (2,4,5), Hannelore Denys (2,5), Jan Van Bocxlaer (1), Pascal de Tullio (6), Geert Berx (2,7), Mark Carey (8), Olivier De Wever (olivier.dewever@ugent.be) (2,4)

(1) Laboratory of Medical Biochemistry and Clinical Analysis, Ghent University, Ghent, Belgium
(2) Cancer Research Institute Ghent (CRIG), Ghent, Belgium
(3) Department of Pathology, Ghent University Hospital, Ghent, Belgium
(4) Laboratory of Experimental Cancer Research, Ghent University, Ghent, Belgium
(5) Department of Medical Oncology, Ghent University Hospital, Ghent, Belgium
(6) Center for Interdisciplinary Research on Medicines (CIRM), Université de Liège, Liège, Belgium
(7) Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium
(8) Department of Obstetrics and Gynecology, University of British Columbia, Vancouver, BC, Canada

Presenting author: Elien De Thaye – Ghent university, Laboratory of Medical Biochemistry and Clinical Analysis, Ottergemsesteenweg 460, 9000 Gent - Elien.DeThaye@UGent.be

Ovarian cancer is associated with the worst prognosis and highest mortality rate from all gynecological cancers. Characterized by an asymptomatic tumor growth and delayed onset of symptoms, most women present with the disease in an advanced stage. At this point, the cancer shows widespread peritoneal carcinomatosis and is difficult to treat. The majority of ovarian carcinomas are serous tumors including the high-grade and low-grade subtype. Low-grade serous ovarian cancer (LGSOC), accounting for about 10% of all serous carcinomas, is different from other ovarian cancers as it affects younger women and is often resistant to chemotherapy.

Characterized by a high prevalence of KRAS mutations affecting the mitogen-activated protein kinase (MAPK) pathway, the use of targeted therapies such as MEK inhibitors remains an active area of investigation in LGSOC. However, targeting MEK can result in acquired resistance through activation of complementary prosurvival pathways such as Akt. Since Akt is a client of the Hsp90 chaperone system, dual inhibition of the MEK and Hsp90 signaling pathways was investigated in clinically relevant KRAS wild type and KRAS (G12V)-mutant patient-derived cell lines from LGSOC primary tumor and peritoneal metastases.

Combined Hsp90 and MEK inhibitor treatment efficiently inhibits cell confluence and colony formation of KRAS mutant but not KRAS wild type cells. In addition, combination of both drugs result in an important decrease of AKT expression and ERK, AKT and mTOR phosphorylation. In vivo, using the combination therapy over single or no therapy resulted in a significant better control of peritoneal metastasis and survival. In conclusion, dual targeting of Hsp90 and MEK proteins may represent a potential promising therapeutic strategy against KRAS-mutant LGSOC.

Abstract n°24

Barcode sequencing experiment in yeast identifies genes important for the response to photon and proton irradiation

Laura Vanderwaeren (1,2,3), Rüveyda Dok (1), Karin Voordeckers (2,3), Kevin Verstrepen (2,3), Sandra Nuyts (1,4)

(1) Laboratory of Experimental Radiotherapy, Department of Oncology, KU Leuven, Leuven, Belgium
(2) CMPG Laboratory of Genetics and Genomics, KU Leuven, Leuven, Belgium
(3) VIB – KU Leuven Center for Microbiology, Leuven, Belgium
(4) Department of Oncology, Leuven Cancer Institute, UZ Leuven, Leuven, Belgium

Presenting author: Laura Vanderwaeren – KU Leuven, Oncology, Herestraat 49, 3000 Leuven - laura.vanderwaeren@kuleuven.vib.be

During their course of illness, up to 50% of all cancer patients are treated with radiotherapy, either alone or in combination with other cancer treatments. The goal of radiotherapy is to kill the tumour cells, but normal tissue can be damaged leading to normal tissue toxicity. Technical improvements in radiotherapy delivery and planning resulted in increased precision and accuracy of radiotherapy delivery. However, toxicity issues to the normal tissue remain a major problem and is particularly challenging for head and neck squamous cell carcinoma (HNSCC) due to the close proximity of critical organ structures like the spinal cord and the swallowing apparatus. Proton beam radiation, because of its unique energy deposition, forms an alternative that can overcome this problem of high toxicity associated with conventional X-rays (photons). However, the underlying radiobiology of proton irradiation is not well understood.

In this research, we assessed the radiobiological response of photon and proton irradiation by a barcode sequencing experiment in the model eukaryote yeast. We used the haploid yeast deletion collection consisting of ~4800 gene deletion mutants with unique barcodes to identify them. These mutants were pooled in equal ratios and irradiated with 50 Gy photons (6 MV) or 50 Gy protons (62 MeV, right before the Bragg peak). We examined how the ratios changed 8 generations after irradiation. This led us to identify genes important for the response to photon and proton irradiation. For both irradiation types, genes involved in cell cycle processes and DNA repair were enriched. The main DNA repair pathway after photon and proton irradiation was homologous recombination. These results suggest similar DNA repair after photon and proton irradiation in yeast. However, the amount of DNA damage and the kinetics of repair can differ and will be further investigated. The strong conservation of basic biological and biochemical pathways as well as gene function between yeast and humans, will allow us to translate and validate these findings in human HNSCC cells.

Abstract n°25

Characterization of pancreatic cancer stem cells using a stem-reporter

Nona Laura Lisa Merckx (1,2)

(1) Instituto Nacional de Medicina Genomica (INMEGEN)
(2) Universitat Autonoma de Barcelona (UAB)

Presenting author: Nona Laura Lisa Merckx - VIB, Center for Brain & Disease Research, Shorenshof 15, 3012 Leuven - nonamerckx@kuleuven.vib.be

Background: Cancer stem cells (CSCs) are an upcoming part of the tumor microenvironment. CSCs have enhanced abilities to drive tumorigenesis and can induce relapse via chemo-resistance. We focus on a relatively new technique to isolate CSCs based on a GFP-stem-reporter controlled by stemness transcription factors SOX2 and OCT4. Isolated CSCs are expected to have self-renewal abilities and high tumor- and metastasis-initiating abilities. We here focus on CSCs in pancreatic ductal adenocarcinoma (PDAC), an aggressive cancer type with an extremely low survival rate of 9% (5-years).

Methods: The GFP-stem-reporter was introduced in a PDAC cell line (AsPC-1) by lentiviral infection. CSCs were analysed by flow cytometry as GFP+ in both 2D and 3D (CSC enriching technique) in vitro culturing technique. Downstream characterisation of GFP+ cells is ongoing by means of in vivo tumorigenic capacities in zebrafish and single cell transcriptome analysis.

Results: A net CSC proportion of 0.42% was determined in 2D cultures. Enrichment for CSCs by 3D culturing increased the proportion to 1.61% which confirms correct vector integration. Downstream gene expression analysis and in vivo xenotransplantation requires optimisation which is ongoing.

Future prospective: This relatively new method to isolate CSCs can be utilised in various downstream applications, including the identification of CSC specific therapeutic targets. The need for novel therapeutic targets in PDAC is high and combining a CSC targeting therapy with conventional chemotherapy is a promising strategy to overcome chemo-resistance.

Abstract n°26

The development of methods to detect the different hallmarks of immunogenic cell death

Jolien De Munck (1), Philipp Kalus (1), Halima Lara Smets (1) Karine Breckpot (2), Joeri Aerts (2)

(1) Neuro-Aging and Viro-Immunology, Vrije Universiteit Brussel, Brussels, Belgium
(2) Laboratory for Molecular and Cellular Therapy, Vrije Universiteit Brussel, Brussels, Belgium

Presenting author: Jolien De Munck – Vrije Universiteit Brussel, Neuro-Aging and Viro-Immunotherapy, Laarbeeklaan 103, 1090 Brussels - jolien.de.munck@vub.be

Immunogenic cell death (ICD) is a form of regulated cell death (e.g. immunogenic apoptosis, necroptosis) that is able to activate both an innate and adaptive immune response. The induction of ICD is an interesting strategy in the development of future immunotherapies. So far, several markers of immunogenic cell death are known and summarized by Galluzzi L. et al. (Cell death and differentiation, 2018), including calreticulin (CRT), ATP, high-mobility group box I (HMGB-1), type I interferon (IFN) and annexin A1 (ANXA1). In this context, we developed and/or optimized different assays to detect these hallmarks using both flow cytometry based and enzyme-linked immunosorbent assays (ELISA). 624-mel, 938-mel (both human melanoma cells) and B16 (mouse melanoma cells) were treated with several known ICD-inducers like staurosporine and doxorubicine and analyzed with different in-house developed and/or optimized protocols. The presence of exposed CRT can be analyzed with a flow cytometry based assay. ATP can be detected after staining of treated cells with quinacrine, a dye that stains intracellular ATP vesicles. Using flow cytometry, it can be shown that the percentage of quinacrine positive cells is decreasing after induction of ICD. Using a reporter cell line, HEK293T cells transduced with a lentiviral vector expressing a reporter gene under the control of an IFN dependent promotor, the supernatant of treated cells can be screened for the presence of type I IFN. The assays for the detection of both HMGB-1 and ANXA1 are currently being optimized. In conclusion, these methods allow to analyze the different markers of ICD and make it possible to fingerprint the type of ICD in different experimental settings.

Abstract n°27

Characterization of the metabolic control of brain metastasis in breast cancer.

Marine CNM Blackman (1), Thibaut Vazeille (1), Loïc Hamelin (1) and Pierre Sonveaux (1)

(1) Pole of Pharmacology and Therapeutics (FATH), Institut de Recherche Expérimentale et Clinique (IREC), Université de Louvain (UCLouvain), Belgium.

Presenting author: Marine Blackman – UCLouvain, IREC, FATH, Avenue Hippocrate 57, Brussels - marine.blackman@uclouvain.be

Introduction: Brain metastases are devastating, affecting up to 50% of triple-negative breast cancer (TNBC) patients.1 We previously found that altered metabolic behaviors of cancer cells can promote the metastatic process.2,3 In line with the seed-and-soil hypothesis,4 we hypothesized that metabolic alterations could also control the tropism of metastases to the brain. For cancer therapy, we aim to identify differentially expressed metabolic enzymes or transporters in cancer cells that control brain-specific metastasis.

Experimental: Our main cellular model consisted of MDA-MB-231 human metastatic TNBC cells and corresponding brain-seeking variants that were generated independently by serial cycles of in vivo selection for brain specificity.5 Comparison was performed using RNAseq, followed by RT-qPCR and WB validation. A CRISPR/Casp9 strategy was then used to test gene functions in brain-specific metastatic variants.

Results: Compared to wild-type MDA-MB-231 cells, brain-specific variants had increased mitochondrial respiration. Using RNAseq, RT-qPCR and WB, we identified increased expression of MBL1 and MBL2 genes*. Brain variants deficient for MBL1 and MBL2 (CRISPR/Cas9) had decreased mitochondrial respiration and a reduced clonogenic potential, whereas their migratory activity was unchanged.

Conclusions: Selective metastasis of TNBC cells to the brain is linked to increased mitochondrial respiration, clonogenic potential, and MBL1 and MBL2 gene overexpression.

References: 1. Lin NU et al. Cancer. 2008;113:2638-45. 2. Payen VL et al. Cell Mol Life Sci. 2016; 73:1333-48. 3. Porporato PE et al. Cell Mol Life Sci. 2016;73:1349-63. 4. Paget S Cancer Metastasis Rev. 1889;8:98-101. 5. Burnett RM et al. Oncotarget. 2015;6:12682-96.

Acknowledgements: We thank P. Steeg and H. Nakshatri for the gift of brain-seeking cell lines. Funding from the Belgian F.R.S.-FNRS, Télévie and French Community of Belgium (ARC 14/19-058). *Names of genes not disclosed for IP protection.

Abstract n°29

Immune cell infiltration in cervical tumor tissue as a marker for response to (chemo)radiotherapy

Lien Lippens (1,2,3), Mieke Van Bockstal (1,4), Emiel A. De Jaeghere (1,2,3), Philippe Tummers (3,5), Amin Makar (5), Sofie De Geyter (1,2), Koen Van de Vijver (4), An Hendrix (1,3), Katrien Vandecasteele (3,6)*, Hannelore Denys (2,3)*

(1) Laboratory of Experimental Cancer Research, Department of Human Structure and Repair, Ghent University, Ghent, Belgium
(2) Medical Oncology, Department of Internal Medicine and Pediatrics, Ghent University Hospital, Ghent, Belgium
(3) Cancer Research Institute Ghent (CRIG), Ghent, Belgium
(4) Pathology, Department of Diagnostic Sciences, Ghent University Hospital, Ghent, Belgium
(5) Gynecology, Department of Human Structure and Repair, Gent University Hospital, Ghent, Belgium
(6) Radiation Therapy, Department of Human Structure and Repair, Ghent University Hospital, Ghent, Belgium
** Last authors contributed equally*

Presenting author: Lien Lippens – Ghent University, Laboratory for Experimental Cancer Research, Corneel Heymanslaan 10, 9000 Ghent - lien.lippens@ugent.be

Introduction. As cervical cancer patients show heterogeneous response to treatment, a marker to assign the most optimal treatment schedule to each patient is needed. We investigated different subtypes of infiltrating immune cells in pre-treatment biopsies and post-treatment resection samples as well as the ratio of both as potential markers for response, metastasis status and survival.

Methodology. Cervical cancer patients treated with (chemo)radiotherapy and subsequent surgery were included in the current study. Pre- and post-treatment specimens were retrospectively analysed via immunohistochemistry. Immune cell markers for T cells (CD3, CD4, CD8, and FoxP3), macrophages (CD68 and CD163), and B cells (CD20), as well as interleukin-33 (IL33) and programmed death-ligand 1 (PD-L1), were analysed. Patients were grouped in the low score or high score group based on the amount of positive cells on immunohistochemistry. Correlations to pathological complete response (pCR), cause-specific survival (CSS), and metastasis development during follow-up were evaluated.

Results. In total, 38 patients were included. In analysis of pre-treatment biopsies, significantly more pCR was seen for patients with CD8=CD3, CD8≥CD4, positive IL33 tumour cell (TC) scores, IL33 immune cell (IC)<TC, and PD-L1 TC≥5%. Besides patients with high CD8 scores, also patients with CD8≥CD4, CD163≥CD68, or PD-L1 IC≥5% had better CSS. In analysis of post-treatment specimens, less pCR was observed for patients with high CD8 or CD163 scores. Patients with decreasing CD8 or CD163 scores between pre- and post-treatment samples showed more pCR, whereas those with increasing CD8 or decreasing IL33 IC scores showed a worse CSS. Meanwhile, patients with an increasing CD3 score or stable/increasing PD-L1 IC score showed more metastasis during follow-up.

Conclusion. The intratumoural immune cell landscape is a tool for prediction of outcome and response to (chemo)radiation.

