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tRNA wobble editing links mRNA translation to metabolic reprogramming and dictates ferroptosis sensitivity in lung cancer

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Reprogramming of mRNA translation is central to cancer development, mediating cancer cell adaptation and supporting tumour progression. tRNAs are highly modified molecules that are essential to correctly translate mRNAs into proteins. Recently, the importance of tRNA-modifying enzymes in promoting cancer development and therapy resistance through codon-specific translation reprogramming has been uncovered. tRNA-specific Adenosine Deaminase 2 (ADAT2) is an evolutionarily conserved enzyme that catalyses the conversion of adenosine to inosine at the wobble position of tRNAs (A34). Here, we found that ADAT2 is up-regulated in human lung cancers and is essential for the growth of lung cancer cells and xenograft tumors. Importantly, genetic deletion of Adat2 in mice lungs strongly impairs tumor development in a KrasG12D/+ model of spontaneous lung cancer. Using a combination of proteomics, ribosome and polysome profiling, we demonstrate that ADAT2 depletion negatively affects global mRNA translation and alters the expression of a specific subset of metabolic enzymes. This results in impaired glutamine metabolism, accumulation of reactive oxygen species and rewiring of lipid metabolism. Accordingly, ADAT2 knockdown triggers ferroptosis activation and sensitizes lung cancer cells to chemical induction of ferroptosis. Finally, we define an ADAT2 translational signature and show that ADAT2 activity correlates with poor outcome in lung cancer patients. Taken together, our data uncover the importance of tRNA wobble editing in controlling cellular homeostasis in lung cancer and highlight new metabolic vulnerabilities to be exploited for future therapies.

TGFβ-dependent extracellular matrix and vascular remodeling in lymph node pre-metastatic niche : new insights into nodal metastases

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Several cancers including cervical cancers and melanomas are known to metastasize primarily to lymph nodes (LN). Recent data demonstrate that LN metastases can seed to distant organs, revealing that LN are worth considering as potential therapeutic target to prevent distant disease and death. The establishment of a dialogue between the primary cervical neoplasm and the sentinel LN in early cervical cancer was previously reported. Based on the combination of an original pre-clinical model and available human samples, we are investigating in-depth the implication of the remodeling of vascular structures and extracellular matrix proteins in the elaboration of a permissive niche for metastatic colonization of LN. The study of this niche is based on the characterization of the tissue remodeling occurring in pre-metastatic and metastatic LN at histological, cellular and molecular levels with a particular emphasis for the understanding of the role of TGF-β in this pathological process. TGF-β bioavailability is controlled by latent TGF-β binding proteins (LTBPs) and GARP, a cell surface TGF-β activator. Recent results obtained by TGF-β blocking antibody injection in a tumorous context showed that TGF-β could play a determinant role at the immune, vascular and matrix level in a permissive niche generation. A deeper understanding of the tumor/LN dialogue and the identification of nodal markers predicting the risk for distant extension are key prognosis variables for patients suffering malignancies that disseminate through the lymphatic vasculature.

Non-Thermal Plasma as an Immunogenic Therapy Addition to the Standard-of-Care for Head and Neck Squamous Cell Carcinoma

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Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide. The current first-line treatment strategy in the recurrent/metastatic setting (R/M HNSCC), immunotherapy alone or in combination with platinum-based chemotherapeutics (PLAT), has limited benefits due to low response rates and severe side effects in already weakened patients. Non-thermal plasma (NTP), an ionised gas containing reactive oxygen and nitrogen species, has been reported to induce immunogenic tumor cell death (ICD). Therapeutic ICD inducers, like NTP, are clinically interesting as they can engage the patient's natural cancer immunity cycle and provide long-lasting anti-tumor immunity. Therefore, the study aim was to investigate a novel combination strategy of NTP with current first-line therapies of R/M HNSCC to improve treatment efficacy and response. We optimised a micro-tissue spheroid model for several HNSCC cell lines to better recapitulate the complex processes in HNSCC and its tumor microenvironment. All experiments were performed using a microsecond-pulsed dielectric barrier discharge plasma system. After tumor kinetics were determined, combination treatments of NTP and PLAT (cisplatin) were analysed for the induction of several membrane-associated and secreted ICD markers. Immunogenicity was tested functionally with dendritic cell (DC) co-culture experiments. Our data show a significant upregulation of the cell-surface exposed ecto-calreticulin, an important 'eat-me signal' for immune cells, along with two heat-shock proteins among multiple HNSCC cell lines at 24h post treatment. In addition, combination therapy improved the release of both the early ICD marker ATP, as the late-stage factor HMGB1. Evaluating immune cell function, DC co-culture experiments demonstrated increased phagocytosis of HNSCC tumor cells of NTP-CIS combined application. These results highlight the potential of NTP to enhance treatment efficacy and tumor immunogenicity. This data was further validated in HNSCC tumor organoids, our patient-derived model that uniquely mimics the phenotypic and genotypic characteristics from the original tumour. This all together will accelerate clinical translation of the obtained results and is the first step towards a rationally designed combination strategy with NTP to improve current first-line HNSCC therapies.

Insights Into The Role Of Endothelial Extracellular Vesicles In Pre-Metastatic Niche Formation And Metastasis In Breast Cancer

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Background
Breast cancer (BC) is one of the most common cancers worldwide with over 2.26 million global cases. While it can be treated, metastatic BC remains incurable. Previously, our research group demonstrated that endothelial cell-derived extracellular vesicles (EVs) enriched in three specific microRNAs (i.e. miR-142-5p, miR-183-5p and miR-222-3p) participate in the polarization of macrophages towards an M2-like phenotype, thus promoting tumour growth in a BC mouse model. However, the impact of these miRs in pre-metastatic niche (PMN) formation and metastasis remains unknown. With this project, we aim to unravel the impact of these microRNAs on PMN formation and metastasis in vitro and in vivo.

Methods
In this study, we isolated EVs from mouse endothelial cell lines using a differential ultracentrifugation method and characterized them using Dynamic Light Scattering (DLS). Next, we evaluated their incorporation into recipient cells using confocal microscopy. After electroporating the isolated endothelial EVs with the three miRs, we added these to macrophage (i.e. RAW264.7) and fibroblast (i.e. MEF) cell lines. To determine the effects of these EVs on cell differentiation, we performed qRT-PCR of several genes that are important in PMN formation. Currently, we are further investigating the role of miR-enriched endothelial EVs in PMN formation in a 4T1-BC mouse model using diverse techniques (e.g. Xenogen approach, flow cytometry).

Results
Using DLS, we determined the average size of the EVs to be in the desired size ranges (i.e. 50-150 nm) that is typical for exosomes. Our confocal images demonstrated that endothelial EVs were successfully incorporated into RAW264.7 and MEFs, which are two major cell types in PMN formation. Furthermore, our qPCR results demonstrated that miR-enriched EVs upregulate the expression of pro-tumorigenic and pro-metastatic genes CSF3 (in macrophages), IL-1β, and CCL3 (in fibroblasts) that play a major role in tumour progression.

Conclusions
Though most results are preliminary, our current findings reveal a previously unrecognized role of miR-enriched endothelial EVs on macrophage and fibroblast differentiation in vitro. These data reveal a first indication for the role of endothelial EVs on PMN formation, which may be further exploited to develop novel therapeutic or diagnostic approaches for BC patients.

Identification of a TAP-independent antigen recognized by a dual T-cell receptor, also recognizing a tumor-specific antigen

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Down-regulation of the TAP transporter has been observed in a wide range of tumors and leads to a dramatic decrease of surface MHC class I. Interestingly, some antigenic peptides called TEIPP (T cell epitope associated with impaired processing) were shown to be presented on TAP-deficient tumors and could represent targets of interest in the frame of cancer immunotherapy. Here, we describe a cytolytic T lymphocyte (CTL), isolated from a melanoma patient and which recognizes an antigenic peptide that is presented when the TAP transporter is not functional. Treatment of TAP-negative cells with a signal peptide peptidase (SPP) inhibitor, ZLL-2-Ketone, resulted in a complete loss of recognition of these cells by the CTL. This suggests that the TAP-independent peptide recognized by the CTL is derived from the signal sequence of a protein. We plan to use an immunopeptidomic approach coupled to mass spectrometry in addition to the use of algorithms described in the literature to identify the TAP-independent peptide recognized by this CTL.

Spatial and circadian regulations of cancer cell (lipid) metabolism: new insights for a better use of metabolism-targeting drugs

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Circadian rhythms are oscillations in the biochemical and metabolic functions of organisms with a periodicity of 24h determined by light/dark cycles. Disruption in the wake/sleep cycle but also in the related alternance of food intake and fasting periods is increasingly associated with the incidence and progression of pathologies where metabolism plays a central role, including cancer. Our lab has identified that the aggressiveness of cancer cells is associated with a preference for fatty acid metabolism (vs. glucose metabolism), making lipid metabolism an attractive therapeutic target.
Here, we examined whether circadian oscillations in metabolism also exist in cancer cells and whether a preferred timing of lipid metabolism-targeting drug administration could be identified to enhance efficacy and possibly reduce side effects. We developed clock luciferase-reporter colorectal cancer (CRC) cells to track the oscillating activities of major clock proteins (incl. Bmal1 and Per2) but also clock-controlled PPAR proteins, the master regulators of lipid metabolism. We could document that different CRC cells undergo circadian oscillations, and that acidic pH was associated with a significant time shift in the oscillations of clock and PPAR proteins. Moreover, using PPAR-α and PPAR-γ antagonists, we found that both drugs induced CRC cell death (an effect further exacerbated in acidic cancer cells) and inhibition of the growth of 3D CRC spheroids. Strikingly, the time in the circadian cycle when the drugs were added, directly influenced the extent of anticancer effects. Finally, we used xenograft mouse models treated with either PPAR antagonist at the beginning of either the fasting or feeding period. While we did not observe differences with PPAR-□ antagonist, tumor growth was more significantly reduced upon exposure to PPAR-γ antagonist during the feeding period. Altogether, these findings provide a strong rationale for the potential application of chronotherapy to target lipid metabolism in CRC.

Targeting LDH self-association as a novel anticancer treatment: towards small molecule LDH disruptors.

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Lactate dehydrogenases (LDHs) are involved in the metabolic adaptations of cancers to sustain cellular proliferation and growth. These tetrameric enzymes catalyse the interconversion of lactate + NAD+ to pyruvate + NADH + H+. LDH1 preferentially catalyses the forward and LDH5 the backward reaction. LDHs sustain several pathogenic functions, including autophagy(1), cancer cell migration and invasiveness(1,2). They therefore constitute promising targets for anticancer therapy. Years of research demonstrated that selectively targeting their highly polar catalytic site is beyond reach, but LDHs are active only in tetrameric states, and previous collaborative works by our groups and others(3) led to pioneering results on the disruption of LDH self-association using peptides(4,5).

Towards therapeutic applications, the aim of the present project is to develop a small molecule inhibitor acting at the oligomeric interface of LDHs in order to disrupt their tetrameric state and block their activities.

To this end, a small molecule library was screened using an original biophysical screening cascade to probe the LDH tetrameric interface. Hence, the use of nano differential scanning fluorimetry (nanoDSF), microscale thermophoresis (MST) and NMR spectroscopy led to the identification of two hits,(6) Maprotiline and Triprolidine, which are FDA-approved drugs currently used for other applications than cancer. Based on structural similarities, additional FDA-approved drugs have been evaluated against LDHs using orthogonal methods (MST, nanoDSF, NMR STD, denaturation assays), leading to promising results, with for example compound F01 that has a Kd of 19 µM for LDHs. X-ray crystallography precisely revealed the F01 binding site at the oligomeric interface of LDHs. This compound is currently evaluated in cellulo against different human cancer cell lines.

Conclusively, we provide strong evidence that the identification of small molecules with promising Kd could constitute a novel framework for the development of new LDH inhibitors with a novel mechanism of action and overcome the historically poor druggability of this enzyme.

