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Unraveling the poly-pharmacology of beta-blockers in neuroblastoma using chemo-proteomics approaches

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Our team has previously shown that common anti-hypertensive drugs, β-blockers, can increase the efficacy of chemotherapy in high-risk neuroblastoma, a deadly childhood cancer. The mechanism(s) involved remains however poorly understood. First, we showed that the enantiomers of propranolol and carvedilol (R-S) were equipotent at increasing the cytotoxic activity of vincristine, a common chemotherapeutic agents used in neuroblastoma. Since the (R)-enantiomers have low affinity for their canonical targets, the β-adrenergic receptors, we concluded that the chemo-sensitizing activity of β-blockers is mediated by non-canonical targets. Therefore, this project aims at revealing these non-canonical targets and improving our understanding of the mechanism of action of β-blockers to optimize their clinical use in oncology.

Using an innovative chemo-proteomics approach coupled with pharmaco-metabolomics, we identified the interactome of β-blockers in neuroblastoma cells and started to unravel their mechanism of chemo-sensitization.

We exploited a biophysical assay called “Thermal Proteome Profiling” (TPP) coupled with label-free quantitative mass spectrometry to evaluate the impact of β-blockers (propranolol and carvedilol) and the microtubule-targeting agent, vincristine alone or their combination, on the thermostability of the proteome of neuroblastoma cells. We applied TPP on cell lysates and in cellulo to discriminate between primary and secondary interactors. Our results highlighted well-known targets of vincristine such as tubulins, but also an enrichment in proteins involved in heme biosynthesis with the β-blockers and combinatorial treatments. We then performed 13C glucose and glutamine tracer experiments and showed an alteration of the pyrimidine synthesis pathways and the tricarboxylic acid cycle under the combinatorial treatment. Finally, by using functional genomics and click-fluorescence approaches to perform co-localization experiments, we will now validate and characterize the identified metabolic targets of β-blockers impacting neuroblastoma chemosensitivity.

Overall, our results show that β-blokers increase the efficacy of chemotherapy in neuroblastoma by interfering with mitochondrial metabolism, independently of beta-adrenergic receptors. This project could help better understand neuroblastoma biology and reveal novel targetable metabolic pathways, that could be therapeutically exploited to develop new treatments and unveil biomarkers for patient selection, thus facilitating clinical trials. This study also establishes a proof-of-concept for the use of TPP-based chemo-proteomics to unravel the mechanism of action of repurposed drugs.

Ezurpimtrostat autophagy blocker, a palmitoyl-protein thioesterase 1 (PPT1) inhibitor, and atezolizumab/bevacizumab triple combination regimen enhances antitumor efficacy in hepatocellular carcinoma

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Background – Immune checkpoint therapies combination with anti-VEGF is the standard-of-care in first-line of hepatocellular carcinoma (HCC). However, only 30 % of patients present a response to first line therapy. Autophagy inhibitors were recently spotted out as a potential robust strategy to promote antigen presentation and therefore reinforce immune checkpoint inhibitors (ICIs) potency conducting to strong anti-tumoral response. Hereafter, we aimed to assess the efficacy of GNS561 (Ezurpimtrostat), an autophagy inhibitor targeting palmitoyl-protein thioesterase-1 (PPT-1) lysosomal enzyme, in combination with atezolizumab/bevacizumab in HCC in a chicken embryos model.

Methods – Chorioallantoic membrane (CAM assay) model was used, inoculated with liver adenocarcinoma Hep3B cell line at day 9 (D9). Between D9 and D18, eggs were treated every two day with GNS561 alone (0.92 mg/kg), or in combination with atezolizumab/bevacizumab (at 1 mg/kg/1mg/kg (R1), or 2 mg/kg/2mg/kg (R2)). At D18, the upper portion of the CAM containing tumor is removed. The tumors are cut away from normal CAM tissue and weighed, and the number of dead embryos is counted. A one-way analysis of variance with post-tests is done. No cytotoxicity was reported.

Results – All regimens induced a significant tumor growth regression compared to the control group, GNS561 alone having a more potent antitumoral effect ($p<0.0004$) compared to both atezolizumab/bevacizumab regimens (R1, $p<0.0147$; R2, $p<0.0071$). Interestingly, atezolizumab/bevacizumab combination regimens exhibited no significant difference between the two tested dose-regimens. A triple combination regimen led to significantly improved anti-tumoral efficacy compared to the two atezolizumab/bevacizumab groups, for comparable dosing (R1, $p<0.0076$, R2, $p<0.0001$), spotlighting triple combination superior benefit. Results further pointed out GNS561 combination with atezolizumab/bevacizumab significant superior antitumor effect at the highest dose in comparison with GNS561 alone, reinforcing the interest of triple therapy ($p<0.0030$).

Conclusion – This study reports that GNS561 potentiates the anti-tumor effect of atezolizumab/bevacizumab combination emphasizing PPT1 inhibitors' therapeutic interest in combination with immunotherapy in HCC. This triple therapy is currently tested in the randomized phase 2 clinical trial ABE-LIVER, in first line setting in advanced HCC (NCT05448677).

Unveiling the Therapeutic Potential of Novel FAK Inhibitors in Overcoming Acquired Resistance to EGFR-TKI in Non-Small Cell Lung Cancer

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Background – The focal adhesion kinase (FAK) signaling pathway has recently emerged among oncogenic signaling associated to resistance to EGFR-tyrosine kinase inhibitors (EGFR-TKIs) in non-small cell lung cancer (NSCLC). In a previous study, we synthesized and evaluated a new series of imidazo[2,1-b][1,3,4]thiadiazole derivatives, and the two most active compounds (5a and 5f) exhibited remarkable cytotoxic activity against pancreatic cancer cells as FAK inhibitors. Therefore, this study aimed at investigating the anti-tumor activity of 5a/5f and combined effect with EGFR-TKIs in isogenic NSCLC cells resistant to second- and third-generation EGFR-TKIs.