Abstract n°30

Combination of targeted therapies including p53 reactivator, PRIMA 1Met and SRC inhibitor, dasatinib in thyroid cancer

Laetitia Gheysen (1), Giuseppe Attardo (1), Dejean Thomas (1), Anne Trelcat (1), Fabrice Journe (1), Sven Saussez (1)

(1) Department of human anatomy and experimental oncology, Faculty of medicine and Pharmacy ,6, Avenue du Champ de Mars 7000 MONS

Presenting author: Laetitia Gheysen – University of Mons, Human anatomy and experimental oncology, Avenue du champs de Mars 6, 7000 Mons - laetitia.GHEYSEN@umons.ac.be

Thyroid cancer records around 2% of total cancers diagnosed worldwide and is the most frequent endocrine cancer. The anaplastic thyroid carcinoma (ATC) is one of the most aggressive types of any cancer merged and represents only 1-2% of all thyroid cancers but the mortality is about 100%. Until now, the clinical management proposed for ATC is a conventional therapy consisting of surgery and radiotherapy and/or chemotherapy, or the combination of dabrafenib and trametinib since 2018. Giving this situation, it is urgent to understand the failures of conventional treatments to purpose better combinations of treatments for each patient depending on his tumor signature.
The present work aims are to understand the mechanisms induced by the combination of dasatinib (multi-kinase inhibitor) and PRIMA-1Met (p53 reactivator) with different MAPK inhibitors as trametinib, vemurafenib,etc... and purpose an efficient treatment depending on the tumor signature.

In vitro assays including apoptosis assays have been performed on 6 different cell lines (8505C, CAL-62, FTC-133, TT2609-CO2, BCPAP, TPC-1). The treatment has been applied on cells during 48h, with a panel of concentrations of the molecules alone or in combination and assess the percentage of apoptosis via the Annexin V staining (FACS analysis). The aspect of proliferation has been evaluated by crystal violet staining. Combination index has been calculated with Calcusyn software (Biosoft, UK). Mechanisms implicated in targeted cellular pathways such as MAPK, PI3K, SRC or p53 have been studied by Western blot and RPPA method (reverse phase protein array).
In vivo assays have been led on an orthotopic mouse model of anaplastic thyroid carcinoma (8505C cell line). Mice were randomized in different groups including no treat, Dasatinib alone, PRIMA 1Met alone, Trametinib alone, Combination Dasatinib + PRIMA 1Met. Doses were administrated 5X/week by intraperitoneal injection. Concentrations of dasatinib (10mg/kg), PRIMA-1Met (50mg/kg), Trametinib (1mg/kg) were administrated. The tumour, lungs, liver and kidneys were collected after the euthanasia. Detection of p53, p21, Erk and galectin are planned to be detected by immunohistochemistry.
In vitro assays demonstrated an improvement of the efficiency with combinatorial treatment. In vivo assays are ongoing and needs more investigations.

Abstract n°31

AZD6738 is an effective radiosensitizer in both HPV-positive and HPV-negative head and neck cancer

Glorieux Mary 1, Dok Ruveyda 1, Nuyts Sandra 1;2

(1) KU Leuven, University of Leuven, Department of Oncology, Laboratory of Experimental Radiotherapy, 3000 Leuven, Belgium
(2) Department of Radiation Oncology, Leuven Cancer Institute, UZ Leuven, 3000 Leuven, Belgium

Presenting author: Mary Glorieux – KU Leuven, Oncology, Herestraat 49, 3000 Leuven - mary.glorieux@kuleuven.be

Background: Majority of all locally advanced head and neck squamous cell carcinoma (HNSCC) are treated with (chemo) radiotherapy (cRT). However, up to 50% of the advanced cancers still develop locoregional relapse. Ataxia telangiectasia and Rad3-related (ATR) plays a critical role in the orchestration of the DNA damage response, acting as apical kinase in response to replication fork stalling and DNA double strand breaks ultimately influencing the cell cycle checkpoint proteins.
Aim: Here, we investigate the relation between ATR inhibition, DNA repair and radiotherapy response both in vitro and in vivo.

Methods: The effect of the ATR inhibitor (AZD6738) was tested in vitro in a panel of 6 HNSCC cell lines, covering both HPV-positive and HPV-negative HNSCC.
The radiosensitizing effect was assessed via sulforhodamine B (SRB) and clonogenic assays. The effect on the cell cycle (PI) and DNA damage (gH2AX) was assessed by flow cytometry. The in vivo effects of AZD6738 were investigated in a CAL27 xenograft model in female nu/nu NMRI mice.

Results/Conclusion: AZD6738 significantly radiosensitized both HPV-positive and HPV-negative HNSCC in a dose-dependent manner as was shown by both SRB and colony assays. Immunoblotting demonstrated that AZD6738 inhibited the downstream phosphorylation of CHK1 thereby influencing the cell cycle which was confirmed by flow cytometry showing that AZD6738 treatment abrogated the RT-induced G2M-arrest. In addition, increased levels of DNA damage were observed after 36h of combination treatment RT with AZD6738. In vivo, combination of AZD6738 with RT significantly suppressed tumor growth in an HPV-negative HNSCC xenograft model for all doses of AZD6738 (25, 50 and 75 mg/kg) without toxic effects based on body weight. Currently, three different schedules are tested to optimize the combination of AZD6738 with RT: neoadjuvant, concurrent or adjuvant. In the future, the molecular mechanisms of radiosensitization will be more investigated focusing on apoptosis and replication stress.

Abstract n°32

The role of Akt in acquired cetuximab resistant head and neck squamous cell carcinoma: in vitro study on a novel combination strategy

Ines De Pauw (1), Hannah Zaryouh (1), Hasan Baysal (1), Patrick Pauwels (1,2), Marc Peeters (1,3), Jan B. Vermorken (1,3), An Wouters (1), Filip Lardon (1)

(1) Center for Oncological Research (CORE), University of Antwerp, Belgium
(2) Department of Pathology, Antwerp University Hospital, Belgium
(3) Department of Medical Oncology, Antwerp University Hospital, Belgium

Presenting author: Ines De Pauw, University of Antwerp, Center for Oncological Research, Universiteitsplein 1, Antwerp - ines.depauw@uantwerpen.be

The epidermal growth factor receptor (EGFR) is a therapeutic target in head and neck squamous cell carcinoma (HNSCC). Resistance to EGFR-targeted therapies, such as cetuximab, poses a challenging problem. This study aims to unravel resistance mechanisms by protein phosphorylation profiling. By doing this, we hope that new combination treatments can be designed to overcome therapy resistance.

Acquired cetuximab resistant HNSCC cell lines (SC263-R and SCC22b-R) were generated by chronically exposing initially sensitive cell lines to cetuximab. In parallel, control cell lines (SC263-PBS and SCC22b-PBS) were established by exposing these cells to the vehicle control (PBS). Proteome Profiler Human Phospho-Kinase Antibody Array kit (R&D Systems) was used to determine the relative levels of protein kinase phosphorylation in sensitive and acquired resistant cell lines after cetuximab treatment. Results were validated using western blot. Based on the protein phosphorylation profile, cytotoxicity of cetuximab plus the Akt-inhibitor MK2206 was assessed using the sulforhodamine B (SRB) assay. Hereby, two simultaneous combination schedules were tested:
(1) Cetuximab plus MK2206 with total treatment duration 72h
(2) Cetuximab for 168h with MK2206 added during the last 72h of treatment
Possible synergism between cetuximab and MK2206 was determined by calculating the combination index (CI) using the additive model. CI < 0.8, CI = 1.0 ± 0.2, and CI > 1.2 indicated synergism, additivity, and antagonism, respectively.

Protein-phosphorylation profiling showed increased phosphorylation of Akt1/2/3 after cetuximab treatment in acquired cetuximab resistant cells compared to cetuximab sensitive cells, which was confirmed by western blotting. Synergy between cetuximab and the Akt inhibitor MK2206 was observed in three out of four HNSCC cell lines (i.e. in SC263-PBS, SC263-R and SCC22b-PBS with CI < 0.783) in both simultaneous treatment schedules.

This study demonstrates that increased Akt1/2/3 phosphorylation might be characteristic for acquired cetuximab resistance in HNSCC cell lines. Our results also show an additive to synergistic interaction between cetuximab and MK2206 in simultaneous treatment schedules. These data support the hypothesis that the combination of cetuximab with an Akt inhibitor might be a promising novel therapeutic strategy to overcome acquired cetuximab resistance in HNSCC.

Abstract n°33

Evaluation of Metastatic Character of Head and Neck Squamous Cell Carcinoma Following Non-Thermal Plasma Treatment in 3D Spheroids and in ovo Tumour Models

Hanne Verswyvel (1,2), Abraham Lin (1,2), Angela Privat-Maldonado (1,2), Annemie Bogaerts (2), Evelien Smits (1)

(1) Center for Oncological Research (CORE), University of Antwerp, Antwerp, Belgium,
(2) PLASMANT, Department of Chemistry, University of Antwerp, Antwerp, Belgium

Presenting author: Hanne Verswyvel – University of Antwerp, FGGW, CORE, Universiteitsplein 1, 2610 Antwerp - hanne.verswyvel@uantwerpen.be

Non-thermal plasma (NTP), a partially ionized gas, is a promising, anti-cancer treatment modality that has been emerging in the last decade. The working mechanisms and cellular effects of plasma are currently being studied, but the impact of NTP on several vital elements of the tumour microenvironment (TME) has been largely overlooked. Several key factors of the hostile TME, like hypoxia and altered cancer cell metabolism, actively participate in the process of cancer cell detachment, and locoregional and distant spread. This invasion of surrounding normal tissue by cancerous cells is a major cause of morbidity and cancer-related deaths for many cancer types, particularly in head and neck squamous cell carcinoma (HNSCC). Therefore, the aim of this study was to investigate plasma-induced changes on the migratory and invasive character of HNSCC. Since conventional 2D monolayer cancer models are inadequate in simulating the complex and dynamic processes in the TME, we performed our study using 3D tumour models.

In this study, we optimised and implemented two 3D tumour models, a micro-tissue spheroid model and a chicken embryo chorioallantoic membrane (CAM) model (in ovo model) for several HNSCC cell lines. To establish 3D tumour spheroids, cells were cultured in ultra-low attachment plates (ULA), forcing cells to grow in non-adherent 'spheroid-shaped' tumours. NTP treatment effects were analysed based on monitoring cell motility, migration profiles and matrix invasion capacities for several days after treatment. Tumour cells were also grown on the CAM of fertilised chicken eggs to create 3D cancerous growth in the egg model. This technique provides essential tumorigenic phenotypes like vascularisation and spontaneous cancer cell invasion without the use of animals. In this model, NTP-induced changes on metastatic spread of tumour cells from the primary tumour site were evaluated via detection of human DNA in several embryonic chicken organs. Altogether, these results will contribute to our current knowledge of NTP effects on tumours, their TME, and their effects on metastasis and will help find a niche for NTP in cancer therapy among existing treatments.

Abstract n°34

Development and application of the CRISPR-Cas9 technique in human cell lines of head and neck cancers infected with human papillomavirus

Sonia Furgiuele PhD student (1) Géraldine Descamps PhD (1), Lionel Tafforeau PhD (2), Fabrice Journe PhD (1), Sven Saussez MD PhD (1)

(1) Department of Human Anatomy and Experimental Oncology, Faculty of Medicine and Pharmacy, University of Mons
(2) Departement of Cellular Biology, Faculty of Sciences, University of Mons

Presenting author: Sonia Furgiuele – University of Mons, Human Anatomy and Experimental Oncology, Rue de la Briqueterie 212, 6140 Hainaut - Sonia.FURGIUELE@umons.ac.be

Head and neck cancers (HNC) are mainly associated with excessive consumption of tobacco and alcohol. However, over the past decade, many studies have reported the emergence of a new type of squamous cell carcinoma related to oncogenic papillomaviruses. Various authors have shown that these HPV-positive tumors differ clinically and molecularly from HPV-negative tumors. Although concomitant chemoradiotherapy remains the standard treatment for these advanced cancers, protontherapy is emerging and is beginning to prove itself as a complement, or even an alternative to these treatments. As such, we would like to characterize the effects and benefits of protontherapy compared to photontherapy. In this project, we will focus on the recruitment of different cells of the immune system into the response to protontherapy alone, or in combination with various treatments. The first step of this research project will be to generate HNC cell lines Knock-Out (KO) for E6 and E7 oncoproteins based on HPV + cell lines available in the laboratory (93VU-147T, UD-SCC -2, UPCI-SCC-154) by the CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats-Cas9) technique. Since its discovery, CRISPR-Cas9 has rapidly emerged as a major molecular tool for producing targeted double strand DNA breaks. These breaks can lead to the specific inactivation of target genes and thus offer a leading tool in the field of genomics. We used products from IDT (Integrated DNA Technologies B.V.B.A. Interleuvenlaan 12A – 3001 Leuven (Belgium)) to generate our KO cell line. A non-homologous and joining (NHEJ) of double-strand breaks was performed on SCC-2 cell line with a ribonucleoprotein (RNP) complex. As soon as the technique will be optimize, we will then be able to compare oncoprotein-expressing lines to their non-expressing ones and evaluate the impact of HPV oncogene expression on cellular behavior, proteomic and metabonomic profiles, and on the secretion of immunomodulatory chemokines. These cell populations will be irradiated by photons and protons alone, as well as in combination with PRIMA-1Met, which reactivates the expression of p53, and nanoparticles that seem to potentiate the effect of protontherapy. These treatments will then be applied in different in vivo models, where an anti-PD-1 immunotherapy treatment will also be evaluated. The comparison of these different results will establish relationships between HPV status, immune status, the expression of checkpoint proteins and tumor development in HNC.

Abstract n°35

Investigation of Epithelial-Mesenchymal Transition in Response to Non-Thermal Plasma Therapy

Abraham Lin (1,2), Eline Biscop (1,2), Hanne Verswyvel (1,2), Angela Privat Maldonado (1,2), Evelien Smits (2), Annemie Bogaerts (1)

(1) PLASMANT, Department of Chemistry, University of Antwerp, Antwerp, Belgium
(2) Center for Oncological Research, University of Antwerp, Antwerp, Belgium

Presenting author: Abraham Lin – University of Antwerp, Chemistry, Universiteitsplein 1, 2610 Antwerp - abraham.lin@uantwerpen.be

Non-thermal plasma (NTP) is an emerging cancer treatment strategy that has recently been reported to induce immunogenic cell death (ICD) [1]. Induction of ICD increases tumor immunogenicity via emission of 'danger signals' and can stimulate the initial steps of the cancer immunity cycle [2]. This is desirable for cancer immunotherapy, where the goal is to stimulate and assist the patient's own anti-cancer immune response to fight against cancer and provide long-term protection.