Refs: 1. Brisson L. et al. Cancer Cell 2016; 30, 418-31
2. Luigi F. et al. Future Med. Chem. 2014; 6(4), 429–445
3. Nadal-Bufi F. Et al. J. Med.Chem. 2021; 64(7), 3767-3779
4. Léopold T. et al. J. Med. Chem. 2020; 63, 9, 4628–4643
5. Léopold T. et al. J. Biol. Chem. 2021; 296, 100422
6. L. Thabault*, C. Brustenga*, et al. Eur J Med Chem 2022, 230, 114102 (*shared 1st authorship)

Induction of antitumor immunity in syngeneic mouse models exposed to X-ray vs high LET proton irradiation

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The induction of an adaptive antitumor immunity is key for a specific and systemic eradication of cancer cells and possibly for the formation of an immune memory. A growing number of studies propose that specific parameters of radiotherapy such as the type of radiation (X-rays vs. charged particles), dose and fractionation may promote immunity. While irradiation with charged particles provides advantages over X-ray irradiation, such as better ballistics and enhanced cancer cell death, it also induces different cellular responses in irradiated cancer cells which could ultimately lead to a stronger immunogenic response.
The aim of this project is therefore to study and compare the immunogenicity induced by X-ray and high LET proton irradiation at different RBE-adjusted doses in syngeneic mouse models of head and neck cancer. The activation of cGAS/STING pathway under different irradiation conditions was analyzed, demonstrating its activation after X-ray and proton irradiations in SCC-VII and MTCQ1 cells. The pathway was activated after a single dose of 8 Gy but even more so with 20 Gy 72h post X-ray and after 3 fractions of 8 Gy.
The second objective of the project, an in vivo vaccination assay comparing X-ray and proton irradiation was performed in order to evaluate the induction of systemic anti-tumor immunity. The secondary tumor development rate as well as the percentage of surviving mice indicate that an effector immune response was established after X-ray as well as proton irradiation. IHC staining was used to analyze and quantify lymphocyte populations in primary and secondary tumors. In the primary tumor, the levels of CD3+ lymphocytes show no significant variation upon irradiation. Conversely, primary tumors formed from cells previously exposed to X-ray or proton radiation exhibited higher numbers of CD8+ T cells. In mice, where vaccination did not work, a larger population of CD8+ T cells is observed in the secondary tumors of mice injected with irradiated cells.
At this state, we succeed to vaccinate mice with injection of irradiated cells. Moreover, different effects were observed according to the cell lines, emphasizing the importance of further investigations.

Circulating extracellular vesicles in the identification of immune checkpoint associated with lung cancer

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Introduction: Immunotherapy has revolutionized cancer treatment. However, not all patients show effective responses to the established selection criteria. This study aims to determine whether extracellular vesicles (EVs) are appropriate tools for detecting and blocking immune checkpoint proteins (ICPs). We developed a new methodology that identifies several ICPs at the surface of EVs in single-liquid biopsies. Based on this technology development, we aim to profile ICPs in lung cancer patients treated by immunotherapy. Having identified novel EVs-derived immune checkpoint candidates, we will determine the efficiency of modified EVs as an immune checkpoint blockade.
Method: We isolated and characterized EVs from plasma by ultracentrifugation method. ICPs were identified through EV surface markers analysis using the MAGPIX platform. Modified endothelial EVs were generated with siRNA targeting programmed death ligand 1 (PD-L1). Effects on pro-tumoral properties were assessed through several functional tests and in vivo using the TC1-xenograft mice model.
Results: As a proof of concept, we designed our assay to detect 6 ICP-EVs in the bloodstream of patients (LAG-3, PD-1, PD-L1, TIM-3, TIGIT, VISTA). We highlighted that PD-L1 downregulation affects the pro-tumoral properties in vitro and in vivo.
Conclusions: We present a new method for circulating ICPs-EVs characterization in lung cancer patients. We aim to use this technology to monitor the ICPs-EVs profile in patients undergoing immunotherapeutic treatment. Furthermore, we confirmed that modified endothelial EVs impair TC-1 tumor model growth.

Casting the Spotlight on Lymph Node Premetastatic Niche: The Dynamic Duo of Fibroblasts and Integrin Alpha11 in Tissue Remodeling

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Lymph node metastasis represents a pivotal event in cancer progression, significantly impacting patient prognosis. While lymphangiogenic growth factors and lymphatic vessel density have been extensively studied, the role of Integrin α11- a collagen binding integrin mainly expressed by Cancer Associated Fibroblasts - in this context remains underex-plored.Furthermore, fibroblasts, crucial contributors to tissue remodeling, are emerging as key players within the premetastatic niche. This research investigates the putative contribution of Integrin α11 in tissue remodeling dynamics within lymph nodes, focusing on murine models at premetastatic and metastatic stages.
To assess the spatial distribution of Integrin α11, we employed the mouse ear sponge assay to explore the premetastatic lymphovascular niche in the draining lymph nodes in C57BL mice. Our RNAscope analyses revealed the expression of the Integrin α11 gene (ITGA11) in both metastatic and premetastatic lymph nodes. Furthermore, we utilize Fluorescent Activated Cell Sorter Cytometry (FACS) to isolate fibroblastic reticular cells (FRCs) for subsequent Single Cell RNA sequencing. This sequencing will allow us to dig into the expression patterns of various targeted proteins within different subpopulations of fibroblasts in lymph nodes at the premetastatic stage. Subsequent in-vitro studies will enable a deeper understanding of the molecular mechanisms underlying Integrin α11's actions and its interacting partners.

Study of the role of microRNAs-encapsulated in extracellular vesicles in osteosarcoma

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INTRODUCTION The tumor microenvironment assumes a crucial role in tumor progression and relies on secreted factors, including extracellular vesicles (EVs). Within the osteosarcoma (OS) microenvironment, EVs participate directly in OS growth and invasion. Notably, EVs have been observed to instigate osteoclast differentiation, stimulate bone resorption activity, and enhance blood vessel formation and the proliferation of endothelial cells. Additionally, numerous studies have found that BMSC-derived EVs can regulate cell proliferation, migration, survival, and OS drug resistance. Currently, studies have highlighted that EVs from adipose-derived stem cells (ASC) decrease OS cell proliferation. Our objective is to elucidate ASC-EVs' impact on OS growth and engineer these EVs to improve their therapeutic potential through modification of cargoes and surfaces.
RESULTS To unravel the therapeutic potential of miRs encapsulated in isolated EVs, we conducted a series of functional assays on OS cells transfected with the five miRs*. Our results show that the miRs could act as tumor suppressors (affecting OS cell migration and proliferation). Furthermore, we conducted co-culture experiments to investigate the repercussions on microenvironmental components through indirect coculture of OS cells transfected with miRNAs* with fibroblasts and endothelial cells.
The results show that this co-culture has an impact on the migratory behavior of fibroblasts and endothelial cells within an angiogenic medium.
Future investigations will evaluate the potential of miR-encapsulated EVs on OS cells. Additionally, to unravel the therapeutic potential of ASC-EVS surface modification, we undertook peptide* grafting experiments on the surface of these vesicles, yielding encouraging results, notably effects on cell viability.
CONCLUSION Collectively, our findings underscore the promise inherent in cultivating ASCs within a three-dimensional scaffold-free extracellular matrix, a process that leads to the enrichment of miRNAs within EVs, giving them the potential for potent anti-tumor activity. Future perspectives will be to determine the clinical viability of employing these EVs in the treatment of osteosarcoma, paving the way for novel therapeutic strategies in the battle against this bone pathology.
*Names are withheld due to patent and publication considerations.

Identification of a mitochondrial control of radioresistance in human breast cancer models

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Radiotherapy is a common treatment for breast cancer but the development of radioresistance often leads to treatment failure. Among the different adaptations that confer resistance, studies showing the connection between metabolism and resistance are increasing. In particular, previous research in our lab showed an increase in oxygen consumption in radioresistant cells.
To investigate how mitochondria control radioresistance, MCF-7 and MDA-MB-231 human breast cancer cell lines were exposed to increasing doses of X-ray irradiation over time to obtain radioresistant (RR) cells. Colony formation assays were used to confirm radioresistance.
Metabolic adaptations were found in RR cells, such as an oxidative switch in MDA-MB-231-RR cells and a glycolytic switch in MCF7-RR cells compared to their parental counterpart. As hexokinases are key rate limiting enzymes of glycolysis, their activity and expression were measured. Despite showing opposite metabolic switches, a common increase in their activity was shared by the two RR cell lines but this was only associated with an increased HK1 and HK2 expression in MDA-MB-231-RR and not in MCF7-RR. As HK2 is connected to stemness, the expression of other stem markers MYC, Oct4, NANOG, SOX2 and CD44 was analyzed. A high upregulation in the transcription factor MYC, which exerts many proto-oncogenic roles in glucose metabolism, was found in MDA-MB-231-RR cells, while MCF7-RR cells showed an increase in CD44 and SOX2 expression. Collectively, our data suggests a potential link between hexokinase activity and radioresistance in breast cancer stem cells. Using these models, further studies are ongoing to establish a causal link between radioresistance and the onset of stemness by inhibiting HK2 with 3-bromopyruvic acid and by HK2 knockout.

Disrupting Arginase-1 Trimeric State: a New Strategy to Increase the Anti-Tumor Immune Response

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Arginase-1 (ARG-1) represents a validated target in cancer immunotherapy. The catalytic site of ARG-1 is small and highly polar, making it a challenging target for inhibitor development. Only a limited number of inhibitors have been explored further due to the difficulty in achieving an acceptable pharmacokinetic profile and concerns about potential target-related toxicity. These challenges have motivated us to explore an alternative strategy for enzyme inhibition: targeting ARG-1 trimeric self-assembly to induce enzyme inhibition. This approach offers several potential advantages, including increased selectivity, promotion of protein degradation, and the potential for substoichiometric inhibition. In this study, we demonstrated that ARG-1 is only active in its trimeric state by producing monomeric ARG-1. The characterization and assessment of the protein's self-association were performed using in silico studies and site-directed mutagenesis, which identified five amino acids as key hotspots. Based on these findings, we designed (stapled)-peptides by mimicking the regions involved in ARG-1 self-association. Subsequent biochemical and biophysical evaluations revealed that one of these peptides disrupted the trimeric state of ARG-1. However, this peptide did not exhibit sufficient affinity to effectively inhibit the enzyme. Various biochemical and biophysical tools, including mass photometry, size exclusion chromatography coupled with multi-angle light scattering (SEC-MALS), cross-linking, chemical and thermal denaturation, and microscale thermophoresis (MST), were employed throughout this study.

Role of MRC2 in lymphatic biology

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Cancer, in its many forms, is now a well-studied pathology and life after cancer is now more and more conceivable. However, post cancer complications are yet a shadowy area. In breast cancer (between others), around 20% of treated patients will develop lymphedema. This condition is caused by a decrease or defect in lymphangiogenesis (i.e. formation of new lymphatic vessels from already established ones) and is characterized by tissue swelling and fibrosis. Lymphedema is not a life-threatening disease per se but highly impact patient quality of life and leads to recurrent erysipelas and cellulitis events, mental health issues (anxiety and depression) and even cancer (sarcoma and lymphoma). Our laboratory has previously identified the endocytic receptor “urokinase plasminogen activator receptor-associated protein’’ (“uPARAP” encoded by MRC2 gene) as negatively regulating lymphangiogenesis. uPARAP interacts with VEGFR2/3 and blocks their heterodimerization and signalization. Recently, we have demonstrated uPARAP interaction with Vascular Endothelial Cadherin (VE-cadherin), the major protein implied in lymphatic endothelial cell junctions. uPARAP orchestrates VE-cadherin recycling or degradation in lysosomes, further participating in correct junction maintenance and vessel permeability. In collaboration with Pr. M. Vikkula (UCL), we identified different uPARAP variants (SNPs) from a primary lymphedema patient cohort. We are now focusing our interest on those variants and their impacts on LEC biology. We have yet successfully demonstrated a decreased interaction of two variants with VE-cadherin by proximity ligation assay (PLA) and coimmunoprecipitation (coIP) in A431 cells transduced with VE-cadherin gene. In order to confirm the interaction of uPARAP with VE-cadherin in a more resolutive assay (10nm VS 40nm for PLA), we designed tools for FRET experiment in A431 cells and primary human lymphatic endothelial cells (hLECs). Model limitation and technical issues have prone us to develop immortalized hLECs (imhLECs) by using co-transduction of hTERT and BMI1 genes. Generated cells are currently under characterization. Altogether, we hope to clarify clinical relevance of MRC2 gene in the context of lymphedema.

PD-1/PD-L1-dependent transfer of Programmed death-ligand 1 from Human Tumor cells to T cells

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Background: Expression of Programmed death-ligand 1 (PD-L1) by cancer cells in the tumor microenvironment is a hallmark of immunosuppression: its interaction with PD-1 expressed by activated Cytotoxic T Lymphocytes (CTLs) negatively regulates the latter leading to their exhaustion and compromising anti-tumor immunity. Although much less studied, PD-L1 was also found on CTLs themselves following antigen recognition on target cells, but the precise mechanism leading to this expression remains poorly understood. Therefore, our study aims to decipher the regulation and the role of PD-L1 expression by CTLs.

Method: We performed in vitro coculture assays between a human melanoma tumor cell line (MEL.A-1) and CD8+ T cells recognizing the MHC-I-restricted MUM3 antigen presented by MEL.A-1 cells and detected PD-L1 on T cells' surface by Flow Cytometry.

Results: Coculture between PD-L1+ tumor cells and CD8+ T cells resulted in the detection of PD-L1 on T cells' surface by flow cytometry, while we failed to detect PD-L1 on T cells after coculture with PD-L1KO tumor cells. The use of tumor cells expressing a tagged PD-L1 demonstrated that PD-L1 was transferred from tumor cells to T cells. PD-L1 transfer requires trans-PD-1/PD-L1 interaction since the addition of PD-1/ PD-L1 neutralizing antibodies in the coculture abolished PD-L1 transfer. Lastly, PD-L1 transfer is accompanied by membrane fragments exchanges, also called trogocytosis.

Conclusion: Our preliminary results uncover that PD-1/PD-L1 interaction not only mediates PD-L1 transfer from tumor cells to T cells but also drives membrane fragments exchanges, (trogocytosis). Further understanding of the mechanism and the role of PD-L1 acquisition by CD8+ T cells, notably in the tumor microenvironment, would bring important new insights in the current knowledge of the PD-1/PD-L1 pathway.

PDGFRA K385 mutants in myxoid glioneuronal tumors promote receptor dimerization and oncogenic signaling

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Myxoid glioneuronal tumors (MGNT) are low-grade glioneuronal neoplasms composed of oligodendrocyte-like cells in a mucin-rich stroma. These tumors feature a unique dinucleotide change at codon 385 in the platelet-derived growth factor receptor α (encoded by the PDGFRA gene), resulting in the substitution of lysine 385 into leucine or isoleucine. The functional consequences of these mutations remain largely unexplored. Here, we demonstrated their oncogenic potential in fibroblast and Ba/F3 transformation assays. We showed that the K385I and K385L mutants activate STAT and AKT signaling in the absence of ligand. Co-immunoprecipitations and BRET experiments suggested that the mutations stabilized the active dimeric conformation of the receptor, pointing to a new mechanism of oncogenic PDGF receptor activation. Furthermore, we evaluated the sensitivity of these mutants to three FDA-approved tyrosine kinase inhibitors: imatinib, dasatinib, and avapritinib, which effectively suppressed the constitutive activity of the mutant receptors. Finally, K385 substitution into another hydrophobic amino acid also activated the receptor. Interestingly, K385M was reported in a few cases of brain tumors but not in MGNT. Our results provide valuable insights into the molecular mechanism underlying the activation of PDGFRα by the K385I/L mutations, highlighting their potential as actionable targets in the treatment of myxoid glioneuronal tumors.

Heterogeneity in experimental parameters influences the biology and function of cultured cancer-associated fibroblasts

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Cancer-associated fibroblasts (CAFs) are abundantly present in the microenvironment of multiple tumor types. CAFs are a heterogeneous population with a prominent role in tumor progression and therapy resistance which sparked a global interest in CAF, resulting in a prolific number of publications. To facilitate community wide data consolidation and sharing, we initiated a crowdsourcing data-repository to map 85 general and CAF-specific experimental, biological and functional parameters from more than 1300 CAF-related publications. Preliminary evaluation of 550 publications revealed a general heterogeneity and underreporting of experimental parameters, which potentially influence CAF biology. Using single-cell RNA sequencing, we investigated the impact of in vitro aging and immortalization on CAF cultures. Comparison of passages revealed a loss of heterogeneity upon immortalization and specific gene and pathway rewiring upon CAF passaging, highlighting the importance of reporting of passage numbers/immortalization procedures. Further preliminary data shows how different CAF isolation procedures result in the generation of CAF cultures with different functional and molecular properties. In conclusion, this CAF knowledgebase combined with empirical evaluation will further support interpretable, reproducible and transparent experimental reporting in CAF research.

Autophagy degrades immunogenic endogenous retroelements induced by 5-azacytidine in acute myeloid leukemia

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The hypomethylating agent 5-azacytidine (AZA) is the first-line treatment for AML patients unfit for intensive chemotherapy. The anti-tumor effect of AZA results in part from T-cell cytotoxic responses against MHC-I-associated peptides (MAPs) deriving from hypermethylated genomic regions such as cancer-testis antigens (CTAs), or endogenous retroelements (EREs). However, clear evidence supporting higher ERE MAPs presentation after AZA treatment is lacking. Therefore, using proteogenomics, we examined the impact of AZA on the repertoire of MAPs and their source transcripts. AZA-treated AML upregulated both CTA and ERE transcripts while only CTA MAPs were presented at greater levels. Upregulated ERE transcripts triggered innate immune responses against double-stranded RNAs but were degraded by autophagy and not processed into MAPs. Autophagy resulted from the formation of protein aggregates caused by AZA-dependent inhibition of DNMT2. Autophagy inhibition had a synergistic effect with AZA on AML cell proliferation and survival, increased ERE levels and triggered pro-inflammatory responses. Finally, autophagy gene signatures were associated with a lower abundance of CD8+ T-cell markers in AML patients expressing high levels of EREs. Altogether, this work demonstrates that the impact of AZA is regulated at several levels and suggests that inhibiting autophagy could improve the immune recognition of AML blasts in patients.

Unraveling the tumor immune microenvironment of colorectal peritoneal metastases

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INTRODUCTION
Current therapies for colorectal cancer (CRC) peritoneal metastasis (PM) lack sufficient efficacy, hence the need for novel therapeutic strategies. Predominantly, the cancer cells are studied and targeted, while the tumor microenvironment (TME) of CRC PM lesions is neglected. Therefore, a detailed characterization of the TME of these CRC PM lesions is required.

METHODS
From patients providing informed consent, fresh surgical PM samples were obtained from three anatomical locations: the abdominal wall, the small bowel mesentery, and the greater omentum. Peritoneal tissue without macroscopic evidence of metastasis was taken from the same patients as control. To unravel the tumor immune microenvironment (TIME) of CRC PM, the resected tumor and peritoneal tissues were analyzed using flow cytometry, single-cell RNA-sequencing (scRNAseq) and cytokine and chemokine analysis of the secretome.

RESULTS
Flow cytometry data revealed the presence of a prominent immune cell population in all three PM locations and in the normal peritoneal samples, representing all major immune cell types (granulocytes, lymphocytes, and myeloid cells). Despite considerable inter- and intrapatient heterogeneity, trends could be observed. In general, the abdominal wall metastases comprised fewer immune cells compared to lesions sampled from the other locations. Granulocytes (especially neutrophils) and macrophages were well represented in the tumor nodules. Within the adaptive immune system, a substantial number of T cells could be observed, with the majority consisting of cytotoxic T cells. Secretome analysis indicated a strong inflammatory TME and a chemokine secretome conductive to recruit immune cells. Using scRNAseq, we further explored the immune population, especially the mononuclear phagocyte and T cell compartments, in which a diverse number of pro- and anti-tumor immune cell clusters could be observed.

CONCLUSION
Our dataset provides unprecedented insights into the TME of CRC PM and demonstrates the presence of a prominent immune cell population, putting immunotherapies forward as a potential novel treatment strategy.

ATR signaling primes acute myeloid leukemia cells for resistance to hypomethylating agents

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Acute myeloid leukemia (AML) is a malignancy characterized by epigenetic aberrations and is currently the most lethal form of leukemia, with survival rates of 40-45% in young patients and less than 10% in the elderly. The latter group constitutes the primary population affected by the disease, with a median age of diagnosis at 68 years. Older patients are typically not eligible for high-intensity chemotherapy, and hypomethylating agents (HMA) are preferred as first-line therapy. Azacitidine (AZA) and decitabine (DAC) are cytidine analogues acting as suicide substrates for DNA methyltransferases, inducing genome-wide hypomethylation. They can also induce double-strand breaks following their incorporation into genomic DNA. While approximately 50% of patients respond to HMAs, almost all of them eventually relapse, raising the need to understand the mechanisms involved in therapy resistance. By performing RNA sequencing on AML cell lines exposed to various concentrations of HMAs, we observed that cells treated with either DAC or AZA presented an enrichment of senescence signatures. Surprisingly, the same signatures were also enriched in patients who did not respond to AZA. Large-scale transcriptomic comparisons of >10,000 gene sets between AZA responders and non-responders pointed to the ATR pathway, regulating genome integrity and senescence entry, as critically involved in AZA resistance. Accordingly, ATR inhibitors showed an impressive synergy with AZA in vitro. They also restored a response to AZA in cell lines in which resistance to AZA was induced by prolonged culture in the presence of the drug. Finally, a significant synergy between AZA and an ATR inhibitor was observed in established disease in immunodeficient mice transplanted with human AML cells. While further research is warranted, our data suggest that senescent-like cells might play a role in HMA resistance and that ATR inhibitors could improve the efficacy of HMAs or restore therapy response in resistant patients.

Myoferlin depletion affects PDAC cell migration through actin cytoskeleton disorganization and focal adhesion proteins abundance modulation

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Myoferlin is a transmembrane protein described as overexpressed in several types of cancer including pancreatic cancer (PDAC). The myoferlin expression correlates with low survival of PDAC patients and we previously showed that myoferlin was required for dissemination to the liver. We performed 2D wound healing assay and 3D Boyden's chambers assay after myoferlin depletion by siRNA in Panc-1 cells. Migration was significantly reduced under myoferlin depletion. We investigated what gene sets related to migration were modulated by myoferlin depletion by analyzing RNA-Seq data. Gene set enrichment analysis (GSEA) pointed at actin cytoskeleton (GO:0015629) gene set as significantly enriched upon myoferlin depletion. We have validated our result in PDAC patients segregated by MYOF expression. In collaboration with Professor Marc Thiry, we analyzed cell ultrastructure by electron microscopy and noticed an accumulation of cytoskeleton patches in myoferlin-depleted PDAC cells. We stained myoferlin-depleted cells with rhodamine-conjugated phalloidin and observed a decrease of actin-F structures. We hypothesized that myoferlin is required for the formation or maintenance of actin structures. Actin cytoskeleton is involved in cancer cell migration and metastatic dissemination in part through its association with focal adhesion proteins. We explored the abundance of multiple focal adhesion proteins. The abundance of several focal adhesion proteins is increasing under myoferlin depletion. Interestingly, our previous RNA-seq data showed a significant enrichment for the cell-substrate junction (GO:0030055) gene set when myoferlin is depleted. These data support the hypothesis that myoferlin regulates cell-substrate adhesion and actin cytoskeleton for migratory capacities.

Study of extracellular matrix remodelling in the lymph node during the premetastatic and metastatic processes

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In several types of cancers, metastases are first established in lymph nodes (LN). However, the main role of these secondary lymphoid organs is immunity. Cancer cells' ability to survive in this harsh environment depends on different mechanisms. Prior to metastasis establishment, several morphological and structural changes occur in the LN, allowing disseminating tumour cells to survive and proliferate. This process is the induction of a premetastatic niche. Different factors can be secreted by primary tumours to initiate a premetastatic niche in the LN. The niche presents several characteristics, such as immunosuppression, vascular remodelling, and extracellular matrix (ECM) remodelling. Understanding how draining lymph nodes evolve under the influence of primary tumours and what structural modifications appear during the metastatic process is important to understand how tumour cells evade immunosurveillance and survive in such a hostile environment.
We use the "Ear Sponge Assay", an in vivo model consisting of cancer cells embedded in collagen coated gelatine sponges implanted in the mice's ears. It allows easy lymph node collection during different stages of the metastatic process, and thus allows to map spatial and temporal changes occurring during the process.
ECM distribution and structure during premetastatic niche establishment, and depending on metastases presence, are evaluated with immunofluorescence on oriented histological slides of draining and sub-draining lymph nodes. To highlight fibroblastic reticular cell (FRC) conduits, we validated staining of collagen 1, 4, 6 and ER-TR7. Other ECM proteins are highlighted with immunofluorescence, including periostin or tenascin. We quantify ECM protein densities with QuPath and identify their relationship with each other. By using GFP+ tumour cells for implantation, we highlight metastases in the lymph node and map their arrival through the lymphatic route. We detect lymphatic vessels by staining of LYVE1. We also focus our attention on High Endothelial Venules (HEV) in LN, with staining of PNAd, which could be highways towards blood circulation for tumour cells.