While current reports on NTP for cancer therapy are promising, there are still many unanswered questions on the fundamental effects of NTP on cancerous cells. One essential gap in knowledge is the effect of NTP on epithelial-mesenchymal transition (EMT). EMT is a normal, morphogenic process involved in organismal development, but in the context of cancer, EMT is exploited to increase metastatic potential [3]. When cells transition to a more mesenchymal-like phenotype, they acquire enhanced migratory and invasive properties, which may thereby cause metastasis, the final, life-threatening manifestations of cancer progression. To date, EMT-induction has been observed as a consequence of a wide spectrum of therapeutic agents [3], and therefore, must be investigated in NTP treatment regimes. In this study, we used previously defined ICD-inducing regimes of NTP to study the effects on EMT. Immunohistochemistry and flow cytometry were used to analyze EMT markers following NTP treatment in both monolayer cultures and 3D tumor spheroids. Migration and invasion assays were also performed to determine the effect of NTP on these hallmarks of malignancy.

References

[1] Lin, Abraham, et al. Advanced Science 6.6 (2019): 1802062.
[2] Chen, Daniel S., et al. Immunity 39.1 (2013): 1-10.
[3] Williams, Elizabeth D., et al. Nature Reviews Cancer (2019): 1-17.

Abstract n°36

Targeting mitochondrial reactive oxygen species (mtROS) in breast cancer: a way to blunt cell migration without affecting the cytotoxic effects of standard therapies

Tânia Capelôa(1), Joanna Krzystyniak(1), Paolo E Porporato(2), Pierre Sonveaux(1)

(1)Pole of Pharmacology and Therapeutics, Institute of Experimental and Clinical Research (IREC), University of Louvain (UCLouvain), Brussels, Belgium;
(2)Department of Molecular Biotechnology and Health Science, Molecular Biotechnology Centre, University of Torino, Torino, Italy

Presenting author: Tânia Capelôa – Insitute of Experimental and Clinical Research (IREC), Pole of Pharmacology an Therapeutics, Avenue Hippocrate 57, 1200 Brussels - tania.demiranda@uclouvain.be

Introduction: Despite significant improvements of cancer therapy, metastasis is still a main cause of cancer patient death. Recent studies of our team indicated that mitochondrial superoxide production induced by metabolic dysfunctions promotes migration, invasion, and metastasis in preclinical cancer models (1). These phenotypes were reverted by using selective mitochondria-targeted antioxidants mitoTEMPO and mitoQ. However, clinically, ROS are directly linked to the cytotoxic effects of many forms of chemotherapy and radiotherapy. We therefore aimed to test whether chemotherapies and radiotherapy used to treat breast cancer promote metastasis, and whether mitoQ could counter this effect.

Experimental: We tested the effects of chemotherapy (cisplatin, doxorubicin, epirubicin, 5-fluoruracyl and paclitaxel) or X-ray radiotherapy ± mitoQ on the metastatic phenotype of highly metastatic human breast cancer cell lines MDA-MB-231 and SKBR3. Crystal violet assays were performed to evaluate cell viability and clonogenic assays for the replication potential. Effects of mitoQ on cell migration were evaluated in vitro (wound healing and chemotaxis assays) and in vivo, using NMRI nude mice.

Results: MitoQ at clinically relevant doses does not interfere with the cytotoxic effects of all forms of chemotherapies used to treat breast cancer cells. It did not impair X-ray effects when the cellular clonogenic potential was evaluated. We further observed in vitro and in vivo that mitoQ significantly inhibited breast cancer cell migration and the number of experimental lung metastasis in mice.

Conclusions: Our results suggest that mitoQ can be consider as a promising therapeutic option to prevent tumor metastasis without interfering with standard of care therapy in established breast cancers.

(1) Porporato PE, et al. Cell Rep 2014;8:754-66.

Abstract n°37

Non-transformed cells respond to fat by inducing a cancer-like glucose metabolism

João André Gonçalves Duarte(1,2), Roberta Schmieder(1,2), Lindsay A. Broadfield(1,2), Dorien Broekaert(1,2), Koen Veys(3), Mélanie Planque(1,2), Kim Vriens(1,2), Yasuaki Karasawa(4,5), Francesco Napolitano(6), Suguru Fujita(4), Masashi Fujii(4,7), Miki Eto(4), Bryan Holvoet(8), Roman Vangoitsenhoven(9), Juan Fernandez-Garcia(1,2), Joke Van Elsen(1,2), James Dooley(10), Rebeca Alba Rubio(11), Jos van Pelt(12_, Thomas G.P. Grünewald(11,13,14), Adrian Liston(10), Chantal Mathieu(9), Christophe M. Deroose(8), Diego Di Bernardo(7,15), Shinya Kuroda(4), Katrien De Bock(16)_ and Sarah-Maria Fendt(1,2)

(1) Laboratory of Cellular Metabolism and Metabolic Regulation, VIB-KU Leuven Center for Cancer Biology, VIB, Herestraat 49, 3000 Leuven, Belgium
(2) Laboratory of Cellular Metabolism and Metabolic Regulation, Department of Oncology, KU Leuven and Leuven Cancer Institute (LKI), Herestraat 49, 3000 Leuven, Belgium
(3) Laboratory of Angiogenesis and Vascular Metabolism, Department of Oncology (KU Leuven) and Center for Cancer Biology (VIB), Herestraat 49, 3000 Leuven, Belgium
(4) Department of Biological Sciences, Graduate School of Science, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, 113-0033 Tokyo, Japan
(5) Department of Neurosurgery, Department of Rehabilitation, University of Tokyo Hospital, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033
(6) Telethon Institute of Genetics and Medicine (TIGEM), System Biology and Bioinformatics lab. and High Content Screening facility, Via Campi Flegrei 34, 80078 Pozzuoli (NA), Italy
(7) Molecular Genetic Research Laboratory, Graduate School of Science, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033
(8) Nuclear Medicine and Molecular Imaging, Department of Imaging and Pathology, KU Leuven, Belgium
(9) Department of Endocrinology, UZ Gasthuisberg KU Leuven, Leuven, Belgium
(10) Department of Microbiology and Immunology, KU Leuven; and Translational Immunology Laboratory, VIB Leuven, Leuven, Belgium
(11) Max-Eder Research Group for Pediatric Sarcoma Biology, Institute of Pathology, Faculty of Medicine, LMU Munich, Thalkirchner Strasse 36, 80337 Munich, Germany
(12) Laboratory of Clinical Digestive Oncology, Department of Oncology, KU, Leuven, Belgium
(13) German Cancer Consortium (DKTK), partner site Munich, Munich, Germany
(14) German Cancer Research Center (DKFZ), Heidelberg, Germany
(15) Department of Chemical, Materials and Industrial Production Engineering, University of Naples Federico II, Piazzale Tecchio 80, 80125 Naples, Italy
(16) Laboratory of Exercise and Health, Department of Health Sciences and Technology, ETH Zurich, Schorenstrasse 16, 8603 Schwerzenbach, Switzerland

Presenting author: Lindsay A Broadfield – KU Leuven –VIB, Center for Cancer Biology, Herestraat 49, 3000 Leuven - lindsay.broadfield@kuleuven.vib.be

In vivo nutrient availability is an important determinant of cellular metabolism. Here, we analyzed the metabolic response of non-transformed mouse livers to increased availability of the nutrient fat. Strikingly, we discovered that these normal cells hyperactivate their glucose metabolism when exposed to fat. This finding contrasts the known effects of fat-induced insulin resistance which dampens glucose metabolism. We further found that glucose contributes to serine, pyruvate carboxylase and lactate metabolism in fat exposed non-transformed mouse livers. Accordingly, we observed that the waist circumference of healthy humans correlates with increased plasma lactate abundance upon glucose administration. Moreover, we found that the changes induced by fat in normal liver cells were similar to the rewiring of glucose metabolism found in hepatocellular carcinoma, independent of diet. This finding suggests that fat is an inducer of metabolic cancer hallmarks in non-transformed liver cells. In conclusion, we show that normal, non-transformed livers respond to fat by inducing a cancer-like glucose metabolism.

Abstract n°38

Effects of cycling hypoxia on macrophages and on the communication between macrophages and endothelial cells in promoting tumor inflammation

Victor Delprat(1), Celine Tellier(1), Olivier Feron(2) and Carine Michiels(1)

(1) URBC, University of Namur (UNamur), Rue de Bruxelles, 61, 5000, Namur
(2) FATH, University of Louvain Medical School (UCL), avenue Mounier, 73, B-1200 Brussels

Presenting author: Victor Delprat – Unamur, URBC, rue de Bruxelles 61, 5000 Namur - victor.delprat@unamur.be

Cycling hypoxia (cyH), also called intermittent hypoxia, occurs in solid tumors and affects different cell types in the tumor microenvironment and in particular the tumor-associated macrophages (TAMs) and endothelial cells. As cyH and TAMs both favor tumor progression, we investigated whether cyH could drive the pro-tumoral phenotype of macrophages. Here, the effects of cyH on human THP-1 macrophages, either unpolarized M0, or polarized in M1 or M2 phenotype were studied. Secondly, the impact of CyH on the communication between macrophages and endothelial cells was investigated. In M0 macrophages, cyH induced a pro-inflammatory phenotype characterized by an increase in TNFα and IL-8/MIP-2 secretion. CyH amplified the pro-inflammatory phenotype of M1 macrophages evidenced by an increased pro-inflammatory cytokine secretion and pro-inflammatory gene expression. Furthermore, cyH increased c-jun activation in human M0 macrophages and highly increased c-jun and NF-κB activation in M1 macrophages. C-jun and p65 are implicated in the effects of cyH on M0 and M1 macrophages since inhibition of their activation prevented the cyH pro-inflammatory effects. We demonstrated that cyH induces or amplifies a pro-inflammatory phenotype in M0 and M1 macrophages by activating JNK/p65 signaling pathway. On the other hand, M0 CyH conditioned media increased the expression and secretion of IL-6 and IL-8 by endothelial cells. Furthermore, M0 CyH conditioned media increased the expression and protein abundance of ICAM-1 by endothelial cells. THP-1 monocyte adhesion on endothelial cells was higher on endothelial cells incubated with M0, M1 or M2 macrophages media exposed to CyH (vs. N). These results highlight a specific role of cyH in the amplification of tumor-related inflammation by modulating the inflammatory phenotype of macrophages and endothelial cells.

Abstract n°40

DNA Methylation Analysis of the PDX1 Gene can be used for PNET Subtyping and has a Possible Prognostic Value

Gitta Boons (1,2), Timon Vandamme (1,2,3,4), Joe Ibrahim (1,2), Anne Schepers (2), Geert Roeyen (5), Ann Driessen (6), Marc Peeters (1,3), Guy Van Camp (1,2), Ken Op de Beeck (1,2)

(1) Center for Oncological Research, University of Antwerp, Antwerp, Belgium
(2) Center of Medical Genetics Antwerp, Antwerp University Hospital/University of Antwerp, Edegem, Belgium
(3) Department of Oncology, Antwerp University Hospital/University of Antwerp, Edegem, Belgium
(4) Section of Endocrinology, Department of Internal Medicine, Erasmus Medical Center, Rotterdam, Netherlands; (5) Department of Hepatobiliary, Endocrine and Transplantation Surgery, Antwerp University Hospital/University of Antwerp, Edegem, Belgium; (6) Department of Pathology, Antwerp University Hospital/University of Antwerp, Edegem, Belgium

Presenting author: Gitta Boons, University of Antwerp, Medical Sciences, Prins Boudewijnlaan 43, 2650 Antwerp - Gitta.Boons@uantwerpen.be

Estimating the prognosis of non-functional pancreatic neuroendocrine tumor (PNET) patients remains challenging. Mutation status of DAXX/ATRX/MEN1, histone modification patterns and immunohistochemistry for relevant transcription factors, including PDX1, have recently been used to perform subtyping and distinguished two main subtypes, A and B. These subtypes are linked to cell-of-origin and associated with clinical outcome. In this study, we assessed whether DNA methylation of PDX1 can be used to identify the A and B subtypes, linked to cell-of-origin, and tested the prognostic value of these subtypes. Therefore, DNA methylation analysis using Infinium Methylation EPIC and 450K arrays (Illumina) was performed on DNA from fresh-frozen tissue of 41 PNETs. Additional DNA methylation data of 42 PNETs and healthy alpha and beta cells were collected through public databases. Methylation values of CpGs in the PDX1 region were extracted and used to perform clustering to identify subtypes for survival analysis. Available clinicopathological and sequencing data were included in the analyses. Clustering analysis identified two separate clusters. One cluster contained the alpha cells and 97% of the mutated PNET samples, suggestive of the A subtype. The other cluster consisted of beta cells and mostly wild type PNETs, suggestive of the B subtype. A significant association was found between mutation status and subtype (Chi-square, $p<0.001$). Furthermore, Kaplan-Meier analysis showed a trend towards longer overall survival (OS) for patients with subtype B ($p=0.058$). Median OS for type A was 11.9 years (95% CI 9.9 years – not reached) and has not been reached for type B. In conclusion, DNA methylation analysis can be used to perform subtyping and links subtype with cell-of-origin by including DNA methylation patterns of alpha and beta cells. This subgrouping might have prognostic properties.

Abstract n°41

Functional impact of anti-PD-L1 treatment on specific lung (tumor) residing myeloid cell subsets

Kirsten De Ridder (1), Miren Zuazo Ibarra (4), Robin Maximilian Awad (1), Julia Neumann (1), Yannick De Vlaeminck (1), Quentin Lecocq (1), Lien De Beck (1), Thomas Ertveldt (1), Hanne Locy (1), Wout De Mey (1), Xenia Geeraerts (2,3), Lea Brys (2,3), Damya Laoui (2,3), Hélène Salmon (5), Karine Breckpot (1) and Cleo Goyvaerts (1)

(1) Lab for Molecular and Cellular Therapy, Department of Biomedical Sciences, Vrije Universiteit Brussel, Belgium
(2) Myeloid Cell Immunology Lab, VIB Center for Inflammation Research, Belgium
(3) Lab of Cellular and Molecular Immunology, Vrije Universiteit Brussel, Belgium
(4) Navarrabiomed, Department of Oncology, Pamplona, Spain
(5) Equipe Salmon, Centre De Recherche, Institut Curie, Paris, France

Presenting author: Kirsten De Ridder – Vrije Universiteit Brussel, LMCT, Laarbeeklaan 103, 1090 Brussels - kirsten.de.ridder@vub.be

Antibodies targeting Programmed Death-1 (PD-1) or its ligand became a first-line treatment option for metastatic non-small cell lung cancer (NSCLC) patients. Unfortunately, about 75% of patients don't show any benefit. The main clinically applied biomarker is PD-L1 expression on tumor cells. However, preclinical observations reveal that not PD-L1 on tumor cells, but on tumor infiltrating myeloid cells represents a major determinant for therapy outcome. Further, the lung represents an immunologic organ packed with myeloid subsets that could interact with anti-PD-(L)1 antibody via Fc receptors and/or PD-(L)1. Still the functional impact of anti-PD-L1 therapy on lung myeloid subsets remains largely unknown.