Neoantigen-targeted dendritic cell vaccine generates long-lived T cell responses exhibiting the full spectrum of differentiation states in lung cancer patients

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Background. Non-small cell lung cancer (NSCLC) is known for high relapse rates despite resection in early stages. Because of frequently high mutational load, immunotherapy targeted at tumor neoantigens constitutes an attractive option to control residual disease in this setting. Here, we present the results of a phase 1 clinical trial in which a novel dendritic cell vaccine (DC) vaccine targeting neoantigens was evaluated in patients with resected NSCLC.
Methods. Neoantigens were identified using a proteogenomics approach. Patients underwent leukapheresis for the manufacturing of monocyte-derived DCs loaded with neoantigen-encoding mRNA (Neo-mDCs). Neo-mDCs were injected intravenously according to an intra-patient dose escalation scheme. Primary endpoint of the trial was safety. Secondary endpoints were feasibility, immunogenicity, and relapse-free survival.
Results. Vaccine manufacturing was feasible in 6 of 10 enrolled patients. Toxicity was limited to grade 1-2 adverse events. Systemic T cell responses were observed in 5 out of 6 vaccinated patients and were dominated by CD8+ T cells, which could be detected ex vivo at high frequencies up to 1.5 years after vaccination. Furthermore, single cell analysis indicated that the responsive CD8+ T cell population was polyclonal and exhibited the near entire spectrum of T cell differentiation states, including a naïve-like state associated with long-lasting memory, but excluding exhausted cell states. Three of 6 vaccinated patients experienced disease relapse during the follow-up period of 2 years.
Conclusion. Adjuvant Neo-mDC vaccination is safe, feasible and induces polyclonal and long-lived neoantigen-specific T cells with a high degree of differentiation heterogeneity in resected NSCLC patients, suggesting clinical potential.

Targeting of Tumor Macrophages with a CD206 Binding Peptide Conjugated to Gold Nanoparticles

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Overcoming the immune-suppressive tumor microenvironment (TME) is a significant challenge. Within the TME, tumor-associated macrophages (TAMs) play crucial roles due to their abundance, multifaceted interactions with the immune system, and context-dependent plasticity. Research focuses on repolarizing TAMs from pro-tumor M2-like to anti-tumoral M1-like phenotypes. Nanoparticles offer a way to influence macrophage polarization, reprogramming, and immunological function, thereby impacting disease progression. We investigated the use of a CD206-binding peptide (fluorescein-mUNO) to deliver polyethylene glycol-conjugated Au nanoparticles (FUNO-PEG-AuNPs) and Poly(vinyl pyridine)-conjugated Au nanoparticles (FUNO-PVP-AuNPs) to M2 TAMs in vitro. We prepared and functionalized NPs and investigated their possible effects on macrophages. M2 macrophages exhibited a higher affinity for FUNO-conjugated NPs than M0 macrophages. Peptide internalization kinetics indicated that peptide-conjugated NPs were internalized over 6 hours, while free peptides peaked at 2 hours, suggesting higher NPs bioavailability. Cytotoxicity tests revealed FUNO's IC50 of 12μM for M2 and 50μM for M0, with no cytotoxicity up to 50μg/ml of NPs based on Au and 4μM FUNO. Future experiments will explore M2 and M0 macrophage polarization in the presence of NPs. In conclusion, our study supports the potential of the CD206-binding peptide for delivering AuNPs to M2 macrophages, presenting a mode of targeting tumor-associated macrophages (TAMs).

Therapy-tolerant persistent cells in colorectal cancer: impact of methylglyoxal stress

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Colorectal cancer (CRC) ranks as the third most frequently diagnosed cancer, and the limited efficacy of current treatments often leads to frequent relapses. Cetuximab (CTX) stands as the most used anti-EGFR targeted therapy for CRC. Mutation of oncogenes is commonly associated with the development of resistance to CTX. However, patients with CRC classified as "quadruple wild type" (4WT), characterized by the absence of mutations in KRAS, NRAS, BRAF, and PIK3CA, also exhibit a resistance to this therapy. The dependency of cancer cells on glycolysis promotes the initiation and progression of tumors. Methylglyoxal (MG), an inevitable by-product of glycolysis, induces glycation on proteins, lipids and DNA. We have previously shown that the depletion of glyoxalase 1 (GLO1), the main MG detoxifying enzyme, induces an endogenous MG stress that enhances growth and metastatic capacity. We observed that GLO1-depleted CRC cells develop resistance to CTX. This project endeavours to elucidate the molecular mechanisms, driven by MG stress, that lead to the development of CTX resistance in 4WT cells.

The use of CRC resistant clones, generated after long term challenge with CTX, let us conclude that MG stress was not a major feature of resistant cells. Therefore, we next focused on the generation of persistent CRC cells, enriched after short challenge with CTX, to better position MG stress during the process of the acquisition of resistance. Persistent cells demonstrated resistance to CTX that was evidenced by a gain in AKT and ERK activation, efficient escape from apoptosis when compared with parental cells. Nevertheless, persistence was not associated with MG stress as shown by unchanged free MG/MG protein adducts levels and GLO1 activity.

Knowing that the sole induction of MG stress in 4WT CRC cells render them resistant to CTX resistance, we next used GLO1-depleted cells to understand how MG triggers acquired resistance. Upon MG stress and CTX challenge, resistant cells displayed enhanced EGFR activation and persistent activation of ERK/MEK pathway when compared with control cells. Taken together, these findings suggest that MG stress may facilitate cellular resistance to anti-EGFR therapy. Ongoing experiments will help demonstrating the use of MG scavengers to potentially resensitize resistant CRC cells to CTX.

Predictors of knowledge, attitudes, and practices associated with Oesophageal cancer risk or prevention in Malawi

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Introduction
Oesophageal cancer (EC) is the seventh most common cancer and the sixth leading cancer-causing death globally. EC is a multifactorial disease influenced by internal and external exposome factors. Specifically for external exposome factors alcohol consumption, tobacco smoke, poor oral hygiene, hot beverage consumption, geophagia, poor diet, and mycotoxin exposure have all been linked to increased EC cases in high-risk regions. Worldwide, Malawi has the highest age-standardized incidence of EC. Contrary to the etiological evidence pointing to the vital contribution of the modifiable EC external exposome factors, data on individuals' knowledge, attitude, and practises (KAP) regarding EC risk factors or their prevention are limited in Malawi.

Methodology
A descriptive cross-sectional study (n = 310) was conducted in Blantyre and Chiradzulu districts to provide a snapshot of predictors associated with EC risk or prevention in Malawi. We hypothesised that participants' knowledge, attitudes, and practises towards EC risk or prevention could be influenced by age, gender, monthly income levels, education backgrounds, marital status, and occupation status.

Results
Preliminary results show that being male significantly reduces the chances of being knowledgeable about EC risk (OR 0.395, 95% CI: 0.227–0.688) in adjusted logistical regression models. Moreover, being self-employed (OR 0.482, 95% CI: 0.225–0.911) or a homemaker (OR 0.402, 95% CI: 0.165–0.976) reduced the odds of having good attitudes towards EC prevention. On the other hand, being divorced increased the odds of having good attitudes towards EC prevention by 17 times (OR 17.120, 95% CI: 1.995–146.884).

Conclusion
Malawi's current EC incidence trends place a strenuous demand on an already dilapidated healthcare system. Regardless, this presents an opportunity to awaken stringent primary prevention strategies to contain EC cases. The preliminary data from this study suggests a vital contribution of masculinity, matrimony, and type of occupation in EC risk or prevention. While the direct relations of these predictors to EC risk reduction have not been ascertained at a large scale, their prospects (and other socio-demographic predictors) warrant further research to inform policy in national cancer control programmes.

Alterations of HLA class I antigen presentation in melanoma: A focus on patients treated with anti-PD-1.

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Background: Prognosis of advanced cutaneous melanoma has drastically improved in patients treated with PD-1 blockade, however 60% of patients present lack of response. Alterations in the Antigen Processing Machinery (APM), such as inactivating mutations of HLA class I or B2M genes, lead to reduced antigen presentation and recognition by immune cells. This results in impaired host's ability to detect and eliminate melanoma cells and undermines the efficacy of immunotherapeutic strategies focused on generating specific cytotoxic T-cell responses targeting melanoma-associated antigens. We aimed at analysing anti-PD-1 treatment outcomes on melanoma patients based on the APM alterations occurring and whether these have an impact on type, abundance, and distribution of immune cells in tumours.
Methods: Formalin-fixed paraffin-embedded (FFPE) samples obtained from melanoma patients were analysed using three multiplex immunofluorescence (mIF) stainings to up to 7 different markers. This allows to simultaneously visualize multiple markers related to APM, immune cells, and the distribution of these markers within tumour sections. Statistical analysis using R was utilized to compare pre- and post-treatment, immune cell infiltration, variation across high-powered magnification views within each sample, and variation between patients.
Results: mIF showed higher HLA and B2M expression in non-cancer cells than in cancer cells. PD-L1 cell expression was overall low. Immune cells were located at the periphery of the tumour instead of infiltrating it. An immunoscore-like adapted from Jerome Gallon's methodology was created and both the proportions of high and low immunoscore were higher in metastasis than in primary tumours, meaning, the immune invasion heterogeneity is far more complex in metastasis. Expression of pSMAD3 (TGFB) and AXL (dedifferentiated melanoma cells) is higher in non-cancer cells than in cancer cells. As expected, higher number of cells expressed both IDO-1 and HLA in melanoma cells compared to healthy cells.
Conclusion: Being IDO-1 a marker for IFN-gamma, which upregulates HLA class I expression, our results indicate that our method of detection is accurate. An optimized and validated mIF process has been successfully developed for the detection of multiple markers in melanoma samples. This preliminary data allows for further investigation into the correlation between anti-PD-1 treatment and APM alterations in cutaneous metastatic melanoma.

Combinatorial poliovirus receptor blockade synergistically enhances cetuximab-mediated antibody-dependent cellular cytotoxicity of natural killer cells against head and neck cancer.

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Introduction: Cetuximab is the only FDA-approved targeted therapy for head and neck squamous cell carcinoma (HNSCC), but is confronted with multiple resistance mechanisms. Interestingly, this epidermal growth factor receptor (EGFR)-inhibiting antibody can induce antibody-dependent cellular cytotoxicity (ADCC) by natural killer (NK) cells. This study aims to identify a potent immunotherapy-based combination partner for cetuximab to further NK cell activity in HNSCC.
Methods: Formalin-fixed paraffin-embedded tissue sections from 50 HNSCC patients were stained for NK cells (NKp46) and immune checkpoint ligands. Data was validated on a larger sample size of 530 HNSCC patients using The Cancer Genome Atlas (TCGA). NK cells from HNSCC patients were analyzed by flow cytometry for immune checkpoint receptor expression. Co-culture assays with our well-established panel of HNSCC cell lines capturing sensitivity, intrinsic resistance and acquired resistance to cetuximab were performed to investigate NK cell activities. Live-cell imaging using the IncuCyte ZOOM system was performed for cytotoxicity analysis, while cytokines secreted by NK cells were analyzed using Mesoscale Discovery electrochemiluminescence. Co-cultures were performed with cetuximab, anti-poliovirus receptor (PVR), and anti-CD16 antibodies.
Results: NKp46-based immunohistochemistry of HNSCC revealed a positive correlation of tumor-infiltrating NK cells with overall survival, underscoring their promise. TCGA analysis of HNSCC and healthy individuals in combination with immunohistochemistry identified PVR as potential partner: expressed in 81.3% of the patients (>60% in 41.7% patients) and correlated to tumor stage (+), overall survival (-), and NK cell infiltration (-). NK cells of HNSCC patients showed elevated expression of PVR's inhibitory receptor TIGIT. Functionally, live-cell imaging and cytokine analysis of cocultures of NK cells with panel of cetuximab sensitive/resistant HNSCC cell lines revealed that combined targeting of EGFR and PVR synergistically enhanced the anti-tumor activity by NK cells, which was completely dependent on cetuximab-mediated ADCC.
Conclusion: We show that blocking inhibitory signaling induced by PVR synergizes with the clinically approved EGFR-targeting cetuximab to generate a very potent NK cell response towards HNSCC cells, irrespective of their cetuximab resistance status. These data provide a preclinical rationale towards combining oncogenic and immune-targeting agents in HNSCC to enhance the clinical efficiency of cetuximab treatment.

High resolution and quantitative spatial analysis reveal intra-ductal phenotypic and functional diversification in pancreatic cancer

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A 'classical' and a 'basal-like' subtype of pancreatic cancer have been reported, with differential expression of GATA6 and different dosages of mutant KRAS. We established in situ detection of KRAS point mutations and mRNA panels for the consensus subtypes aiming to project these findings to paraffin embedded clinical tumour samples for spatial quantitative analysis.
We unveiled that, next to inter-patient and intra-patient inter-ductal heterogeneity, intra-ductal spatial phenotypes exist with anti-correlating expression levels of GATA6 and KRASG12D. The basal-like mRNA panel better captured the basal-like cell states than widely used protein markers. The panels corroborated the co-existence of the classical and basal-like cell states in a single tumour duct with functional diversification, i.e., proliferation and epithelial to mesenchymal transition, respectively. Mutant KRASG12D detection ascertained an epithelial origin of vimentin-positive cells in the tumour. Uneven spatial distribution of cancer-associated fibroblasts could recreate similar intra-organoid diversification.
This extensive heterogeneity with functional cooperation of plastic tumour cells, puts extra challenges to therapeutic approaches.

Keywords: molecular heterogeneity, mutation detection, spatial subtyping, pancreatic cancer, plasticity

Short Title: Spatial subtyping to investigate intra-ductal heterogeneity in pancreatic cancer

Methylglyoxal stress: a novel role in tumor immunity?

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The Warburg effect, describing the glycolytic switch occuring in tumor cells, has a strong hidden consequence: the spontaneous production of methylglyoxal (MG), an oncometabolite able to glycate proteins, lipids and DNA thus inducing MG cellular stress. These advanced glycation end products (MG-AGES) confer pro-cancer features and favor breast tumour growth and metastasis in vivo as previously demonstrated by our team and others. Using the MMTV-PyMT breast cancer spontaneous model, we observed using immunohistochemistry (IHC) an accumulation of MG-AGES from adenoma to late carcinoma lesions. In these tumors, we observed an increased recruitment of specific immunosuppressive cells called granulo-myeloid derived suppressor cells (g-MDSCs) that is efficiently blocked upon treatment with carnosine, an MG scavenger. G-MDSCs contribute to the proliferation of tumor cells notably by limiting cytotoxic T cell response. We observed the same results in 4T1 breast cancer model. Consistently, 4T1 mouse breast cancer cells injected orthotopically in mice treated with carnosine showed a lower proportion of infiltrated g-MDSCs population when compared to untreated mice. To prove that this change was due to MG stress, we developed 4T1 cells stably depleted for the enzyme detoxifying MG thus inducing an endogenous MG stress. Coherently with our previous results, we observed an accumulation of g-MDSCs in the microenvironment of MG-stressed tumors. Mechanistically, our findings could be explained by the alteration of the cytokines landscape under MG stress. Indeed, a cytokine array showed that MG stress increased the production of cytokines known in the literature to induce an immunosuppressive environment and able to recruit immunosuppressive cells. Besides pointing to a novel link between methylglyoxal and the immune tumor microenvironment, these data indicate a potential therapeutic benefit of carnosine treatment in breast cancer.

Deciphering the interplay between circadian rhythms and metabolic preferences in colorectal cancer cells and 3D spheroids

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While disruption of circadian rhythms is increasingly associated with cancer incidence, whether alterations in clock machinery influence the progression of established tumors is poorly explored. Given the crosstalk between circadian rhythms and various metabolic paths in healthy organs, we aimed to examine the expression of circadian clock proteins in cancer cells and to evaluate how drugs targeting them influence tumor metabolism.
For this purpose, we generated clock reporter colorectal cancer (CRC) cells to track the oscillating activity of two core clock proteins BMAL1 and PER2 and performed immunofluorescence studies in 3D CRC spheroids to combine spatial and temporal dimensions in our analyses.
While we identified oscillations in the expression of BMAL1 and PER2 in 2D CRC cell cultures, we failed to document time-dependent changes in the expression of either clock protein in 3D spheroids. A heterogeneous spatial distribution was however observed, with PER2 expressed at the periphery but not in the center of spheroids. We next used KL001, an activator of CRY, a protein that dimerizes with PER2 to co-repress BMAL1. We found that KL001 dose-dependently induced CRC cell death and, when used at non-cytotoxic doses, lengthened the period and reduced the amplitude of BMAL1 oscillations. In CRC spheroids, high dose KL001 exerted cytostatic effects while lower doses induced re-oxygenation of the spheroid center, an observation further supported by the capacity of KL001 to inhibit oxygen consumption rate in CRC cells. Measurements of PARP cleavage and propidium iodide confirmed that KL001-induced increase in pO2 did not result from cell death. Finally, we found that low dose KL001 led to a redistribution of PER2 from the rim to the entire spheroids.
Altogether, our data shed a new light on the tumor expression of clock genes and the possibility to impact cancer cell growth and metabolism by modulating the activity of regulators of circadian rhythms.

Quantitative In-Cell Western Immunofluorescence Assay: a novel tool to assess methylglyoxal stress in cancer cells

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Alteration of cellular energetic metabolism is a typical event in cancer. Cancer cells predominantly produce energy through glycolysis even in presence of oxygen (the so-called Warburg effect). Aggressive breast cancer subtypes such as triple negative breast cancer (TNBC) tumors are known to undergo such metabolic reprogramming and to be highly glycolytic. Increased glycolytic rate in tumor cells inevitably results in the production of a toxic by-product named methylglyoxal (MG). In all mammalian cells, MG is detoxified by glyoxalase 1 (GLO1) thus controlling MG stress level. We have previously shown that GLO1-depleted TNBC cells show enhanced growth and metastatic capacity.
Proteins are primary targets for MG-mediated non enzymatic post-translational glycation resulting in altered function and/or stability. To date, the detection and quantification of MG glycated proteins in cancer cells remain a real challenge. This study is aimed at establishing a standardized protocol for the detection of MG adducts using immunofluorescence (IF) technique and specific antibodies directed against the most abundant glycated proteins that are methylglyoxal-hydro-imidazolones (MGHs).
To set up IF conditions, we first used GLO1-depleted MDA-MB-231 breast cancer glycolytic cells that produce and accumulate endogenous MG. Another model consisted in the addition of exogenous MG to cultured cells to induce the formation of MGHs. For comparison, we also analyzed non glycolytic MCF7 breast cancer cells. Our pilot IF experiments showed a good detection of MGHs basal level in cultured glycolytic cells that was increased upon GLO1 depletion and MG treatment. Once IF conditions are optimized, we plan to perform in-cell western blot IF assays, a technique that combines the specificity of a western blot and the high-throughput of ELISA technique. We will quantify MGHs in cells cultured in microplates and we will test the inhibitory effects of potent MG scavengers such as carnosine and metformin.
We expect that the detection and the quantification of MGHs will represent a useful and reliable readout of MG stress in preclinical models of breast cancer.

Intrinsic resistance to CDK4/6 inhibitor is associated with lack of CDK4 phosphorylation in head and neck squamous cell (HNSCC) carcinomas.

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CDK4 and CDK6 phosphorylate and inactivate the tumor suppressor pRb. The T172-phosphorylation of cyclin D-bound CDK4 determines the cell cycle commitment in pRb-proficient cells. Its level varies in breast, thyroid tumors or mesotheliomas and in corresponding cell lines. Its absence informs on their insensitivity to CDK4/6 inhibitors. As the RB1, CCNE1 or CCNE2 genes are generally intact while the CDKN2A gene is inactivated in HPV-negative HNSCC tumors, CDK4/6 inhibitors may potentially be useful to treat them.
The aim of this work was to determine if the level of CDK4 T172-phosphorylation also varies in HNSCC tumors and preclinical models, whether it informs on their sensitivity or resistance to CDK4/6 inhibitors and whether the gene expression-based tool developed to predict the CDK4 profile in breast tumors works on HNSCC tumors.

We observed that the level of CDK4 phosphorylation is variable in HNSCC tumors, cell lines and PDTX models. Samples with active proliferation but without CDK4 phosphorylation include tumors or models with defective pRb, elevated E2F1 or CCNE1 expression as well as HPV-positive tumors or models, except one HPV-positive cell line with mutated CDKN2A locus and absent p16 expression. Phosphorylated CDK4 was detected in all sensitive cell models but not in insensitive ones, except the CDKN2A-mutated HPV-positive cell line. CDK4/6 inhibitors did not affect the growth in vivo of 5 out 11 tested PDTX models and of xenografts of the SCC9 cell lines sensitive in vitro, despite phosphorylated CDK4 detection. A gene expression-based CDK4 phosphorylation prediction tool developed for breast tumors was adapted to HNSCC tumors, This required to take two factors into account: CDKN2A mutation and high expression of CDKN2A locus exclusively due to expression of the p14-coding mRNA. The prediction accuracy reached 95% in the exploratory tumor cohort (IJB) and 89% or 100% in the validation cohorts of tumors (UCL) or PDTX models (Crown Biosciences).
Phosphorylated CDK4 does not inform on the capacity of HNSCC tumors or models to escape the effects of CDK4/6 inhibitors, but its lack informs on the irreversible intrinsic resistance to them. Its efficient prediction may guide inclusion of CDK4/6 inhibitors in the treatment of HNSCC tumors.

The effect of oxidation on the antigenic peptide repertoire

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Cytolytic T lymphocytes recognize peptides presented at the cell surface by MHC class I molecules. These peptides originate from the degradation of cellular proteins by the proteasome, a large protease complex mainly located in the cytoplasm. Four subtypes of proteasome exist, which differ in their catalytic subunits and display different proteolytic activities: the standard proteasome (ß1ß2ß5), two intermediate proteasomes (ß1ß2ß5i and ß1iß2ß5i) and immunoproteasome (ß1iß2iß5i). Previously, by performing in vitro digestion experiments with purified proteasomes, our lab has shown that oxidized proteins are more efficiently degraded by ß5i-containing proteasome. This suggests that cells exposed to oxidative stress might produce a different peptide repertoire, which originate from the degradation of these oxidized proteins. This is of particular interest in the field of cancer, because the tumor environment is often low in oxygen, and this state of hypoxia has been shown to increase the production of ROS. We therefore plan to use a mass-spectrometry-based approach to analyze the peptide repertoire of tumors cells exposed to an oxidative agent or grown in hypoxia. Analysis of the peptidome will be coupled to a more general analysis of the proteome and of the transcriptome to understand how these treatments affect tumors and to understand the origin of any potential change in the peptidome. This study might lead to the identification of new peptides, which could represent potential targets for anti-tumor CTL and could therefore be used as immunotherapeutic cancer vaccines

Tumor metabolic acidosis: a key piece in the puzzle of colorectal cancer heterogeneity

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Colorectal cancer (CRC) is one of the most frequent and aggressive cancers worldwide. Besides inter- and intra-tumor heterogeneities at both genetic and transcriptomic levels, tumor microenvironment (TME) is recognized to support intratumoral heterogeneity and disease progression. Acidosis is a common feature of TME in solid tumors, including CRC, and it has been shown to shape more aggressive cancer cell phenotypes. We hypothesize that targeting acidosis-induced phenotypic traits, in particular metabolic preferences, may serve as a “one-size-fits-all” therapeutic strategy to thwart tumor aggressiveness in CRC, regardless of their initial genotype. Four CRC cell lines (DiFi, LIM1215, HCT-116, HT-29), with distinct genotypes, have been adapted to chronic acidosis (pH 6.5) and then characterized by genomic, transcriptomic, metabolomic and phenotypic analyses. A common signature of 10 up- and down-regulated genes has been identified in the 4 acidosis-adapted CRC cells, as well as increased migratory capacity and anoikis resistance. Acidosis-adapted CRC cells display reduced glycolytic rate while mitochondrial respiration is increased, and impairment of mitochondrial respiration reduces their viability as well as resistance to anoikis. Another common metabolic feature is increased intracellular levels of proline (linked to a pro-migratory phenotype) and serine amino acids. The relevance of our findings is strengthened by the observation that inhibiting serine biosynthesis pathway, with a PHGDH inhibitor, reduces the viability of acidosis-adapted CRC cells. While data are now being validated in pre-clinical CRC models, including patient-derived tumor organoids, these preliminary results open new perspectives in the identification and further therapeutic targeting of metabolic preferences driven by tumor acidosis.