To evaluate the abundance of specific PD-(L)1+ myeloid subsets during NSCLC progression, C57BL/6 mice were intravenously challenged with Lewis Lung Carcinoma (LLC) cells. Next, mice were treated every three days with anti-PD-L1, four times in total. NSCLC engraftment and PD-(L)1 expression was evaluated on weeks 1, 2 and 3 using immunohistochemistry and flow cytometry. Sorted myeloid subsets were functionally evaluated using an ex vivo 3D killing assay, qPCR for alternative checkpoints, arginase-1, nitric and super oxide evaluation.

Only a marginal therapeutic benefit without increase in CD8+ T cell infiltration was observed upon anti-PD-L1 therapy. In contrast, the fraction of Ly6G+ neutrophils decreased during anti-PD-L1 therapy while MHCIIlo F4/80+ macrophages ('M2'), Ly6C+ inflammatory (IM) and Ly6C- non-classical monocytes (RM) decreased after therapy. Systemically, the ratio of M2/M1 and amount of RMs decreased in bone marrow or/and spleen, respectively. In contrast, PD-L1+ neutrophils and macrophages decreased in blood and spleen while PD-L1+ monocytes only decreased in spleen. Functional evaluation of sorted myeloid subsets showed that in vivo anti-PD-L1 treatment increased: arginase-1 expression in RMs, superoxide production in M2 and the CD8+ T-cell stimulating potential of M1 and RMs.

These findings could provide novel rationales for potent combination therapies.

Abstract n°42

New considerations of lymphatic endothelial cells as key players of a pro-tumoral microenvironment

Marie Ebroin (1), Maureen Van de Velde (1), Tania Durré (1), Marc Joiret (2), Lionel Gillot (1), Blacher Silvia (1), Liesbet Géris (2), Frédéric Kridelka (1,3) and Agnès Noël (1).

(1)Laboratory of Tumor and Development Biology, GIGA-Cancer, Sart-Tilman, 4000, Liege, Belgium
(2)Biomechanics Research Unit, University of Liège, Sart-Tilman, 4000, Liège, Belgium.

Presenting author:Marie Ebroin - Liege Universtity, GIGA-Cancer, LBTD – Avenue de l'hopital 1, 4000 Liège - marie.ebroin@uliege.be

Metastases are associated with a poor prognosis for patients. Cancer cells spread from the primary tumor to distant organs via blood and/or lymphatic vessels. Lymphangiogenesis, the formation of new lymphatic vessels and their impact on tumor microenvironment is less studied than the angiogenic process. The lymphatic vessels are highly permeable and are often viewed as simple "tubes" where tumor cells passively transit to form metastasis. However, lymphatic endothelial cell (LEC) exhibit plasticity and heterogeneity that are worth exploring for understanding their implication in tumor microenvironment. Our hypothesis is that LEC can be activated by tumor cells and therefore act as active stromal players in the complex metastatic process.

The direct confrontation of a LEC monolayer to different human carcinoma cell lines (HaCat II4, HaCat A5RT3) disturbs the endothelium integrity and impairs LEC-LEC junctions (VE-cadherin internalization). A 3D migration assay (spheroid) showed an increase of tumor cells migration in presence of LEC. Moreover, we observed an increase of tumor cell proliferation in presence of medium conditioned by activated LEC (previously exposed to tumor cell conditioned medium). Furthermore, tumor cells induced a modification of LEC secretome as assessed by RT-PCR. Cytokine array and ELISA revealed that medium conditioned by tumor cells induced significant modulations of secreted mediators such as IL6 produced by LEC. These LEC-secreted factors may provide a favorable pro-inflammatory environment for tumor growth and invasion. These results demonstrate that LEC confronted to tumor cells adapt their secretome and contribute to tumor invasion by secreting pro-inflammatory cytokines.

Our work sheds a new light on reciprocal tumor cells-LEC crosstalk that are beyond the intravasation of tumor cells into lymphatics. LEC are thus new key actors of the tumor microenvironment that can be activated by tumor cells to secrete cytokines and that in turn provide a permissive microenvironment for tumor cells.

Abstract n°43

Pragmatic subtyping of pancreatic ductal adenocarcinoma with markers and histological features

Sandrina Martens (1), Pierre Lefesvre (2), Tatjana Arsenijevic (3), Christelle Bouchart (3,4), Francesco Puleo (3,5), Jean-Luc Van Laethem (3,6)*, Ilse Rooman (1)*

(1) Laboratory of Medical and Molecular Oncology, Vrije Universiteit Brussel, Brussels, Belgium;
(2) Department of Pathology, UZ Brussel, Brussels, Belgium;
(3) Laboratory of Experimental Gastroenterology, Université Libre de Bruxelles, Brussels, Belgium;
(4) Department of Radiotherapy, Institut Jules Bordet, Brussels, Belgium;
(5) Department of Medical Oncology, Institut Jules Bordet, Brussels, Belgium;
(6) Department of Gastroenterology and Digestive Oncology, Erasme Hospital, Brussels, Belgium;
** These authors share senior authorship.*

Presenting author: Sandrina Martens – Vrije Universiteit Brussel, LMMO, Laarbeeklaan 103, 1090 Brussels - Sandrina.Martens@vub.be

Pancreatic ductal adenocarcinoma is a lethal cancer with a 5-year prognosis of less than 10%. In the past decade, researchers have focused on characterizing molecular subtypes to more accurately define prognosis and therapy. Two main molecular subtypes, basal-like and classical, have been described in various papers. Aside from that, it is also possible to characterize tumours based on their histological appearance.

In this study, we aimed to determine whether the molecular subtypes can be characterized by a histological phenotype or by specific markers. We based the histological appearance on a paper of Schlitter et al. and used samples of 81 patients from the molecular subtyping paper of Puleo et al. The HE slides of these patients were evaluated by multiple pathologists and categorized in a specific histological subtype. The markers were tested using either immunohistochemistry or multiplex fluorescent RNAscope (in situ hybridization). These markers were tested on the same samples, and the stainings were scanned at high resolution. This was then quantified using the HALO software.

We found that certain histological appearances correlate with molecular subtypes. For the basal-like subtype, the molecular subtype with the worst prognosis, we found that these tumours showed a gyriform pattern, which is a less differentiated histology. For classical tumours, which have a better prognosis, tumours often showed either a classical appearance or a papillary appearance, both more differentiated histologies. We also found certain markers using the multiplex fluorescent RNAscope that were expressed more in the basal-like subtype compared to the classical subtype.

These findings could indicate that we can determine the molecular subtypes based on either the histological phenotype or using markers, which would be both time- and cost-efficient. However, these findings should be tested on a larger cohort of patients, and on samples that don't belong to one sequencing experiment. Determining a subtype quickly could aid in the prognosis of a patient and to determine the therapy regimen more specifically.

Abstract n°44

Glucose-dependent effects on the response of hormone and IGF-IR/IR mediated growth of breast cancer spheroids.

Eva Blondeel (1,2), Glenn Wagemans (1), Elly De Vlieghere (1,2), Herman Depypere (2,3), Olivier De Wever (1,2)

(1) Laboratory of Experimental Cancer Research, Department of Human Structure and Repair, Ghent University
(2) Cancer Research Institute Ghent (CRIG), Ghent, Belgium
(3) Breast and Menopause, Ghent University Hospital

Presenting author: Eva Blondeel – Laboratory of Experimental Cancer Research Ghent, Department of Human Structure and repair, Corneel Heymanslaan 10, 9000 Ghent - enblonde.blondeel@ugent.be

In recent years the importance of three-dimensional (3D) cell cultures has been shown. 3D cultures provide more physically relevant and predictive data that overcome the correlation mismatch between the preclinical and clinical situation. However the growth conditions used in most cancer-related research does not resemble the physiological tumor microenvironment. Intriguingly blood glucose levels under fasting conditions are 5.5mM and do not reach higher than 10mM even under diabetic conditions, questioning the use of media using supra-physiological (25mM) levels of glucose. Our research group examines the influence of glucose concentration and different media on MCF-7 breast cancer spheroid size, metabolic activity, cytokine and chemokine secretion, density and response to stimuli and inhibitors of the Estrogen Receptor (ER) and Insulin-like Growth Factor I/Insulin Receptor (IGF-IR/IR). Remarkably, spheroids grown in physiological glucose concentrations are larger, have a higher ATP production, have a different cytokine and chemokine profile, are less dense and differ in response to stimuli and inhibitors compared to spheroids in supraphysiological glucose concentrations. Furthermore, these spheroids, grown under physiological glucose concentrations, expulse a core of dying cells within one week of culture. From the expulsed core a number of viable cells are able to form new spheroids, resembling metastatic cells on the search for better grounds after nutrient depletion. These data emphasize further the importance of physiological culture methods and should be taken into consideration in cancer research.

Abstract n°45

Cetuximab-induced antibody-dependent cellular cytotoxicity in HNSCC: role of expression and internalization of the EGFR receptor.

H. Baysal (1), I. De Pauw (1), H. Zaryouh (1), M. Peeters (1, 3), J.B. Vermorken (1, 3), E. Smits (1, 4), F. Lardon (1), J. Jacobs (1, 2), A. Wouters (1)

(1) Center for Oncological Research (CORE), University of Antwerp, Belgium
(2) Department of Pathology, Antwerp University Hospital, Belgium
(3) Department of Oncology, Antwerp University Hospital, Belgium
(4) Center for Cell Therapy and Regenerative Medicine, Antwerp University Hospital, Belgium

Presenting author: Hasan Baysal – University of Antwerp, Medicine and Health Sciences, Universiteitsplein 1, 2610 Antwerp - hasan.baysal@uantwerpen.be

The epidermal growth factor receptor (EGFR)-targeted therapies and immunotherapies are now at the forefront for treatment of head and neck squamous cell carcinoma (HNSCC). However, resistance to cetuximab, an anti-EGFR monoclonal antibody (mAb), is a major clinical problem, limiting the clinical benefit of treatment. Besides inhibition of downstream signalling, cetuximab can also induce antibody-dependent cellular cytotoxicity (ADCC). The aim of this study was to investigate the role of baseline EGFR expression and EGFR internalization following treatment with cetuximab as possible mechanisms that may explain differences in cetuximab-induced ADCC between HNSCC cell lines.
Ten HNSCC cell lines, comprising cetuximab sensitive as well as intrinsically and acquired resistant cell lines, differing in HPV-status, were used. ADCC was assessed using the xCELLigence RTCA system, by co-culturing HNSCC cells with natural killer cells from healthy volunteers (E:T; 5:1). Baseline EGFR expression was flow cytometrically determined, following staining with an EGFR PE-conjugated antibody or a PE-conjugated isotype control. Experiments were performed under normoxic and hypoxic (1% O2) conditions. To determine EGFR internalization, cetuximab and the corresponding isotype control were stained with FabFluor-pH reagent prior to incubation with HNSCC cells. Phase-contrast and red fluorescence kinetics were quantified using the IncuCyte Zoom.
We demonstrate that cetuximab-induced ADCC was highest in intrinsically cetuximab resistant cell lines compared to the acquired resistant and cetuximab sensitive cell lines. Under hypoxic conditions, a reduced ADCC was observed only in the cetuximab sensitive HNSCC cell lines. Baseline EGFR was expressed in a cell line-dependent manner and was not correlated with cetuximab sensitivity or HPV-status. Moreover, there was no correlation between baseline EGFR expression and the amount of cetuximab-induced ADCC. In contrast, the degree of EGFR internalization positively corelated with cetuximab-induced ADCC. Importantly, the intrinsically cetuximab resistant cells demonstrated the highest level of EGFR internalization compared to the acquired resistant and cetuximab sensitive cell lines.
In conclusion, we showed that induction of ADCC is maintained, independent of EGFR expression. Furthermore, internalization of EGFR might play a role in cetuximab resistance and ADCC-induction. Future research further unravelling this mechanism will provide a better understanding of mAb-based anti EGFR therapies in the future.

Abstract n°47

Investigation of combined ROS inducing therapies in 2D and 3D glioblastoma cell cultures

Jinthe Van Loenhout (1), Christophe Deben (1), Laurie Freire Boullosa (1), Jorrit De Waele (1), Delphine Quatannens (1), Marc Peeters (1), Annemie Bogaerts (2), Evelien Smits (1)

(1) Center for Oncological Research, University of Antwerp, Belgium
(2) Research group PLASMANT, University of Antwerp, Belgium

Presenting author: Jinthe Van Loenhout – University of Antwerp, Center for Oncological Research, Universiteitsplein 1, 2610 Antwerp - jinthe.vanloenhout@uantwerpen.be

Glioblastoma multiforme (GBM) cells often display a disturbed intracellular redox balance at basal conditions due to an imbalance of oxidants and antioxidants, leading to oxidative stress. Although GBM cells have developed mechanisms to restore their redox balance, this also makes them more vulnerable to reactive species compared to the surrounding healthy tissue and cells.

Both exogenous and endogenous factors can trigger reactive species generation. Recently, cold atmospheric plasma as ionized gas is investigated as new anticancer treatment modality. This plasma generates exogenously reactive oxygen species (ROS), which can lead to an increase of oxidative stress, causing cell death. ROS can also accumulate due to inhibition of endogenous antioxidant system in cancer cells. A well-known antioxidant system responsible for clearance of ROS is thioredoxin/thioredoxin reductase. This system can be inhibited by an FDA approved compound named auranofin. A combination of plasma as exogenous ROS source and auranofin as endogenous ROS source can be beneficial because the oxidative threshold in cancer cells will be reached more easily due to accumulation of ROS, which will consequently trigger more cancer cell death.

For this study, we used two different plasma sources (kINPenIND® and COSTjet) to treat GBM cells, because they both produce different amounts of ROS. We studied their ability to elicit cell death in 2D and 3D cell cultures of four different GBM cell lines. We showed a cell line dependent sensitivity after plasma treatment with the kINPenIND® device in 2D cultures. Remarkably, this treatment was incapable to affect the viability of 3D cultures. Therefore, we used the COSTjet to treat 3D cultures, because this device is known to induce higher amounts of ROS. Furthermore, we investigated if auranofin is able to sensitize the GBM cells towards plasma treatment with the kINPenIND® device, which was confirmed with a synergistic response in 2D cell cultures of the four GBM cell lines. Finally, we are currently studying which type of cell death is induced after treatment with both plasma and auranofin. Altogether, these results will give more insight in the anticancer effect of combining different ROS inducers for the treatment against GBM.

Abstract n°48

Immunogenic properties of chemotherapeutic agents in the treatment of non-small cell lung cancer

Tal Flieswasser (1,2), Jinthe Van Loenhout (1), Laurie Freire Boullosa (1), Astrid Van den Eynde (1), Jorrit De Waele (1), Jonas Van Audenaerde (1), Filip Lardon (1), Evelien Smits (1), Julie Jacobs (1,2), Patrick Pauwels (1,2)

(1) Center for Oncological Research, University of Antwerp, Belgium
(2) Center of Pathology, Antwerp University Hospital, Belgium

Presenting author: Tal Flieswasser – University of Antwerp, Center for Oncological Research, Universiteitsplein 1, 2610 Antwerp - tal.flieswasser@uantwerpen.be

Introduction
Accumulating evidence suggests that the clinical success of chemotherapy is not only attributed to direct tumor cell toxicity, but also relies on its anti-tumor effects by immunomodulation. In this regard, the concept of immunogenic cell death (ICD) has emerged as a cornerstone of therapy-induced anti-tumor immunity. To this end, we assessed different chemotherapeutic agents on their ability to induce ICD in vitro and in vivo.

Material and methods
NSCLC cell lines (NCI-H1975, A549, NCI-H1650 and LLC) were treated with the IC50 of different chemotherapeutic agents: docetaxel (DOC), carboplatin (CARBO), cisplatin (CDDP), oxaliplatin (OXA) and mafosfamide (MF). In addition, combinations of DOC (IC50) with CARBO (IC40) or CDDP (IC40) were included. Release of important damage-associated molecular patterns (DAMPs) was evaluated: ATP (bioluminescence), ecto-CRT (flow cytometry) and HMGB1 (ELISA) after 24h, 48h and 72h of treatment, respectively. In addition, phagocytosis and maturation status of dendritic cells (DCs) were assessed. Finally, a vaccination assay was performed to validate in vitro findings using 6-week old female C57BL/6J mice (5 mice/condition). Mice were vaccinated twice (1×10^6 treated cells/mouse) before receiving the challenge (5x10^4 live cells/mouse).

Results
Three out of four NSCLC cell lines (NCI-H1975, A549 and LLC) showed significant higher levels of ATP, ecto-CRT and HMGB1 after treatment with DOC, DOC+CARBO and DOC+CDDP compared to vehicle. In addition, phagocytosis of treated tumor cells and maturation (CD86) of dendritic cells (DCs) were significantly increased in all three human NSCLC cell lines after treatment with the above-mentioned chemotherapeutic regimens. Furthermore, murine LLC cells treated with DOC, MF, DOC+CARBO and DOC+CDDP resulted in a significant release of all three DAMPs in vitro, as opposed to treatment with OXA. Along similar lines, 0%, (DOC+CDDP), 20% (MF and DOC+CARBO) and 80% (OXA) of the mice developed a tumor at the challenge site in vivo. This was not the case for treatment with DOC, which resulted in tumor growth at the challenge site in 60% of the mice.

Overall, these findings demonstrate the immunostimulatory effects of clinically relevant chemotherapeutic regimens, especially DOC+CARBO and DOC+CDDP, making it worthwhile to investigate these agents in combination strategies with immunotherapy in NSCLC.

Abstract n°49

RIPK4 maintains epidermal homeostasis and prevents skin cancer

Giel Tanghe (1,2), Corinne Urwyler-Rösselet (1,2,5), Michael Devos (1,2), Barbara Gilbert (1,2), Kelly Lemeire (2), Cédric Blanpain (3,4), Peter Vandenabeele (1,2), Wim Declercq (1,2)

(1) Molecular Signaling and Cell Death Unit, VIB-UGent Center for Inflammation Research, Ghent, Belgium
(2) Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium
(3) Université Libre de Bruxelles (ULB), Stem Cells and Cancer Laboratory, 1070 Brussels, Belgium
(4) WELBIO, Université Libre de Bruxelles (ULB), 1070 Bruxelles, Belgium
(5) Current address: Department of Biology, Institute of Molecular Health Sciences, ETH Zurich, Zurich, Switzerland

Presenting author: Giel Tanghe – VIB-UGent Center for Inflammation Research, Department of Biomedical Molecular Biology, Technologiepark 71, 9000 Ghent - giel.tanghe@irc.vib-ugent.be

The skin is a fast renewing organ with the continuous commitment of proliferative progenitor keratinocytes into a terminal differentiation program forming the stratified epidermis. Previous studies demonstrated that RIPK4, a serine/threonine kinase, is crucial for proper barrier formation during epidermal development. Keratinocyte-specific RIPK4 ablation causes cleft palate, epithelial fusion, aberrant differentiation and a defective barrier leading to perinatal death. Since RIPK4 is reported to be frequently mutated in aggressive cutaneous SCC, we addressed the functions of RIPK4 in adult mouse skin homeostasis and carcinogenesis. Tamoxifen-inducible K14-CreER-driven RIPK4 deletion in adult mouse epidermis caused significant hyperplasia due to the expansion of proliferative basal keratinocytes marked by high p63 expression. Using ex vivo cultures of primary keratinocytes we found that RIPK4 enables cell cycle exit by suppressing p63 expression in a keratinocyte autonomous manner. Additionally, RIPK4 down-regulates EGFR expression and its downstream ERK and STAT3 mitogenic signaling pathways in a kinase-dependent manner. Although RIPK4-depleted epidermal keratinocytes eventually commit to differentiation, the barrier is dysfunctional witnessed by increased transepidermal water loss and a 'subclinical' immune response characterized by an epidermal infiltrate of RORγt-positive CD8 T cells that produce IL17A. Upon aging loss of RIPK4 leads to spontaneous tumor formation, with reduced latency by additional deletion of tumor suppressor p53. Furthermore, RIPK4 serves as a brake on tumor growth driven by an oncogenic KrasG12D knockin transgene. Together, our work demonstrates that RIPK4 fulfills a central role in maintaining the homeostatic balance between keratinocyte proliferation and differentiation by suppressing proliferative signaling.

Abstract n°50

Tracking the origins of metastatic seeding in de novo metastatic prostate cancer

Kim Van der Eecken* (1,2), Evan W Warner* (3), Nicolaas Lumen (2,4), Matti Annala (3,5) Andrew J Murtha (3), Elena Schönlau (3), Nicolette Fonseca (3), Cameron Herberts (3), Jan Vanwelkenhuyzen (2,6), Welsey Verla (2,4), Sarah Buelens (4), Sofie Verbeke (1), Jo Van Dorpe (1,2), Alexander W Wyatt ǂ (3), Piet Ost ǂ (2,7)

(1) Department of Pathology, Ghent University Hospital, Ghent, Belgium
(2) Cancer Research Institute Ghent (CRIG), Ghent, Belgium
(3) Vancouver Prostate Centre, Department of Urologic Sciences, University of British Columbia, British Columbia, Canada
(4) Department of Urology, Ghent University Hospital, Ghent, Belgium
(5) Institute of Biosciences and Medical Technology, University of Tampere, Tampere, Finland
(6) Center for Medical Genetics Ghent, Ghent University Hospital, Ghent, Belgium
(7) Department of Urology, Ghent University Hospital, Ghent, Belgium; (7) Department of Radiation Oncology and Experimental Cancer Research, Ghent University Hospital, Ghent, Belgium
** These authors shared first authorship.*
ǂ These authors shared last authorship.

Presenting author: Kim Van der Eecken – Ghent University, Department of Pathology, Ghent University Hospital, Corneel Heymanslaan 10, 9000 Ghent - kivdreec.vandereecken@ugent.be

Introduction & Objectives: Patients with metastatic prostate cancer (mPCa) at diagnosis have been shown to exhibit an aggressive clinical course, yet the how and when tumoral clones start spreading to surrounding tissues have been difficult to determine because of a lack of access to primary and metastatic tissues. To address this, we analyzed multiregional data from distinct primary and metastatic tumor sites.

Material & Methods: Samples for analysis were collected from 10 prostatectomy de novo mPCa patients from the Ghent University hospital and were taken prior to initiation of systemic therapy. From each prostatectomy specimen, spatially and pathologically distinct regions (n=89) were sampled and interrogated with custom targeted sequencing, along with matched synchronous metastatic lymph nodes (n=48) from 9 patients, prostate biopsy specimens (n=21) from 4 patients and one bone metastasis. Mutation profiles and similarity matrices were used to reconstruct the tumor subclonal architecture and their phylogenetic trees.

Results: The median follow-up was 25 months. All patients had an acinar subtype of prostate adenocarcinoma. 2 patients had visceral (M1c) metastatic disease, 3 patients had bone (M1b) metastases and in 5 patients the tumor has spread to distant lymph nodes (M1a). All patients showed somatic alterations in driver genes associated with advanced disease, such as TP53 and RB1. Phylogenetic reconstruction demonstrated branched rather than linear evolutionary tumor growth in most patients, with clonal diversity both within primary tumors and also between primary and metastatic sites. In 3 patients the primary tumor and metastatic samples were genetically homogeneous. Interestingly, in one case we found a high number of somatic mutations concomittent to mismatch repair defective etiology. This patient may benefit from immune checkpoint inhibitors such as pembrolizumab.

Discussion: Our findings elucidate the complex patterns of metastatic spread in de novo mPCa and warrant further investigations into the intra-patient clonal diversity at time of diagnosis.

Abstract n°51

Immunomonitoring of anti-RANKL therapy for cervical cancer: preliminary results

Yannick Verhoeven(1,2), Jorrit De Waele(1), Julie Jacobs(1), Koen Van de Vijver(1,3,4), Martin Lammens(2,4), An Wouters(1), Evelien L.J. Smits(1,5), Filip Lardon(1), Xuan Bich Trinh(1,2,6), Peter van Dam(1,2,6)

(1) Center for Oncological Research (CORE), University of Antwerp, Wilrijk, Belgium
(2) Multidisciplinary Oncologic Centre Antwerp (MOCA), Antwerp University Hospital, Edegem, Belgium
(3) Department of Pathology, Cancer Research Institute Ghent (CRIG), Ghent University Hospital, Ghent, Belgium
(4) Department of Pathology, Antwerp University Hospital, Edegem, Belgium
(5) Center for Cell Therapy and Regenerative Medicine, Antwerp University Hospital, Edegem, Belgium
(6) Multidisciplinary Breast Cancer and Gynaecologic Oncology Unit, Antwerp University Hospital, Edegem, Belgium

Presenting author: Yannick Verhoeven – University of Antwerp, Center for Oncology Research (CORE), Universiteitsplein 1, 2610 Antwerp - yannick.verhoeven@uantwerpen.be

Background: Conventional treatments for cervical cancer (CC) have reached a plateau and only limited progress for targeted therapy has been made over the last decades, resulting in a meager five-year survival rate of only 17% for the advanced stages. To improve long-term benefits for the patient, a promising hot field of research in oncology that opens new perspectives is immunotherapy. Even though CC has shown to be immunogenic, only a minority of patients respond to this type of treatment. In recent years, the RANKL/RANK signaling pathway has been implicated as a key immune modulating factor in the tumor microenvironment, allowing the cancer cells to evade the immune response by disrupting the immune-intrinsic crosstalk. Both RANKL and RANK are highly co-expressed in CC, which correlates with inferior clinicopathological parameters and an increased risk of death. Targeting this pathway may therefore be of great value in the treatment of CC and the quest to release the brakes on the immune system, thereby reinvigorating the tumors' susceptibility to immunotherapy. Hence, we aim to elucidate the effects of anti-RANKL therapy on the tumor-immune microenvironment in CC.

Methods: Two cervical biopsies were taken before and after anti-RANKL therapy in CC patients. One fresh biopsy was immediately processed to a single cell suspension for flow cytometry using enzymatic digestion, while the other was formalin-fixed and paraffin-embedded for immunohistochemistry (IHC). The samples were then stained with different markers for RANK/L signaling, the immune infiltrate and immune checkpoints. Flow cytometry was performed on a BD FACSAria II· cytometer and analyzed with FlowJo, while IHC staining was performed on a Ventana Benchmark Ult
ra and Ventana Discovery Ultra and scored by a pathologist or by HistoScientist using Visiopharm.
Results: Our results show a relative decrease in T-cells, particularly in the CD4+ population, while a trend is observed in increased lymphocyte activation after anti-RANKL therapy. The latest results will be presented in more detail at the conference.

Conclusion: Preliminary findings indicate that anti-RANKL therapy modifies the tumor-immune microenvironment in CC. Higher patient accrual will allow to dissect targets for combination therapy with anti-RANKL to further optimize this treatment strategy.

Abstract n°52

Establishment of a patient-derived tumor and healthy organoid biobank and development of advanced high-throughput in vitro screening assays.

Christophe Deben (1), Laurie Freire Boullosa (1), Maxim Le Compte (1), Jonas RM Van Audenaerde (1), Patrick Pauwels (2), Paul Van Schil (3), Geert Roeyen (4), Marc Peeters (1,5), Evelien Smits (1), Filip Lardon (1)

(1) Center for Oncological Research (CORE), University of Antwerp, Belgium
(2) Department of Pathology, Antwerp University Hospital, Belgium
(3) Department of Thoracic and Vascular Surgery, Antwerp University Hospital, Belgium
(4) Department of Hepatobiliary, Endocrine and Transplantation Surgery, Antwerp University Hospital, Antwerp, Belgium
(5) Department of Oncology, Multidisciplinary Oncological Centre Antwerp, Antwerp University Hospital, Antwerp, Belgium

Presenting author: Christophe Deben – University of Antwerp, Center for Oncological Research, Universiteitsplein 1, 2610 Antwerp - christophe.deben@uantwerpen.be

Patient-derived organoids (PDOs) represent a more physiologically relevant in vitro model and are revolutionizing translational cancer research. PDOs can be grown with high efficiency from patient-derived healthy and tumor tissues, potentially enabling patient-specific drug testing and the development of individualized treatment regimens (1).

The goal of this study is to establish a PDO biobank of different tumor types, including matched healthy organoids allowing for selectivity studies, from patients undergoing surgery at the Antwerp University Hospital in collaboration with the Tumorbank@uza. To date, we have established PDOs from several non-small cell lung cancer adenocarcinoma and squamous cell carcinoma patients, including normal bronchial organoids, and new patients are still being recruited. Furthermore, we are in the progress of optimizing culturing methods for colorectal cancer lung metastasis and pancreatic cancer PDOs. Phenotypically verified PDO lines will be subjected to whole-exome-sequencing to allow for screening of mutation specific targeted therapies and biomarker studies.

Currently, the CellTiter-Glo 3D cell viability endpoint assay is most frequently used in PDO drug screening assays, but it cannot distinguish between cytostatic or cytotoxic responses. Therefore, we aim to develop more advanced high-throughput multiplex real-time and endpoint assays to improve the prediction of responses to existing and novel therapeutic strategies. In addition, this approach allows for more in-depth studies of the molecular mechanisms underlying these responses. These assays will be developed on the new state of the art Tecan Spark Cyto.

A more detailed composition of the continuously expanding PDO biobank will be presented as well as novel experimental approaches for the use of these PDO for preclinical research. By these means, we aim to set up new collaborations to maximize the use of this biobank.

(1) Drost, J. et al. Nat Rev Cancer 18, 407-418,(2018).