Metastasis: a side effect of low-dose ionizing radiation in breast cancer cells?

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X-ray radiotherapy is standard clinical practice for women with breast cancer (BC), but dose deposition is heterogeneous, with some cancer cells receiving low dose ionizing radiation (LDIR) typically at the tumor margin and in diffusive infiltrates. Since mitochondrial DNA (mtDNA) is a primary target of radiation and because mitochondria dysfunction can trigger metastasis, we hypothesized that subcytotoxic LDIR could induce mitochondrial defects, cancer cell migration, invasion and metastasis.
Here, using human MCF-7 and MDA-MB-231 BC breast cancer cells as working models, we report that a single dose of radiation elicits maximal BC cell migration in Transwells at 0.5 Gy and 0.125 Gy, respectively, i.e., within the LDIR range. These doses also increased mtDNA content detected by multiplex qPCR, basal cell respiration by Seahorse oximetry and mtROS production via dihydroethidium and MitoSOX staining. Our observations directly implicate mitochondria in LDIR-induced migration, as specific mtROS inhibitors MitoQ, SOD2 and mitochondrial-targeted catalase (mCAT) overexpression all abolished LDIR-induced migration of the two cell lines. As a blockade of mtH2O2 had the greatest suppressive effect on LDIR-induced migration, we further elucidated that overexpression of mtH2O2 using the mtHyPer-DAAO system was sufficient to induce migration, thereby recapitulating the effects of LDIR. At the molecular level, we report that LDIR-induced migration is regulated by transcription factors NF-κB in MCF-7 and the AP-1 family in MDA-MB-231 BC cells.
Collectively, our data indicate that BC cell migration could be a side effect of radiotherapy when cytotoxic levels are not reached. This study provides an incentive to evaluate mitochondria-targeted antioxidants as inhibitors of LDIR-induced pro-metastatic responses and to elucidate targetable mtROS pathways that contribute to these responses.

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Validation of anti-PD-1 monocolonal antibody binding to novel Immune checkpoint based Chimeric Antigen Receptors for better anti-tumor activity in glioblastoma patients.

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Background. Glioblastoma (GB) is the most aggressive and common malignant primary brain tumor in adults. Limited impact of current treatment options and high tumor recurrence make GB an incurable disease. (Pre)clinical studies using neo-adjuvant anti-PD-1 Immune checkpoint blocking antibodies (Ab) showed overall-survival benefits for GB and encouraging results for combination therapies. In parallel, various efforts were made in the field of chimeric antigen receptor (CAR) therapy to overcome the scarcity of tumor-specific T-cells in GB patients. This study aims to generate a novel CAR-type which can be activated after binding with Nivolumab (anti-PD-1 monoclonal Ab) to tune T-cells for better anti-tumor activity. With this, we would combine immune checkpoint blockade and CAR-T-cell therapy to overcome their individual limitations towards a synergistic T-cell product.
Methods. Novel CAR lentiviral vectors with an extracellular conventional PD-1 or mutated PD-1 (mPD-1) domain capable of binding to Nivolumab were produced. The mPD-1-CAR (K78a point mutation) was generated to avoid clinical on-target, off-tumor toxicity interactions with PD-L1+ cells. A 2D co-culture assay with a 1:1 (CAR transduced model-T-cell/PD-L1+ cells) ratio was performed to evaluate their intrinsic binding capabilities to PD-L1 via flow-cytometry. Nivolumab binding and intracellular CD3ζ-signal activation were demonstrated by addition of various concentrations of immobilized (0-700µg/mL) and soluble (0-150µg/mL) Nivolumab to a CAR transduced model-T-cell monoculture using flow-cytometry and Incucyte live-cell imaging.
Results. Model-T-cells, with intracellular GFP read-out after CAR-mediated CD3ζ-signaling, expressing the novel CAR-T constructs were generated. The PD-L1 co-culture assay confirmed the loss of PD-L1 binding capacity and retention of the Nivolumab binding capacity for the mPD-1-CAR. Intracellular CD3ζ-signal activation was quantified, and an optimal Nivolumab concentration of 150µg/mL for in vitro assays was established. Despite this, intracellular CD3ζ-signal activation was only noticeable for immobilized Nivolumab, while cross-linkage between CAR and soluble Nivolumab remained inadequate.
Conclusion. Our data shows the ability of both the (m)PD-1 CAR constructs to be activated by crosslinked (immobilized) Nivolumab. However, soluble Nivolumab was unable to trigger CAR-mediated CD3ζ-signaling, questioning the further use of this concept. Nevertheless, a possible continuance of this concept would be the inclusion of a conditional heterodimerization domain, removing the soluble Nivolumab crosslinking limitation.

High-Plex Co-Detection of RNA and Protein to Explore Tumor-Immune Interactions Utilizing RNAscope With Imaging Mass Cytometry

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The next breakthroughs in immuno-oncology will be driven by high-plex tools that decipher the spatial arrangement of different cell types within the tumor microenvironment (TME). Imaging Mass Cytometry™ (IMC™) is a proven tool for the study of complex cellular interactions in the TME. It utilizes CyTOF® technology for simultaneous assessment of 40-plus protein markers at subcellular resolution without spectral overlap or background autofluorescence, thus providing unprecedented insight into the organization and function of the TME. Despite this, some protein targets are challenging to include in IMC as they have very few or no commercial antibodies available. Moreover, although cellular identity can easily be deciphered through detection of protein targets, knowledge of the cell's transcriptome improves understanding of cellular function and activation state. Here, we present a robust and reliable workflow that combines the highly sensitive and specific RNAscope™ technology for RNA detection with the multiplexing capability of IMC to visualize key RNA and protein markers in the same tumor samples. The RNAscope HiPlex v2 assay was combined with protein detection using IMC to evaluate expression of both RNA and protein targets in formalin-fixed, paraffin-embedded (FFPE) tumor tissue microarray (TMA).

MECHANISTIC CONTRIBUTION OF DIFFERENT CELL DEATH MODALITIES TO SQUAMOUS CARCINOGENESIS.

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Squamous cell carcinoma (SCC) is a tumour type with surging incidence that can arise at different body sites. Metastatic SCCs represent a major unmet medical need as the 5-year survival rate is lower than 50%.
Previous research in my team identified excessive death of keratinocytes -the cell-of-origin of SCCs- as a driver of squamous carcinogenesis (Hoste et al., 2019). While many anti-cancer drugs aim to induce cell death, little is known about the effects of the elicited cell death on inflammation and proliferation in the tumour stem niche. Apoptotic cell death is known to have a dual role in carcinogenesis, being protective against tumour initiation and promoting tumour progression. Recent evidence indicates that, besides apoptosis, dysregulation of other cell death programs, such as necroptosis and pyroptosis, can impact on carcinogenesis (Hoste et al., 2021).
The aim of my project is to investigate the contribution of distinct cell death modalities and their downstream signalling events to SCC tumour initiation and progression. For this, we make use of mice lacking key cell death mediators selectively in keratinocytes. These mice are subjected to tumour models inducing oral and skin squamous carcinomas. Our findings will contribute to the understanding of the link between cell death and carcinogenesis, not only in skin and oral mucosa, but also in other tissues.

Practice patterns, time trends and quality of care for uterine cancer in Belgium: an analysis of the EFFECT database

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Objectives
To investigate the practice patterns and quality of care for uterine cancer on a national level in Belgium, including trends in clinical practice over the period 2012-2016.

Methods
Quality indicators were measured on a national level and studied in time by using the EFFectiveness of Endometrial Cancer Treatment (EFFECT) database, a nationwide clinical database covering 56% of all uterine cancer patients diagnosed in Belgium during the period 2012-2016. Multivariable logistic mixed regression was used to estimate risk-adjusted odds ratios (aORs) and 95% confidence intervals (CIs) for associations between the quality indicators and year of diagnosis.

Results
Of all patients, 97.6% (n=4077) were discussed in at least one multidisciplinary team meeting, increasing from 96.4% (n=726) in 2012 to 98.5% (n=767) in 2016 (aOR=2.95; 95% CI=1.43-6.08). A preoperative biopsy was performed in 87.9% (n=3212) of all endometrial carcinoma (EC) patients who had surgery, increasing from 85.5% (n=568) in 2012 to 90.0% (n=624) in 2016 (aOR=1.65; 95% CI=1.14-2.39). Minimally invasive surgery (laparoscopic or robotic-assisted) was used in 61.6% (n=1359) of all patients who had surgery for clinical stage I EC, increasing from 52.9% (n=192) in 2012 to 66.4% (n=285) in 2016 (aOR=1.99; 95% CI=1.35-2.95). At least pelvic lymph node staging was performed in 69.0% (n=363) of all patients with clinical stage I, high-grade EC; and in 63.9% (n=177) of all patients with clinical stage I-II serous carcinoma, clear cell carcinoma or carcinosarcoma. The latter increased from 48.8% (n=21) in 2012 to 77.2% (n=44) in 2016 (aOR=4.68; 95% CI=1.64-13.37). Adjuvant radiotherapy was administered to 33.5% (n=107) of all patients with pathological stage I EC at high-intermediate or high risk of recurrence; and adjuvant chemotherapy to 64.4% (n=270) of all patients with pathological stage III-IVA EC.

Conclusions
While Belgian hospitals were found to perform excellently on the quality indicators regarding diagnostic work-up, pathology and staging, this study indicates room for improvement in the surgical and adjuvant treatment of uterine cancer in Belgium. However, improvements were already observed over the period 2012-2016, which may have continued since.

Enhancing Glioblastoma Sensitivity to Immune Checkpoint Blockade through Metabolic Reprogramming

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Glioblastoma (GBM) patients are in dire need of an effective therapy. GBM rewires crucial metabolic programs for proliferation and survival, thereby creating a hostile tumor microenvironment that impairs tumor immunosurveillance and response to immunotherapy. Our study investigates the potential of targeting mitochondrial respiration and glutaminolysis with various drugs to reprogram the metabolic environment of GBM tumors, with the aim of sensitizing them to immune checkpoint blockade (ICB) in vivo.

We examined the therapeutic potential of metabolic inhibitors in the orthotopic GL261 GBM murine model. Specifically, we tested the efficacy of Metformin and IACS-01759, which inhibit oxidative phosphorylation, and JHU-083, which targets glutaminolysis, in GL261 tumor-bearing mice. Mice were randomized based on tumor volume determined via magnetic resonance imaging. Metformin was administered via drinking water, while IACS-01759 was given orally at either a low or high dose daily. JHU-083 was administered orally either daily at a lower concentration or every four days at a higher concentration. Concurrently, mice were injected intraperitoneally with anti-PD-1. Survival was used as the primary read-out to evaluate the therapeutic effect, with humane endpoints for brain tumors being considered.

The administration of metformin and JHU-083 did not yield significant improvements in survival outcomes. However, we observed a significant improvement in overall survival when a low concentration of IACS-01759 was combined with anti-PD-1 compared to both vehicle control and IACS-01759 monotherapies. These preclinical findings offer promising evidence that targeting mitochondrial respiration using IACS-01759 has the potential to increase the sensitivity of GBM tumors to ICB therapy. Further research is necessary to determine the optimal administration strategy for this approach.