Abstract n°53

Characterization of EMT-shifted coagulant CTC

Genna A (1), Vanloo S (2), Francart M (1), Lambert J (1), Vanwynsberghe A (1), Gilet T (2) and Gilles C (1).

(1) Laboratory of Biology of Tumor and Development, Liège
(2) Microfluidic Lab, Liège

Presenting author: Anthony Genna – Giga Cancer, University Liège, Avenue Hippocrate 13, 4000 Liège - anthony.genna@uliege.be

Generating cells with enhanced invasive and survival properties, Epithelial-Mesenchymal-Transitions(EMTs) would particularly contribute to the biology of Circulating Tumor Cells (CTCs). Considering that not every CTCs possess a metastatic potential, studies are converging on the idea that CTCs encompass multiple phenotypes. Previous works in our lab identified Tissue Factor (TF), a key molecular activator of the coagulation cascade, as a protein induced by EMTs that provides CTCs with coagulant properties favoring their abilities to accomplish early metastasis. Our hypothesis is that EMT-shift coagulant CTCs are an aggressive subpopulation of CTCs that may initiate metastasis formation. We thus aim at developing a device that would allow the identification and isolation of such coagulant CTCs in order to perform an unprecedented cell characterization of human CTCs based on their coagulant properties. This will be done by combining a coagulation assay and droplet microfluidics, a promising cell isolation method for single-cell analysis.

In collaboration with Dr. VanLoo and Pr. Gilet (Ulg), a prototype µfluidic chip has been designed for our application that is composed of 3 different modules dedicated to (i) encapsulate cells in droplets, (ii) detect droplets containing a "labeled" cell by a software-based on fluorescence and (iii) sort the positive and the negative cell-containing droplets. So far, each module has been successively developed and tested separately. In parallel, we are also developing an in vitro functional coagulation assay label coagulant. Indeed, different coagulant cocktails will be tested to put in highlight the coagulant properties of MDA-MB-231 cell lines in vitro that could be transposed in the microfluidic device to isolated coagulant cancer cells in cancer patient blood samples. We included Platelet-Poor-Plasma(PPP) or recombinant factors such as FVII and FX (activated or not activated), Ca++ and GPRP that we combined to a specific fluorescent component to reveal coagulant cells. We have so far used a quenched coagulation peptide substrate for coagulation factors. A kinetic study revealed that the tumor cells may cleave the substrate at a concentration of 100µM within 15 min. Further analyzes are in progress to verify toxicity. Once the coagulant test validated in vitro, tumor cell spiking experiments in blood samples of healthy donors will first be realized to validate our complete coagulant CTC assay before blood samples from breast cancer patients.

Thus, the development of a droplet microfluidic device based on the functional coagulant properties of the cells may put in the limelight a new subpopulation of pro-metastatic CTCs that will be molecularly characterized and may help to establish a clinical significance of this coagulant CTC subpopulation in cancer management.

Abstract n°55

Modeling Hematopoietic malignancies in Xenopus tropicalis by CRISPR/Cas9.

Dionysia Dimitrakopoulou (1), Dieter Tulkens (1,2), Pieter Van Vlierberghe(1,2,3),Kris Vleminckx (1,2,3).

(1) Department of Biomedical Molecular Biology, Ghent University, Ghent, Belgium
(2) CRIG cancer research institute Ghent, Ghent, Belgium
(3) Department of Biomolecular medicine, Ghent University, Ghent

Presenting author: Dionysia Dimitrakopoulou – Ghent University, Department of Biomedical Molecular Biology, Technologiepark 71, 9000 Ghent - Dionysia.Dimitrakopoulou@irc.UGent.be

Acute Leukemias (ALs) are the most common cause of childhood cancer worldwide that originate from malignant transformation of lymphoid and myeloid lineage cells within the bone marrow. There are two types of ALs, acute T- or B- lymphoblastic leukemia (T-ALL or B-ALL) and acute myeloid leukemia (AML). ALLs account for 85% of ALs, whereas AML for 15% of cases. Current chemotherapeutic approaches demonstrate good cure and survival rates in patients. However, prognosis of relapsed patients is still dismal. Therefore, there is an urgent need to identify new targets for the development of more effective therapeutic compounds. Genetic alterations associated with these neoplasias are activating mutations in NOTCH1 and loss of function mutation (LOF) for PTEN. Our goal is to generate novel ALs models in Xenopus and identify new targets through CRISPR/Cas9 approaches.
Xenopus tropicalis embryos were co- injected with notch1* and pten sgRNAs or pten sgRNA alone. After 5-7 weeks, 27 - 30% of notch1*+ pten animals exhibited lethargic behavior and vascular malformations. Dissection of these animals revealed expanded spleen. RT-qPCR analysis of spleens demonstrated statistically significant increased expression of myeloperoxidase (MPO) a myeloid lineage marker, especially in animals with enlarged spleen. In this subset of animals, CD3, a T lymphoid marker, was significantly decreased compared to healthy counterparts. Furthermore, low expression of additional B- and T- lineage markers (cd19, cd79a, lck) was noticed. Spleen immunohistochemical analysis of livers and kidneys revealed increased presence of cytoplasmic MPO (cMPO). RT-qPCR analysis of blood from diseased animals, showed significant increased MPO expression, compared to control animals. Symptomatic animals had increased white blood cell counts. The overrepresentation of MPO and the lack or reduced T- and B-cell lineage markers expression, implies the expansion of myeloid lineage cells in these animals and probably the presence of AML. This novel AML model needs to be further validated by NGS, FACS, and transplantation assays.
Our tumor model will provide opportunities for identification of novel driver mutations and targets for therapeutic intervention and will offer a unique experimental platform that can be easily plugged into the research lines of several groups active in cancer research field.

Abstract n°56

Membrane type-matrix metalloproteinases (MT-MMPs) are known as key regulators of cancer progression/metastasis

Ali Jalili, Shohreh Fakhari, Bahram Nikkhoo, Bayazid Ghaderi, Sako Mirzaie

Cancer & Immunology Research Center, Kurdistan University of Medical Sciences, Sanandaj Iran

Presenting author: Ali Jalili – Kurdistan University of Medical Sciences, Cancer & Immunology Research Center, Pasdaran St, Sanadaj, Kurdistan - Ali130@gmail.com

Membrane type-matrix metalloproteinases (MT-MMPs) are known as key regulators of cancer progression/metastasis. However, their roles in the growth and progression of multiple myeloma (MM) have not been yet elucidated. In this study, we screened 6 MT-MMPs in MM and B cell lines, normal B lymphocytes, mononuclear cells (MNCs) and polymorphonuclear cells (PMNs). Our data showed that MT2-MMP is preferentially expressed at both mRNA and protein levels in MM cell lines and primary MM cells as detected by qRT-PCR and flow cytometry. Moreover, our immunohistochemistry data demonstrated that MT2-MMP is expressed on 100 % of bone marrow (BM) biopsies from patients with MM, but not on any of biopsies from BM of normal individuals. To determine the role of MT2-MMP in MM cells, U266 cell line was transfected with either scramble siRNA or MT2-MMP siRNA. We observed that MT2-MMP knockdown resulted in a significant (i) reduction of adhesion, invasion, and migration, (ii) decreased pro-MMP-2 and pro-MMP-9 activation and (iii) diminish 3D cell proliferation in U266 cells. More importantly, MT2-MMP suppression remarkably prevented xenograft growth of MM cells in nude mice. In conclusion, we found that MT2-MMP is strongly expressed in MM cells and plays vital roles in invasion/progression of these cells in vitro and in vivo.

Abstract n°57

Feasibility of dose painting radiation therapy in a rat model for glioblastoma

Sam Donche (1), Jeroen Verhoeven (2), Charlotte Bouckaert (3), Benedicte Descamps (4), Karel Deblaere (1), Tom Boterberg (5), Robrecht Raedt (3), Christian Vanhove (4), Ingeborg Goethals (1)

(1) Department of Diagnostic Sciences, Ghent University, Ghent, Belgium
(2) Department of Pharmaceutical Analysis, Ghent University, Ghent, Belgium
(3) Department of Head and Skin, Ghent University, Ghent, Belgium
(4) Department of Electronics and Information Systems, Ghent University, Ghent, Belgium
(5) Department of Human Structure and Repair, Ghent University, Ghent, Belgium

Presenting author: Sam Donche – Ghent University, Diagnostic Sciences, Corneel Hemanslaan 10, 9000 Ghent - sam.donche@ugent.be

Purpose
Glioblastoma (GB) is the most common malignant primary brain tumour. Even with the current state-of-the-art treatment of postoperative combined radiation therapy (RT) and chemotherapy, the median survival is only 12-14 months. Therefore, there is an urgent need for alternative treatment strategies in glioblastoma patients. In this work, our goal was to incorporate molecular imaging information, obtained from amino acid (AA) PET, into the RT plan to deliver a non-uniform dose to the tumour volume in a GB rat model.

Methods
F98 GB cells were inoculated in the brain of Fischer rats and tumour growth was evaluated using MRI. When the desired tumour volume was reached, the rats were allocated into three different groups. Group 1 received MRI-based RT conform to clinical practice. A contrast-enhanced T1-weighted MRI scan was acquired. Subsequently, the animal was moved to the small animal radiation research platform (SARRP), where a planning CT was acquired that was co-registered to the MRI scan. Using three non-coplanar arcs, a dose of 20Gy was delivered to the gross tumour volume (GTV). Group 2 received MRI-PET-based RT. An AA PET and contrast-enhanced T1-weighted MRI scan were acquired after the intravenous injection of [18F]FET. Once again, a dose of 20Gy was delivered to the GTV. Additionally, a sub-volume boost of 8Gy was delivered to the PET region with maximum tumour uptake using three additional arcs. Group 3 received RT entirely based on PET imaging. A [18F]FET scan was acquired. A dose of 20Gy was delivered to the biological tumour volume (BTV) defined as 60% of the maximum tumour uptake. Additionally, 8Gy was delivered to the PET region with maximum tumour uptake.

A multimodality bed was used to transport the animals between the different imaging modalities and SARRP. To simplify the co-registration, a spirally coiled tubing filled with contrast agent and/or radioactive solution was attached to the multimodality bed.

Results & conclusions
The feasibility of incorporating PET into RT planning was investigated in a GB rat model. Tumour heterogeneity could be clearly visualized using preclinical PET imaging, allowing to easily define BTV and the target the region for dose escalation.

Abstract n°59

Improving radiosensitization in colorectal cancer cells by combining olaparib with floxuridine

Hui Wang (1), Freek Rosseel (1), Heng Jiang(2), Sven de Mey (1), Kalun Law (1), Thierry Gevaert (2), Inès Dufait (2), Mark De Ridder (1,2).

(1)Vrije Universiteit Brussel, Radiotherapy, Brussels, Belgium.
(2)Universitair Ziekenhuis Brussel, Department of Radiotherapy, Brussels, Belgium.

Presenting author: Hui Wang, Vrije Universiteit Brussel, TROP, Department of Radiotherapy, Laarbeeklaan 103, 1090 Brussels - hui.wang.ecnu@gmail.com

Background and Hypothesis
Floxuridine (FdUrd) is commonly used within the standard treatment regimen for patients with colorectal cancer by disrupting DNA metabolism as well as activating the base excision repair pathway. However, more effective treatments are needed for patients with diseases progression. Poly ADP ribose polymerase (PARP) is activated in the event of DNA damage and promotes DNA repair. Inhibition of PARP is an effective radiosensitizing strategy which leads to impaired DNA repair, causing an increase in DNA damage and consequently cell death. We hypothesized that olaparib, a PARP inhibitor, will synergize with FdUrd in combination with radiation in colorectal cancer cells.

Material and Methods
We examined the radiosensitizing effect by colony formation assay of olaparib and FdUrd respectively in human colorectal cancer cells DLD-1 in both aerobic and hypoxic conditions. Next, to unveil the synergistic radiosensiting effect, cells were treated with concentrations of FdUrd, ranging from 0 nM to 1 μM, in combination with different concentrations of olaparib, ranging from 0 μM to 1 μM, for 24 hours. ROS production, DNA damage, and cell cycle arrest were assessed using flow cytometry to reveal the underlying mechanisms.

Results
Firstly, we found that FdUrd does not synergize the toxicity of olaparib in DLD-1. Secondly, FdUrd and olaparib enhanced radiosensitivity of tumor cells at various concentrations. Next, after optimization, we found that, FdUrd at 30 nM and 100 nM synergize the radiosensitizing effect of olaparib at 0,3 μM. Finally, the radiosensitizing effect of the combination is linked to ROS-mediated increase of DNA damage, and G1 cell cycle arrest. Further mechanisms are still under investigation.

Conclusion
In conclusion, our study suggests that olaparib is a promising radiosensitizer, which can be integrated in the standard regime of colorectal cancer treatment.

Abstract n°60

Metabolic switch in melanoma cells with acquired resistance to targeted therapies

Laura Soumoy (1) , Mohammad Krayem (2), Vanessa Tagliatti (3), Ghanem Ghanem (2), Sven Saussez (1), Fabrice Journe (1,2)

(1) Department of Human Anatomy & Experimental Oncology, University of Mons, Belgium;
(2) Laboratory of Oncology and Experimental Surgery; Institut Jules Bordet, ULB, Belgium;
(3) Department of Human Biology & Toxicology, University of Mons, Belgium.

Presenting author: Laura Soumoy – Umons, Human Anatomy & Experimental Oncology, Avenue du champ de Mars 6, 7000 Mons - laura.soumoy@umons.ac.be

Background and objectives
Melanoma affects a large number of young adults and its incidence has been steadily increasing for more than 20 years. The diagnosis of melanoma at metastatic stage is associated with a very poor patient prognosis in relation to a very high mutational potential of such cancer cells. Advanced melanoma has also a high capacity to activate alternative signalling pathways for cancer cell survival leading to the development of acquired resistance to targeted therapies. Combinations of targeted therapies are proposed as the most promising way to overcome these resistances. However, while many combinations have been evaluated in preclinical settings, only few of them have been approved for clinical use, mainly targeting the same MAPK pathway (BRAF and MEK) but with a limited remission rate or stabilization of the disease.
In our work, we aim to study the mechanisms involved in the development of acquired resistance in melanoma cells. In order to find a new way to overcome these resistances, we particularly focused on the metabolic changes in sensitive versus resistant cells.

Methods
We performed our different experiments on a panel of cells either sensitive or with acquired resistance to the MAPK inhibitors used in the treatment of melanoma patients.
We performed 1H-NMR experiments to compare metabolites between sensitive and resistant cells regarding their consumption and production.
We also studied ROS levels in each cell line by FACS and finally we studied mitochondrial morphology by staining cells with Mitotracker.

Results
Our 1H-NMR experiments highlighted the implication of glutaminolysis in the metabolism of the resistant cell lines. We hypothesis that a metabolic switch occurred during the development of resistance to targeted therapies. As glutaminolysis is linked to mitochondria, we also studied the mitochondrial pool in our cells. Our analysis showed an increase in the mitochondrial pool in the resistant cells. Finally, our experiments also indicated an increase in ROS levels in case of resistance, but these cells had also better defenses against oxidative stress.