Preclinical evaluation of a novel microtubule stabilizing agent in gynaecological tumours

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The microtubule stabilizing agent (MSA) paclitaxel is currently used as a first line therapy in gynaecological cancers. However, major obstacles are associated with the use of paclitaxel, such as poor water solubility, neurotoxicities and therapy resistance. To overcome these problems, a novel MSA with increased water solubility and a different microtubule binding site from paclitaxel was designed at Ghent University.
We aimed to evaluate the effect of the novel MSA in both monolayer cultures and spheroids from breast and ovarian cancer cell lines.

The novel MSA dose-dependently affected spheroid metrics such as size and ATP content. Normalized growth rate inhibition metrics showed a cytotoxic or cytostatic effect in SKOV3 and MCF7 cells respectively. Treatment with the novel MSA results in morphological changes, particularly cell rounding in monolayer cultures and disintegration of spheroids, but also a G2/M cell cycle arrest. Western blot unveiled that the novel MSA induced acetylation of tubulin. In addition, mass-spectrometry assisted proteomics analysis revealed increased presence of TPPP, a tubulin-specific deacetylase inhibitor which contributes to microtubule bundling. All our data show high concordance between paclitaxel and the novel MSA and confirm a mode of action mediated by a mitotic block due to microtubule stabilization.
Furthermore paclitaxel resistance is a major course of treatment failure. We showed that the novel MSA overcomes paclitaxel resistance. Finally, bridging the gap to the in vivo situation, we demonstrated that ex vivo treatment of patient derived tissue fragments locks these tissue fragments in a metabolic inactive morphotype. Analysis of proliferation markers and a microtubule stabilization marker in patient derived tissue is currently under evaluation.

In addition to providing preclinical proof of concept for a novel MSA, our work confirms it proposed mechanism of action through complementary biochemical assays. The novel MSA overcomes paclitaxel resistance and is a promising compound for second line chemotherapy treatment of gynaecological cancers.

Patient-derived Organoids as a State-of-the-Art 3D Tumor Model for Novel Combination Therapy of HNSCC

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Patients with advanced head and neck squamous cell carcinoma often face relapse, metastasis (R/M HNSCC), and detrimental outcomes. First-line treatment with immunotherapy alone or combined with platinum-based chemotherapeutics (CIS) has limited benefits due to low response rates and severe side effects. Therefore, well-tolerated therapeutic strategies to improve currently established therapies are very much required. As non-thermal plasma (NTP), an ionized gas containing reactive oxygen and nitrogen species, has been reported as an inducer of immunogenic cell death, our goal was to evaluate the immunogenicity of a novel combination strategy of NTP with the R/M HNSCC first-line therapies. With the purpose of clinical introduction, this study was performed in advanced in vitro 3D tumor models for head and neck cancer.
Common 2D in vitro models lack the ability to reflect essential components of the complex tumoral context, impairing in vivo translation and clinical implementation. Therefore, our lab implemented a state-of-the-art, 3D in vitro model, the 'patient-derived organoid model' (HNSCC-PDO), that originates from resection fragments or biopsies obtained during surgical intervention on patients. This model uniquely mimics the phenotypic and genotypic characteristics from the original patient's tumor, providing a powerful platform for the screening of novel therapies, validation of treatment results, and translation into clinical applications. Currently, we succeeded to set up an HNSCC-PDO bank from 6 patients, enabling an in-depth screening of therapies against a diverse panel of HNSCC characteristics.
To validate treatment efficacy previously obtained in a 3D spheroid model, an optimized NTP-CIS combination was tested in the HNSCC-PDOs. For the analysis of these therapeutic effects, our lab employed a state-of-the-art kinetic drug screening platform – ORBITS. We demonstrated that our 'multi-organoid-per-well' model can be used to evaluate PDO killing, with increased tumor cell death in the NTP-CIS regimes compared to both monotherapies. Evaluation of immune engagement by our optimized NTP-CIS treatment is ongoing.
In summary, the PDO model is a highly advanced 3D model with unique potential towards clinical translation. Validation of results in this model is of great scientific and medical relevance and has the potential to drastically accelerate the introduction of novel therapies, like NTP, into the clinic.

Evaluation of therapy-induced senescence, therapy resistance and senolytic efficacy in non-small cell lung cancer using a high-throughput labeling strategy.

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Introduction
Non-small cell lung cancer (NSCLC) remains a leading cause of cancer-related deaths with long-term survival still hard to achieve. Cellular senescence is found to be induced by several anticancer therapies (i.e., therapy-induced senescence (TIS)), and is able to affect the surrounding TME through their distinctive senescence-associated secretory phenotype (SASP), ultimately leading to cancer relapse and metastasis. To better understand the role of cellular senescence in cancer and to evaluate potential senolytic therapies, adequate and high-throughput detection of senescence in preclinical and clinical cancer research is becoming increasingly important.
Material and Methods
A high-throughput analysis method was developed that labels cells based on the presence or absence of senescent phenotypic features: (i) an enlarged nucleus; (ii) cytoplasmic senescence-associated β-galactosidase; and (iii) an enlarged cell size. This labeling strategy was used to evaluate the percentage TIS in A549 NSCLC cells after a 5-day treatment with 5 standard of care (SOC) chemotherapies. To evaluate therapy resistance of senescent NSCLC cells, senescent and control A549 cells were treated with several SOC chemotherapies (0 - 90 µM) for 96h, after which cell viability was assessed (Tecan Spark®Cyto). Alternatively, senescent and control A549 cells were treated with the Bcl-2 inhibitors Navitoclax and Venetoclax (0 - 90 µM, 96h) to evaluate their senolytic potency.
Results
TIS induction was observed in A549 cells, using all SOC compounds, with the highest levels of TIS using cisplatin (64%), carboplatin (61%) and pemetrexed (54%). Next, we demonstrated that senescent A549 cells are significantly more resistant to SOC chemotherapies compared to control cells (e.g., for 7.732 µM Vinorelbine: 81.2 □ 2.8 % survival for senescent A549 cells vs. 27.0 □ 1.2 % survival for control A549 cells; p<0.0001). Furthermore, our results indicate that Navitoclax and Venetoclax are significantly able to selectively eliminate senescent A549 cells (p<0.0001).
Conclusion
We have successfully developed an accurate and high-throughput strategy to label senescent NSCLC cells. We further demonstrated that SOC NSCLC therapies induce TIS in in vitro conditions, that senescent cells are resistant to SOC chemotherapeutic treatment and that senolytics are capable of selectively killing senescent NSCLC cells.

Lipid metabolism reprogramming supports escape to anti-EGFR therapy in head and neck cancers

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Despite the implementation of multimodal treatment strategies in the clinical management of patients with advanced head and neck squamous cell carcinoma (HNSCC), overall prognosis remains very poor due to frequent tumor relapses. For instance, while anti-EGFR targeted therapy (cetuximab) leads to the killing of drug-sensitive cells and tumor volume reduction, the effect is often transitory and the emergence of drug-resistant cells almost invariably leads to clinical relapses. In the current project, we reason that to prolong the response to EGFR blockade, concomitant or sequential therapies should ideally target the drug-tolerant persister (DTP) cells that withstand the initial anti-EGFR treatment and sustain residual tumor burden.
Here, we used several biological models, including 2D and 3D cultures of cetuximab-sensitive and -resistant HNSCC cells, as well as patient-derived tumor xenografts, in combination with transcriptomic, proteomic and metabolomic studies to unravel metabolic changes in HNSCC cells during anti-EGFR treatment. We revealed a metabolic switch from glycolysis to lipid metabolism in HNSCC DTP cells upon cetuximab treatment. More precisely, the gene encoding the fatty acid transporter (FA) CD36 was consistently upregulated in residual HNSCC and this was associated with increased FA uptake, oxidation and storage capacities in these cells. Importantly, we observed that such FA metabolism reprogramming was also critical to support growth of long-term cetuximab-resistant HNSCC cells. This study points out lipid metabolism rewiring as a non-genetic resistance-causing mechanism in HNSCC that may be therapeutically targeted to overcome acquired resistance to anti-EGFR therapy.

TF/FVIIa-mediated shedding of CD44 in EMT-shifted human breast tumor cells.

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Epithelial-Mesenchymal Transitions (EMT) are well described to impact Circulating Tumour Cells (CTC) biology. In accordance with extensive literature linking coagulation to cancer, our team previously contributed to show that EMT induces the expression of Tissue Factor (TF, F3, a membrane-associated activator of the coagulation cascade), triggering increased coagulant properties that facilitate niching abilities in experimental metastasis assays. Importantly, a subpopulation of CTCs isolated from breast cancer patients and co-expressing TF and vimentin was identified.

Aiming to identify new TF protein interactants, we performed a Mass Spectrometry analysis on TF immunoprecipitates from EMT+ cells and identified the stem cell marker CD44 as a potential TF-protein partner. This TF/CD44 interaction was confirmed in various EMT+ cellular models by co-immunoprecipitations and proximity ligation assays. Co-immunostainings, FACS analyses and membrane protein biotinylation assays also revealed that TF/CD44 interactions mainly occur at the plasma membrane. Since TF is the receptor of the first serine protease of the coagulation cascade Factor VIIa (FVIIa), we reasoned that CD44/TF interactions could bring FVIIa proteolytic activity around CD44 in TF+/EMT+ cells. Accordingly, incubating EMT+/TF+ cells with FVIIa induces the cleavage of CD44 and the release of a N-terminal cleavage product around ~45kDa in the conditioned medium, which could, according to preliminary results, have a chemoattractant effect on fibroblasts. Modulating TF in EMT+ models, demonstrated the requirement for TF in the process. Incubating cells with BB94, a broad range inhibitor of several canonical sheddases, emphasized the specificity of the cleavage generated by TF/FVIIa. We moreover succeeded to inhibit TF/FVIIa-mediated cleavage of CD44 by introducing a single point mutation in a putative cleavage site in the N-terminal part of the protein. Such a new cell model should help us confirm/infirm the biological effect of CD44 N-terminal cleavage product on surrounding cells.

We are currently deciphering molecular mechanisms involved in this specific cleavage of CD44 by TF/FVIIa and examining the potential of specific CD44 cleavage products as blood-based biomarker using the CHU Sart-Tilman (Liège, Belgium) plasma biobank of breast cancer patients.

Search for markers involved in lymph node remodeling in the pre-metastatic stage by metabolomic analysis.

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Background
Our laboratory has provided evidence for the existence of a premetastatic niche in the sentinel lymph node (LN) draining a human cervical neoplasm, which is characterized by a specific lymphangiogenic, immune and extracellular matrix profile. Metabolomics is a promising approach that provides an opportunity to link the metabolome with physiological or pathological status. The aim of this project is to identify additional markers of the arrival of metastases in the LN by applying a metabolomic approach to human and murine samples.

Methods
To identify metabolic pathways modulated in LN, we benefit from a preclinical mouse model, the “ear sponge assay”, which reproduces each step of the metastatic cascade. The mice’s lymph nodes are analyzed by NMR-based metabolomics and histological procedure. Furthermore, we will use a cohort of patients suffering from advanced cervical cancer and we will analyze different samples issued from the same patient.

Results
Different analyses performed on mice LNs highlight a clear discrimination between the cervical LN (draining the tumor) and the other LNs (mandibular, sub-draining LN, and axillary/inguinal, control LN) issued from mice bearing a tumor. Our data show that the tumor development impacts the environment of the draining LN, but not that of distant LNs.

Conclusion
Our results highlight metabolomics’s interest in investigating LN remodeling during the metastatic process. Indeed, the tumor microenvironment impacts the cervical lymph node at a metabolite level. These results will be confirmed by additional experiments in order to perform further analysis on the discriminating metabolites.

Preclinical study on the potential of PI3K/Akt pathway inhibition in overcoming cetuximab resistance and inducing immunogenic cell death using 2D and 3D models

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Head and neck squamous cell carcinoma (HNSCC) poses a significant clinical challenge due to the resistance against the epidermal growth factor receptor (EGFR)-targeted agent cetuximab. Emerging evidence indicates that dysregulated signaling of the PI3K/Akt pathway may contribute to this resistance. Therefore, this study investigated the efficacy of a novel therapeutic strategy co-targeting EGFR using cetuximab and PI3K using buparlisib. Additionally, we explored whether this combination could induce immunogenic cell death (ICD), which promotes an immune response against cancer cells, potentially enhancing antitumor effects.

Five human papilloma virus (HPV)-negative HNSCC cell lines with different sensitivities to cetuximab were used. Cells were grown as 3D multispheroids for synergy analysis and as 2D monolayers for immunogenic cell death assays. Patient-derived organoids from two different HPV-negative HNSCC patients were characterized for their cetuximab resistance status after 168h of cetuximab treatment and used to validate synergy results. Cytotoxicity of cetuximab plus the PI3K inhibitor buparlisib at different concentrations was assessed using Tecan's Spark Cyto live-cell imaging and analyzed using the in-house developed Orbits image and data analysis platform. Possible synergism between cetuximab and buparlisib was determined. The ICD hallmarks ATP (24h), calreticulin and HMGB1 (72h) were measured in vitro after treatment.

Buparlisib inhibited the growth of all HNSCC spheroids and exhibited cytotoxic effects at high concentrations (>1.65µM). Combining buparlisib with cetuximab accelerated the cytotoxic effect in all except the SCC22b-R cell line. Combination treatment was synergistic in SCC22b-S, and additive to synergistic in the other cell lines. Combined treatment also increased the release of ICD markers ATP and HMGB1 in most cell lines. Moreover, calreticulin-positive cells were the highest following exposure to the combination of cetuximab and buparlisib across all tested cell lines. Both HNSCC organoids were cetuximab-resistant, but synergy analysis revealed a synergistic interaction at different concentrations of buparlisib when combined with 500nM of cetuximab in both organoids.

In conclusion, this study reveals a synergistic interaction between cetuximab and buparlisib, accompanied by the induction of ICD hallmarks. These findings suggest that combining cetuximab and buparlisib could overcome cetuximab resistance and induce immunomodulatory effects, offering promising prospects for improving treatment efficacy in HNSCC patients.

ATP1A1 is a new promising target for melanoma treatment that can be inhibited by its physiological ligand bufalin to restore targeted therapy efficacy

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Despite recent advances in the treatment of metastatic melanoma, many patients do not respond to targeted therapies and develop acquired resistance. Our research focuses on the ATP1A1 subunit of the sodium pump which is associated to cancer development in many studies. We aimed to evaluate the prognostic value of ATP1A1 in melanoma patients and to assess the effect of its ligand bufalin in melanoma cell lines in vitro and in vivo.
First, we scored ATP1A1 levels in patient biopsies (IHC, qPCR) and in melanoma cell lines (WB, qPCR), and found that high ATP1A1 expression was correlated with short patient OS, and that resistance to BRAF inhibitor was associated with elevated ATP1A1 levels in patient biopsies and cell lines. Then, we found that high ATP1A1 expression is positively correlated with markers of differentiation/pigmentation using patient TCGA databases, and that ATP1A1 is present in the Verfaillie proliferative gene signature examining cell lines. We also showed that bufalin targeted ATP1A1 within the caveolae (interaction with Cav1, PLA) and affected SRC phosphorylation (WB), thus interfering with different signalling pathways, further identified by phosphokinase array. We reported that bufalin inhibited cell clonogenicity and induced apoptosis by acting on ATP1A1 (gene silencing), thus affecting cell survival. Finally, we developed a mouse xenograft model transplanted with an osmotic pump and demonstrated the inhibitory effect of chronic delivery of bufalin on tumour development.
In conclusion, ATP1A1 could be useful as a prognostic marker of patient survival and as a predictive marker of response to a BRAF inhibitor. By targeting ATP1A1, bufalin inhibits cell proliferation and induces apoptosis in vitro, and slows down tumour development in mice. Hence, our results strongly support ATP1A1 as a new target for therapy and propose its natural ligand bufalin as a mean to affect its tumour-promoting activity.

Novel therapeutic approaches for EGFR-mediated drug delivery using engineered peptides in anaplastic thyroid cancer

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Anaplastic thyroid carcinoma (ATC) represents the most aggressive and deadliest thyroid cancer in humans, presenting a high proliferative and metastatic potential. Mostly affected signaling pathways in ATC are those of MAP kinase and PIP3/AKT/mTOR. The median survival of patients with ATC is of about 4 months after diagnosis and the mortality rate attains almost 100%. Currently, there is no cure for this cancer, which is highly invasive and resistant to conventional therapies. Accordingly, targeted therapies and delivery, such as those studied in the present work, should be considered to improve the prognosis of patients. Our targeted therapy aims to inhibit the PI3K/AKT/mTOR signaling pathway, thereby inducing apoptosis of target cells with a therapeutic peptide (TP) developed in our laboratory. The epidermal growth factor receptor (EGFR) is commonly studied in oncology as it is overexpressed in cancer cells and is actively investigated in the framework of receptor-mediated drug delivery. Therefore, an EGFR-targeted peptide (vector peptide, VP) was also developed by our group and coupled to TP in a peptide complex (PC) to enable the specific drug delivery to ATC cells. Our results show that EGFR is overexpressed and overactivated in ATC. VP is endocytosed independently of the EGF presence and without activating the EGFR. Within cells, VP is colocalized with EGFR, following its trafficking pathway. Moreover, 10 µM of PC induces cell apoptosis after 1h of incubation. To conclude, our studies confirmed that VP is a good EGFR-targeting candidate to deliver TP to cancer cells. In addition, this VP is able to induce endocytosis of EGFR and thus to deliver TP intracellularly to induce apoptosis.

Using gold nanoparticles and protons to improve immune responses in radiotherapy

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Radiosensitivity of cells has been observed to be enhanced by the use of metallic nanoparticles via an increase in the intracellular reactive oxygen species (ROS) and emitted secondary electrons and can potentially improve the outcome of radiotherapy for cancer patients. Gold nanoparticles (AuNP) are also capable of independently increasing the ROS in cells, thereby blocking pro-tumor M2 polarization in macrophages. Combining these strategies, this study employs AuNP stabilized and functionalized with different polymers, polyethylene glycol (PEG) or functionalized polyvinylpyrrolidone (PVP), of sizes 15 or 50 nm in diameter. These AuNPs were found to have a high coating density (60-70% w/w) and stability. On adding these AuNPs to M2 macrophages, no toxicity was observed even at high concentrations, and the AuNP-PVP were readily endocytosed by the macrophages, but not AuNP-PEG. We also found that AuNP-PVP aids in promoting the M1 pro-inflammatory phenotype, while AuNP-PEG promotes the M2 phenotype. Ongoing work includes exposing the AuNP-internalized M2 cells to X-ray (5 and 10 Gy) and proton irradiation followed by assessment of ROS and phenotype alteration. In vivo effects will then be investigated on C57BL/6J mice to ascertain the translation of the AuNP only and AuNP+radiation approaches in the treatment of cancer.

Macrophage Profiling in Head and Neck Cancer to Improve Patient Prognosis and Assessment of Cancer Cell-Macrophage Interactions Using Three-Dimensional Coculture Models

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Tumor-associated macrophages are key components of the tumor microenvironment (TME) and have been shown to play important roles in the progression of head and neck cancer. As a result, novel treatment approaches are focused on reprogramming M2 macrophages to adopt the M1 phenotype. First, a scoring system based on the high or low density of M1 CD80+ and M2 CD163+ macrophages and on the tumor-infiltrated phenotype was developed in a clinical series of 54 head and neck squamous cell carcinoma patients. Interestingly, this macroscore was found to be more powerful than TNM criteria and p16 status and also significantly associated with poor prognosis for these patients. Additionally, a 3D coculture model was established to analyze the influence of cancer cells on monocyte recruitment and their polarization. This model demonstrated that cancer cells are responsible for monocyte recruitment and M2 polarization, resulting in an immunosuppressive microenvironment with an increased production of IL8 and IL10 cytokines. Finally, we focused on a new compound found in toad venom. Bufalin is an endogenous cardiotonic steroid with reported anti-cancer and immunomodulatory properties. Our data indicated that bufalin reprogram M2 macrophages towards the M1 phenotype underlining its potential as an antitumor immune modulator. Overall, this research highlights the power of the macroscore as a new valuable prognostic biomarker and sheds light on the immunosuppressive tumor microenvironment. Moreover, it indicates that modulating macrophages in the tumor microenvironment using bufalin could be a promising immunotherapeutic strategy for the treatment of cancer

Determination of the genes involved in intrinsic resistance to proton beam therapy for the treatment of glioblastoma.

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Glioblastoma multiforme (GBM) is the most common and aggressive malignant primary central nervous system tumors and account for 60% of brain tumors. GBMs have a very poor prognosis with less than 2-years survival rate. The GBM standard treatments include surgical resection of the tumor combined with fractioned radiotherapy and temozolomide (TMZ) followed by adjuvant TMZ therapy. Despite advances in treatment modalities, the overall prognosis remains poor, and long-term survival is rare due to intrinsic or acquired resistance to anti-cancer therapies. In this context, the main goal of this project is to identify potential genes implicated in this resistance of GMBs in order to bypass it. To reach this objective, we are currently performing a genome-wide CRISPR-Cas9 screen using the GeCKO v2 pooled single-guide RNA libraries in a GBM cell line, T98G. The latter is known to express a high level of a DNA repair protein called MGMT resulting in resistance to alkylating drugs like TMZ. After the validation of candidate genes, the mechanisms underlying the resistance induced by these genes will be further characterized to sensitize the glioblastoma cells to the current treatments. This screening has already been carried out previously in the laboratory on another GBM cell line, U87. Unlike T98G, this cell line is sensitive to TMZ and has low basal MGMT expression. It resulted in the identification of some potential resistance genes that are currently under investigation. Therefore, one of the objectives of this project is to compare the different resistance mechanisms common to these two GBM cell lines. In parallel, the results on 2D cell culture model will be transposed to GBM patient-derived organoids. 3D cultures are a physiologically relevant in vitro model for cancer research and a powerful tool in the prediction of patient treatment responses. Finally, the implication of candidate genes in GMBs resistance will also be assessed by using proton irradiation in comparison with X-rays resistance. Indeed, the use of high energy charged particles like protons offers many benefits over conventional radiotherapy. Its unique physical properties allow to irradiate the tumor more precisely and therefore spare surrounding healthy tissues.

Development of personalized neutron capture therapy using theranostic carriers

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Given the growing interest in targeted therapies, boron neutron capture therapy (BNCT) is receiving renewed attention as a particle radiotherapy based on the interaction between a non-radioactive boron-10 (10B)-labelled compound and low-energy thermal neutrons (Figure I). This interaction leads to the production of α particles and 7Li particles, which are high linear energy transfer (LET) particles more effective at inducing cell death than conventional X-rays and with a short range (about 5 - 9 µm) in tissue, damaging only boron-containing cells (1). Therefore, if sufficient quantities of boron compounds can be selectively accumulated in cancer cells, BNCT has the potential to become an ideal selective and targeted radiotherapy since the two individual components of this binary treatment, the 10B-containing compound and the thermal neutrons, have little or no biological effect of their own (2).
This project aims to initiate fundamental research to better understand the underlying radiobiological basis of BNCT, influencing the design of subsequent clinical trials that will start at the Institut Jules Bordet in 2026. To achieve this, the advantage of using theragnostic agents 157Gd-BPA and 19F-BPA (10B-p-boronophenylalanine) in BNCT treatments will be studied. Being well-established contrast agents in MRI, they will allow, on one hand, the imaging and measuring the heterogeneity of 10B loading in tissues, inducing a personalized medicine approach. Furthermore, they could increase the dose deposition following the neutron capture by emitting electrons, more uniformly distributed throughout tumors. The optimal timing for BNCT treatment will be assessed by studying the pharmacokinetics of these BPA derivatives by MRI as well as their potential therapeutic benefit compared to BPA alone. Finally, the interaction of BPA and their derivates with thermal neutrons will be studied. The chemical synthesis of new compounds and the assessment of various in vitro biological endpoints (toxicity, activity assays, …) and in vivo validation (irradiation, biodistribution kinetics …), both head and neck cancer models are ongoing.
(1) Penninckx., S et al, " Radiation Research 2019; 192(1), 1-12. (2) Sauerwein WAG,. et al.,. Life. 2021; 11(4):330.