Conclusion
Our comparison of the metabolism in our panel of sensitive and resistant cells to various MAPK inhibitors highlighted the involvement of mitochondria, glutaminolysis and phosphorylating oxidation in the development of resistances.

Abstract n°62

Tyrosine induced-oxidative stress promotes phenotype switching in melanoma cells and affects the establishment of primary cultures

Ahmad Najem (1), Mohammad Krayem (1), Jasper Wouters (2), Florian Rambow (3), François sales (1,4), Ahmad Awada A (1,4), Chris Marine (3), Stein Aerts S (2), Ghanem E. Ghanem (1).

(1) Laboratory of Oncology and Experimental Surgery (LOCE), Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium.
(2) Laboratory of Computational Biology, VIB Center for Brain & Disease Research, KU Leuven, Leuven, Belgium; Department of Human Genetics, KU Leuven, Leuven, Belgium
(3) Laboratory for Molecular Cancer Biology, VIB Center for Cancer Biology, KU Leuven, Leuven, Belgium; Department of Oncology, KU Leuven, Leuven, Belgium.
(4) Department of Internal Medicine, Institut Jules Bordet, Université Libre de Bruxelles, 1000 Brussels, Belgium

Presenting author: Ahmad Najem – Institut Jules Bordet, ULB, Laboratory of Oncology and Experimental Surgery (LOCE), Rue Héger Bordet 1, 1000 Brussels - ahmad.najem@bordet.be

Background and objectives: Melanoma cells have high phenotypic plasticity with two main states described so far: "melanocyte-like" and "mesenchymal-like". Tyrosine is the precursor and stimulator of melanogenesis/pigmentation, a main feature in melanocytes. The latter can increase oxidative stress and thus affect melanoma phenotype. In our study, we aimed to investigate the effect of tyrosine-induced oxidative stress on phenotype switching and identify related mechanisms regulating phenotypic plasticity and their consequences.

Results: Seven Primary cultures with different mutation status derived from a different patient were each splited and propagated in 1) Ham's F10 (low tyrosine) and 2) Ham's F10 supplemented with tyrosine, RPMI or DMEM (high tyrosine). We found that high tyrosine promotes a phenotypic switch towards a mesenchymal-like (switchers: 4/7) or senescence-like (non-switchers: 3/7) phenotype. Also, we identified that, unlike pigmented cultures, unpigmented primary cultures have an inherent invasive/undifferentiated phenotype. Both this invasive state and the phenotype switching induced by tyrosine are associated with resistance to MAPKi but vulnerability to RTKi (TKI). Interestingly, our data revealed that ROS and the functional antagonism between RTK and cAMP/MITF axis drive cell-state transitions and play a critical role in regulating cell plasticity. Moreover, our findings suggest that the choice of culture medium is crucial during the establishment of melanoma primary cultures in order to preserve a cell phenotype that is the closest to the corresponding donor tumor tissue.

Conclusion: One aminoacid, tyrosine, can alone promote phenotype switching in melanoma. It occurs early while establishing cell cultures in media containing high tyrosine with immediate consequences such as resistance to drugs. This may have had dramatic impact on published data knowing that the vast majority of melanoma cultures uses high tyrosine media.

Abstract n°63

The Impact of optimized mRNA on cancer vaccination

Wout de Mey (1), Quentin Lecocq (1), Kris Thielemans (1), Lorenzo Franceschini (1)

(1) Laboratory for Molecular and Cellular Therapy (LMCT), Vrije Universiteit Brussel, Laarbeeklaan 103, B-1090 Brussels.

Presenting author: Wout de Mey – Vrije Universiteit Brussel, LMCT, Laarbeeklaan 103, 1090 Brussels - wout.de.mey@vub.be

Messenger RNA (mRNA) based therapeutics have been in the last 10 years extensively exploited as attractive treatment against cancer. However, in vivo delivery of in vitro transcribed (IVT) mRNA can cause rapid production of type I interferon and trigger a counteracting effect. On one hand a strong innate immune response is induced, on the other this is known to reduce mRNA translation and thus possibly decrease the mRNA vaccine efficiency. Here, we enhanced mRNA biological activity improving mRNA translatability in the cell and lowering the in-vivo molecular immunogenicity. This might compensate the negative effect of mRNA-triggered type I interferon response. Furthermore, we could rescue the immune response by using "helper" adjuvant mRNA in combination with our vaccine. Our data showed that the optimized mRNA together with the 'helper' adjuvant mRNA is a promising improvement for cancer vaccination therapy.

Abstract n°64

Novel therapeutic combination strategies in non-BRAF mutant melanoma

Sabbah M (1), Krayem M (1), Najem A (1), Simon S (2) , Vanderkerkhove C (2) , Van gestel D (2), Awada A (3), Ghanem GE (1)

(1) Laboratory of Oncology and Experimental Surgery, Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium
(2) Department of Radiation Oncology, Institut Jules Bordet, Université libre de Bruxelles, 1000 Brussels, Belgium
(3) Medical Oncolgy Clinic, Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium

Presenting author: Malak Sabbah – ULB, Insitut Jules Bordet, Laboratory of Oncology and Experimental Surgery, Rue Héger-Bordet 1, 1000 Brussels - malak.sabbah@ulb.ac.be

Metastatic melanoma can be a mutinous disease, often requiring drug combinations. Currently, a selective mutant BRAF/MEK inhibitors with immunotherapy are used with efficacy in BRAF mutant melanoma patients. Non BRAF mutant melanoma patients have limited treatment with only 30% responding to check-point inhibitors. Thus drug combination studies are needed for this particular group of patients. Radiotherapy may be one of the options despite that Melanoma is commonly regarded as radioresistant. Indeed, when used as adjuvant treatment for patients suffering from advanced disease, it reduced the risk of local and distant recurrences. It is also known that ionizing radiations activate RTKs and hence regulates DDR proteins and proteins involved in DNA repair mechanisms, thus promoting cell proliferation and survival. In this study, we aimed to assess the benefit of combining RTK inhibition to RT in non-BRAF mutant melanoma cells.
We found that RT upregulates the expression of several RTKs (EGFR, HGFR , IGF1R and c-Kit) in a dose dependent manner 2 days after a single irradiation. In a next step, we added different RTK inhibitors (Afatinib/Crizotinib/sunitinib) and found with all a significant enhancement of the radiosensitivity of all melanoma cell lines used. We then assessed the underlying mechanisms and the first was DNA repair. We found an increase in PARP under RT that can be reversed when combined with RTKi (Crizotinib and afatinib) and targeting PARP with olaparib radiosensitises melanoma cells. Intersetingly, its combination with RTK inhibitors is able to eliminate all residual cells even at low doses of RT clearly showing a potentiating effect.
In conclusion, we provide evidence that RT-induced RTKs may promote DNA repair mechanisms by activating PARP and that targeting RTKs and PARP when using RT may be a sound therapeutical approach in non- BRAF mutant melanoma.

Abstract n°65

Dicarbonyl stress induces an epigenetic deregulation leading to a pro-migratory phenotype in breast cancer.

Assia Tiamiou(1), Gaurav Dube(2), Justine Bellier(1), Martin Bizet(2), Rachel Deplus(2), Tom Wissocq(1), Olivier Peulen(1), Vincent Castronovo(1), François Fuks(2) and Akeila Bellahcène(1)

(1) Metastasis Research Laboratory, GIGA-Cancer, University of Liège, Liège, Belgium
(2) Laboratory of Cancer Epigenetics, U-CRC, Université Libre de Bruxelles, Brussels, Belgium

Presenting author: Tiamiou Assia – University of Liege, Mestastasis Research Laboratory GIGA Cancer, Avenue Hippocrate 13, 4000 Liege - assia.tiamiou@uliege.be

Methylglyoxal (MGO) is a very reactive dicarbonyl molecule derived from glycolysis. MGO interacts with DNA, lipids and proteins to form Advanced Glycation Products (AGEs).
Our previous studies have demonstrated that MGO glycating stress triggers enhanced tumor growth and metastasis in breast cancer. The glyoxalase system, glyoxalases (GLO1 and GLO2), is present in all mammalian cells and is one of the major defenses against MGO stress. We generated stable GLO1-depleted breast cancer cells that present with an endogenous MGO stress. Transcriptomic analysis of GLO1-depleted cells revealed a pro-metastatic MGO signature notably comprising the regulation of extracellular matrix and invasion related genes.
A comprehensive genome wide methylation analysis performed on GLO1-depleted cells demonstrated a significant global hypermethylation. This study aims to explore the potential connection between the acquisition of MGO stress pro-metastatic phenotype and the deregulation of the methylation machinery. Interestingly, transcriptomic analysis revealed an increase of DNMT3A expression, one of the main de novo DNA methyltransferases, in GLO1-depleted cells. In fact, DNMT3A and DNMT3B protein levels were increased upon exogenous MGO treatment and decreased in presence of MGO scavengers thus confirming the link with MGO stress. The specific inhibition of DNMTs in GLO1-depleted breast cancer cells induced a significant inhibition of their migratory potential. Ongoing analysis aimed at the integration of gene expression data with gene methylation status in cancer cells has highlighted the down-regulation of several anti-metastatic genes associated with a hypermethylation of their promoters under MGO stress.
Altogether these observations combine to suggest the existence of a novel link between MGO stress and epigenetic regulation in human breast cancer cell lines sustaining their acquisition of pro-migratory and pro-metastatic properties.

Abstract n°66

Myoferlin Contributes to the Metastatic Phenotype of Pancreatic Cancer Cells by Enhancing Their Migratory Capacity Through the Control of Oxidative Phosphorylation

Gilles Rademaker 1, Brunella Costanza 1, Sandy Anania 1, Ferman Agirman 1, Naïma Maloujahmoum 1, Emmanuel Di Valentin 2, Jean Jacques Goval 3, Akeila Bellahcène 1, Vincenzo Castronovo 1 and Olivier Peulen 1

(1) Metastasis Research Laboratory, Giga Cancer, University of Liège, Liège, Belgium
(2) Viral Vector Platform, Giga Cancer, University of Liège, Liège, Belgium
(3) Imaging Platform, Giga Cancer, University of Liège, Liège, Belgium

Presenting author: Gilles Rademaker – Uliège, Metastasis Research Laboratory, Rue lescours 10, 4653 Liège - g.rademaker@gmail.com

Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest malignancies with an overall survival of 5%, and is the second cause of death by cancer, mainly linked to its high metastatic aggressiveness. Accordingly, understanding the mechanisms sustaining the PDAC metastatic phenotype remains a priority. In this study, we have generated and used a murine in vivo model to select clones from the human PANC-1 PDAC cell line that exhibit a high propensity to seed and metastasized into the liver. We showed that myoferlin, a protein previously reported to be overexpressed in PDAC, is significantly involved in the migratory abilities of the selected cells. We first report that highly PANC-1 metastatic clones expressed significantly higher myoferlin level than the corresponding low metastatic ones. Using scratch wound and Boyden's chamber assays, we show that cells expressing high myoferlin level have higher migratory potential than cells characterized by a low myoferlin abundance. Moreover, we demonstrate that myoferlin silencing leads to a migration decrease associated to a reduction of mitochondrial respiration. Since mitochondrial oxidative phosphorylation has been shown to be implicated in the tumor progression and dissemination, we were able to associate myoferlin expression with high mitochondrial respiration in high metastatic cell lines. Our data identify myoferlin as a valid potential therapeutic target in PDAC and suggest that PET-scan is a limited technic to identify early PDAC metastases.

Abstract n°67

Methylglyoxal stress contributes to immunosuppression in breast cancer

Tom Wissocq (1), Pascale Hubert (2), Assia Tiamiou (1), Muhammad Zaeem Noman (3), Olivier Peulen (1), Vincent Castronovo (1), Bassam Janji (3), Akeila Bellahcène (1)

(1) Metastasis Research Laboratory, GIGA-Cancer, University of Liège, Belgium
(2) Laboratory of Experimental Pathology, GIGA-Cancer, University of Liège, Belgium
(3) Tumor Immunotherapy and Microenvironment (TIME) Group, Department of Oncology, Luxembourg Institute of Health, Luxembourg

Presenting author: Tom Wissocq – University of Liege, GIGA Cancer, Mestastasis Research Laboratory, Avenue de l'hôpital, Tour de Pathologie B23, 4000 Liège - t.wissocq@uliege.be

Growing interest in cancer energy metabolism, and in particular in the so-called glycolytic switch, points to methylglyoxal (MGO) as a new oncometabolite which accumulation sustains specific pro-cancer functions. MGO is a very reactive dicarbonyl with high protein glycating capacity, leading to the formation of advanced glycation end products (AGEs). We have previously demonstrated that MGO stress triggers enhanced breast tumor growth and metastasis in vivo. In this study, we undertook to understand the dynamics of the microenvironmental interactions of breast cancer cells under MGO stress with immune cells. We have first characterized MGO stress along spontaneous breast cancer tumor growth and metastasis in mice. Using MMTV-PyMT model, we showed a significant cytoplasmic accumulation of MGO AGEs from adenoma to late carcinoma lesions indicating the occurrence of MGO stress during tumor progression. In good accordance, we observed a significant reduction of lung metastatic foci upon mice treatment with carnosine, a potent MGO scavenger dipeptide. In order to characterize the immune infiltration associated with tumor progression, we performed a FACS screening and characterization of the whole immune cell population in MMTV-PyMT experimental tumors. Interestingly, we observed a significant trend to a decrease of specific immunosuppressive cell subtypes upon carnosine treatment. Taken together, these preliminary data point to an important role of MGO stress in the recruitment of immunosuppressive cells, which could be reversed using carnosine and potentially enhance the susceptibility of breast tumors to various therapeutic regimen.

Abstract n°68

Role of myoferlin in mitochondrial dynamics and metabolic fitness of pancreas cancer

Sandy Anania (1), Gilles Rademaker (1), Tom Wissocq (1), Akeila Bellahcène (1), Vincent Castronovo (1), Olivier Peulen (1)

(1) Metastasis Research Laboratory, Uliege

Presenting author: Sandy Anania – Uliege, Metastasis Research Laboratory, Avenue de l'hôpital, tour de pathologie B23, 4000 Liege - Sandy.Anania@uliege.be

Pancreatic cancer is the 7th most common cause of cancer mortality in the world. It is predicted to become the 2nd leading cause of cancer-related death in 2030. In the majority of cases, due to a late diagnosis, the tumor is not resectable and already disseminated. Therefore, new specific biomarkers providing early diagnosis for pancreatic cancer are needed. In addition to the lack of specific and early biomarkers, chemotherapies (gemcitabine and folfirinox) poorly improve the overall survival of Pancreatic Ductal Adenocarcinoma (PDAC) patients. Hence, a better understanding of physiopathological processes underlying PDAC is required in order to offer more effective treatments.

Myoferlin is a 230 kDa protein with multiple C2 domains known to interact, through calcium binding, with negatively charged phospholipids. This protein was first described in myoblast fusion. Interestingly, Myoferlin is also overexpressed in several cancers, including pancreatic cancer, where it plays a role in endocytosis, exocytosis, and has been located in exosomes. Recently, our team showed a fragmentation of the mitochondrial network in PDAC cells when myoferlin was depleted using siRNA. Understanding the mechanism underlying this mitochondrial disruption would be of great interest as mitochondria are major actors in cancer development, progression and resistance.

Owing to the known role of myoferlin in membrane fusion, we assessed its direct involvement in the mitochondrial fusion machinery. Indeed, if myoferlin is a part of the mitochondrial fusion machinery, its silencing together with an unopposed fission would lead to mitochondrial fragmentation. First, we performed immunofluorescence to colocalize myoferlin and a mitochondrial outer membrane 65kDa protein. Colocalization studies showed no significant colocalization. We then performed immunofluorescence to stained myoferlin and the main factor of mitochondrial fusion mitofusin-1/2 (MFN1/2). Colocalization image analysis revealed a 60% colocalization between both proteins. Those results were further confirmed by PLA (Proximity Ligation Assay). Finally, to evaluate a direct protein-protein interaction, we performed a co-immunoprecipitation assay. The main isoform of myoferlin appeared to coimmunoprecipitate with MFN1/2, suggesting a direct interaction between these proteins.

Abstract n°69

Epithelial HMGB1 delays skin wound healing and drives tumor initiation by priming neutrophils for NET formation

Esther Hoste(1,2), Christian Maueroder(1,2), Lisette van Hove(1,2), Leen Catrysse(1,2), Hanna-Kaisa Vikkula(1,2), Ignacia Fuentes(3), Francis Palisson(3), Kodi Ravichandran(1,2), Yvan Saeys(1), Robert Felix Schwabe(4) and Geert van Loo(1,2)*

(1)VIB Center for Inflammation Research, B-9052 Ghent, Belgium
(2) Department of Biomedical Molecular Biology, Ghent University, B-9052 Ghent, Belgium
(3) Fundación DEBRA Chile, Santiago, Chile
(4) Department of Medicine, Columbia University, New York, NY 10019, USA

Presenting author: Esther Hoste – VIB-UGent, Center for Inflammation Research, Technologiepark 71, 9052 Ghent - Esther.Hoste@irc.vib-ugent.be

Regenerative responses predispose tissues to tumor formation by largely unknown mechanisms. High-mobility group box 1 (HMGB1) is a danger-associated molecular pattern contributing to inflammatory pathologies. Here, we show that HMGB1 derived from keratinocytes, but not myeloid cells, delays cutaneous wound healing and drives tumor formation. In wounds of mice lacking HMGB1 selectively in keratinocytes, a marked reduction in neutrophil extracellular trap (NET) formation is observed. Pharmacological targeting of HMGB1 or NETs prevents skin tumorigenesis and accelerates wound regeneration. HMGB1-dependent NET formation and skin tumorigenesis is orchestrated by TNF and requires RIPK1 kinase activity. NETs are present in the microenvironment of keratinocyte-derived tumors in mice and lesional and tumor skin of patients suffering from Recessive Dystrophic Epidermolysis Bullosa, a disease in which skin blistering predisposes to tumorigenesis. We conclude that tumorigenicity of the wound microenvironment depends on epithelial-derived HMGB1 regulating NET formation, thereby establishing a mechanism linking reparative inflammation to tumor initiation.

Abstract n°70

Investigating immune activation upon beta targeted radionuclide therapy using anti-CD20 single domain antibody fragments in melanoma

Thomas Ertveldt (1), Yana Dekempeneer (2), Lien De Beck (1), Cleo Goyvaerts (1), Quentin Lecocq (1), Yannick De Vlaeminck (1), Robin Maximilian Awad (1), Nick Devoogdt (2), Marleen Keyaerts (2,3), Matthias D'Huyvetter (2,Δ); Karine Breckpot (1,Δ), Ahmet Krasniqi (2,Δ)

(1) Laboratory of Molecular and Cellular Therapy, Vrije Universiteit Brussel, Belgium
(2) In Vivo Cellular and Molecular Imaging, Vrije Universiteit Brussel, Belgium
(3) Department of Nuclear Medicine, UZ Brussel, Belgium
(Δ) Shared last authorship

Presenting author: Thomas Ertveldt – Vrije Universiteit Brussel, LMCT, Laarbeeklaan 103, 1090 Brussels - thomas.karel.ertveldt@vub.be

Introduction: Targeted radionuclide therapy (TRT) is a systemic treatment with radiolabeled cancer-specific probes purposed to selectively hit diseased cells. As radioactive labels, beta--emitters, such as 90Yttrium and 177Lutetium, are studied to cause irreparable DNA damage. Beta--TRT using camelid single domain antibodies (sdAbs) is studied at the VUB, showing high potential in controlling tumor growth. Recently, immune activation after beta--TRT was reported. Therefore, we studied stimulation of CD8+ T cells and upregulation of inhibitory immune checkpoints after sdAb-mediated beta--TRT.

Materials and methods: C57BL/6 mice were inoculated with B16-melanoma cells expressing human CD20 and ovalbumin. After engraftment, mice received fractionated treatment with non-targeting sdAb or anti-CD20 sdAbs, labeled with the radionuclide 177Lutetium. Different readouts were evaluated: (1) tumor volume, measured by caliper, (2) gene expression profiling of the tumor microenvironment (TME), evaluated using RT-qPCR, (3) immune cell composition of the TME, evaluated using flow cytometry and (4) systemic immune responses, evaluated by stimulation of CD8+ splenocytes with tumor antigens.

Results: A reduced tumor progression was observed upon treatment of CD20+ melanoma-bearing mice. Despite this tumor control, we were not able to document immune activation. Gene expression and flow cytometry analysis did not reveal changes in CD8+ T cells or the inhibitory immune checkpoint PD-1/PD-L1 in the TME. Moreover, CD8+ splenocytes did not show specificity for the antigen ovalbumin, which serves as a surrogate tumor antigen.

Conclusion: Although beta--TRT with 90Yttrium coupled to a tumor-targeting alkylphosphocholine in a model non-Hodgkin Lymphoma was shown to induce immune responses, we were not able to show similar immune activation with 177Lutetium-coupled anti-CD20 sdAbs in a melanoma model. Further research to confirm and study the reason for these contradicting results is required.

Abstract n°72

Cancer cell addiction to fatty acids in tumor acidic compartment supports a link between dietary lipids and cancer progression.

E. Dierge (1, 2), C. Corbet (1), L. Mignard (2), Y. Larondelle (2) and O. Feron (1)

(1) Pole of Pharmacology and Therapeutics (FATH), Institut de Recherche Expérimentale et Clinique (IREC), UCLouvain, Brussels, Belgium
(2) Louvain Institute of Biomolecular Science and Technology, UCLouvain, Louvain-la-Neuve, Belgium.

Presenting author: Emeline Dierge – IREC, Pharmacology and Therapeutics, Avenue Hippocrate 57, 1200 Brussels - emeline.dierge@uclouvain.be

Not accepted for publication.

Abstract n°73

Effects of non-invasive vagal nerve stimulation on radiation-induced inflammation and cancer prognosis

Eva Reijmen (1), Sven de Mey (2), Helena Van Damme (3), Thierry Gevaert (2), Christine Collen (2), Lore Decoster (4), Marijke De Couck (5), Damia Laoui (3), Karine Breckpot (1), Jacques De Grève (4), Mark De Ridder (2), Yori Gidron (6), Cleo Goyvaerts (1)

(1) Laboratory of Molecular and Cellular Therapy, Department of Biomedical Sciences, Vrije Universiteit Brussel, Brussels, Belgium
(2) Department of Radiotherapy, Oncology Centre University Hospital Brussels (UZ Brussel), Brussels, Belgium
(3) Myeloid Cell Immunology Lab, VIB Center for Inflammation Research, Brussels, Belgium; Lab of Cellular and Molecular Immunology, Vrije Universiteit Brussel, Brussels, Belgium
(4) Laboratory of Medical and Molecular Oncology (LMMO), Department of Medical Oncology, Oncologisch Centrum, UZ Brussel, Vrije Universiteit Brussel (VUB) Brussels, Belgium
(5) Department of Public Health, Mental Health and Wellbeing Research Group, Faculty of Medicine and Pharmacy, Vrije Universiteit Brussel, Brussels, Belgium; Faculty of Health Care, University College Odisee, Aalst, Belgium
(6) Faculty of Social Welfare and Health Sciences, University of Haifa, Haifa, Israel

Presenting author: Eva Reijmen – Vrije Universiteit Brussel, LMCT, Laarbeeklaan 103, 1090 Brussels - eva.nina.reijmen@vub.be

Background: Radiotherapy (RT) has an established role in the treatment of patients with non-small-cell lung cancer (NSCLC). Unfortunately, tumor irradiation upregulates the expression of various inflammatory cytokines which contribute to radioresistance with subsequent tumor progression and metastasis. Emerging evidence suggests that myeloid-derived suppressor cells (MDSC) play a key role in this response as they contribute to an immunosuppressive tumor microenvironment (TME) and tumor relapse after irradiation. Recent studies indicate that the vagal nerve is an immunomodulator and that its stimulation (VNS) can inhibit the inflammatory process through the so-called 'cholinergic anti-inflammatory pathway'. Moreover, an experimental murine study demonstrated that the efferent vagal pathway is an important suppressor of MDSC expansion in colorectal cancer.
Aim: This study tends to investigate if non-invasive VNS could suppress RT-induced tumor promoting inflammation and revert the immunosuppressive TME in lung cancer.
Methods: Preclinically, lung tumor-bearing C57Bl/6 mice were treated with VNS alone, fractionated RT (2.4 Gy, 4 consecutive days) plus VNS (2x/day, 25HZ, 5 consecutive days) or RT plus sham VNS. Tumor volumes were monitored via bioluminescent imaging. In addition, murine spleens and lungs were subjected to immunological analysis via flow cytometry. Furthermore, NSCLC patients (n=6) were enrolled in a blind randomized clinical trial. Patients were subjected to VNS (2x/day, 20Hz) or sham (control arm) in combination with conventional chemo-RT. Blood was collected in both setups before, during and at the end of treatment for further flow cytometry-based analysis and ELISA.
Results: Preliminary data show that the orthotopic lung cancer model allows evaluation of therapeutic RT efficacy. Furthermore, we observed immune modulation upon VNS, more specifically an increase in conventional type 2 dendritic cells. Accordingly, clinical results show VNS-mediated upregulation of dendritic cells, natural killer cells and CD8+ T cells in blood. In contrast both the granulocytic and monocytic MDSC populations decreased over time.
Conclusions: Though most results are preliminary, we observe trends towards the amelioration of antitumor immunity with reduction of tumor promoting MDSC. VNS is a safe and non-invasive treatment option, therefore it could have a positive impact on (immune)therapy strategies for a broad range of cancer patients.

Abstract n°74

Inhibition of the protein arginine methyltransferase PRMT5 in high-risk multiple myeloma as a novel treatment approach

Philip Vlummens (1,2), Stefaan Verhulst (3), Kim De Veirman (1), Eline Menu (1), Fritz Offner (2), Karin Vanderkerken (1), Jérôme Moreaux (4), Elke De Bruyne (1), Ken Maes (1)

(1) Department of Hematology and Immunology, Vrije Universiteit Brussel, Brussel, Belgium
(2) Department of Clinical Hematology, Ghent University Hospital, Ghent, Belgium
(3) Liver Cell Biology Lab, Vrije Universiteit Brussel, Brussel, Belgium
(4) Laboratory for Monitoring Innovative Therapies, CHU de Montpellier, Montpellier, France

Presenting author: Philip Vlummens – Vrije Universiteit Brussel, Hematology and Immunology, Laarbeeklaan 103, 1090 Brussels - philip.vlummens@vub.be

Introduction
Multiple myeloma (MM) is an incurable clonal plasma cell malignancy. Subsets of patients have high-risk features linked with dismal outcome. Therefore, the need for effective therapeutic options remains high. Here, we used bio-informatic tools to identify novel targets involved in DNA repair and epigenetics and which are associated with high-risk myeloma.

Results
The prognostic significance of the target genes was analysed using publically available gene-expression data of MM patients (TT2/3 and CoMMpass cohorts). Hence, protein arginine methyltransferase 5 (PRMT5) was identified as a promising target. In the CoMMpass trial, high PRMT5-expression was linked with decreased progression free survival (112.7 vs 189.9 weeks, p=0.003). Druggability was assessed in OPM2, JJN3, AMO1 and XG7 human myeloma cell lines using the PRMT5-inhibitor EPZ015938. EPZ015938 strongly reduced the total symmetric-dimethyl arginine levels in all cell lines and lead to decreased cellular growth, supported by cell line dependent changes in cell cycle distribution. At later time points, apoptosis occurred, as evidenced by increased AnnexinV-positivity and cleavage of PARP and caspases. Transcriptome analysis revealed a role for PRMT5 in regulating alternative splicing, nonsense-mediated decay, DNA repair and PI3K/mTOR-signaling, irrespective of the cell line. PRMT5 inhibition reduced the expression of upstream DNA repair kinases ATM and ATR, which may in part explain our observation that EPZ015938 and the DNA-alkylating agent, melphalan, have combinatory effects. Of interest, using a low-dose of mTOR-inhibitor, we observed that cell viability was (partially) rescued from the effects of EPZ015938, indicating a role for mTOR-related pathways in the anti-myeloma activity of EPZ015938.

Conclusion
PRMT5 is involved in high-risk disease and is important for MM cell growth and survival. Transcriptome analysis revealed a cross-talk between PRMT5 and alternative splicing, DNA repair and PI3K/mTOR signaling. Further research is needed to understand how these pathways contribute to high-risk MM disease to optimize therapy.

Sponsors

The BACR wishes to express their gratitude to all the sponsors, who made it possible to organize the BACR Annual Meeting 2020.

With support of

Vrije Universiteit Brussel
UZ Brussel
European Asociation for Cancer Research
Doctoral School Life Sciences and Medicine at the VUB

Gold sponsor

Miltenyi Biotec

Silver sponsors

Bio–techne
PEPROTech
VWR
Sartorius
Led techno
Sanbio
Promega
Enzo Life Sciences
Biolegend
BD Biosciences
Merck
Sysmex

With support of

Gold sponsor

MACS

Miltenyi Biotec

Silver sponsors

PEPROTECH®
OUR SUPPORT, YOUR DISCOVERY

vwr™
part of avantor

sartorius

Led techno
lab solutions

Sanbio
RESEARCH & DIAGNOSTICS

Promega

Enzo

BioLegend®
Enabling Legendary Discovery™


BD
MERCK
sysmex
Lighting the way
with diagnostics