Methods – H1975 Osimertinib-resistant (OR) and PC9 Afatinib-resistant (AR) cells were established using a dose escalation method and their viability and migration ability were measured by Sulforhodamine-B, colony formation and wound-healing assays. Pharmacologic interaction was studied using the combination index method, while effects on cell-cycle and apoptosis were studied with AnnexinV/PI stainings. PCR arrays on 84 key genes involved in oncogenic pathways and kinome profiles of 144 tyrosine or serine/threonine peptides were performed to investigate the potential relationship of FAK and TKI-resistance. ELISA evaluated the expression of P-FAK in cells and immunohistochemistry evaluated the expression of P-FAK in tissues.

Results – FAK was overexpressed and activated in EGFR-TKI resistant NSCLC cell lines and patients. In addition, PCR and PAMchip kinase arrays showed that FAK was a hub gene in EGFR-TKI resistance pathways. Compounds 5a and 5f displayed remarkable antiproliferative, antimigratory and pro-apoptotic activities against both EGFR-TKI sensitive and resistant NSCLC cells, harbouringEGFR-T790M mutation. Remarkably, a synergistic effect was observed in resistant OR and AR NSCLC cells, and 5a and 5f enhanced the sensitivity of these resistant NSCLC cells to EGFR-TKIs by suppressing FAK.

Conclusion – Our results suggest that elevated FAK expression plays a key role in the development of acquired resistance to EGFR-TKI, and support the rational development of new FAK inhibitors, such as compounds 5a and 5f, as a novel therapeutic option for patients who develop resistance to novel EGFR-TKIs.

Glyco-Humanized polyclonal antibody, first in class immunotherapy against cancer

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In addition to these immune-check point modulating monoclonal antibodies (mAb), several approach such as antibody drug conjugate (ADC), chimeric antigen receptor T cells (CAR-T) and more recently bispecific antibodies are being actively explored and successfully introduced as innovative weapon in cancer treatment arsenal. However, for many cancers, there is still a lack of effective treatments, especially treatments that can result in long-term cancer-free survival and lower metastatic and relapse risks.

The emergence of cancer resistance could be minimized by drug combination or by multi-targeting of tumor cells. Anti-cancer polyclonal antibody (pAb) can target several tumors associated antigens simultaneously and would be more efficient than a conventional mAb only binds one specific target. Polyclonal antibody offers an opportunity to reduce cost in terms of development, production clinical trials, and regulatory reviews, compared to the single antibody-based agents developed in combination.

XON7, is a first in class glyco-humanized polyclonal antibody (GH-pAb), targeting multiple tumors associated antigens. It showed selective antitumor activities in vitro and in vivo models against several tumor types. in addition, it showed a positive synergy with Anti-PD-1, significant increase of anti-tumor response rate, increase macrophage M1 in tumor and decrease of metastasis when XON7 is associated with anti PD-1 in NSCLC in vivo CAM model.

XON7 pharmacokinetics and safety was also assessed in mice and marmoset after single and repeated IP and IV dosing up to highest tested dose of 60mg/kg. It is characterized by high tolerance profile and satisfactory exposure.

XON7 recently received EMA approval (France and Belgium) to start FIH escalating and extension clinical trial in solid tumor patients. FPI is expected in Nov2023.

In conclusion, Immunotherapy with XON7 represents an innovative promising therapeutic approach to fight against solid and hematological malignancies.

Using high-throughput drug screening to reveal therapeutic vulnerabilities in patient-derived diffuse midline glioma models

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The power of high-throughput drug screening comes from the ability to test a large number of molecules on multiple models in a very short time. Our approach is based on the use of a custom-made library of 110 drugs divided into four main groups (chemotherapies, epidrugs, targeted therapies and repositionable agents) with at least two compounds of the same therapeutic family. It is then possible to identify the most efficient compounds for a given type of tumor models.

This sensitivity to a particular therapeutic class enables rationally deducing and identifying the molecular vulnerabilities of a given cell model, depending on the target(s) of the hit compounds. This weakness can then be exploited to better understand tumor pathophysiology and also develop innovative therapies. In the current study, we applied our methodology to a fatal type of pediatric brain tumor called Diffuse Intrinsic Pontine Glioma (DIPG). Approximately 80% of DIPG have a recurrent somatic mutation on histone 3 (i.e., H3K27M), resulting in a major epigenetic dysregulation.

Recently, a compound called ONC-201 has demonstrated therapeutic benefit for patients with H3K27M mutated tumors and is currently being evaluated in several clinical trials. The objective of this project is to quickly identify new therapeutic vulnerabilities that can be targeted to increase the efficacy of ONC-201 and its derivatives ONC-206 and ONC-212. To achieve this, we performed a high-throughput drug screening using our 110-drug library in combination with ONC-201 or its derivatives in 8 different patient-derived primary models of diffuse midline glioma with distinct molecular features.

Amongst the 330 tested pairwise combinations, the association of ONC compounds with two types of metabolic inhibitors were identified as most potent drug combinations, highlighting potential therapeutic vulnerabilities. Using a matrix of 6x5 different drug concentrations, we then proceeded to validating the potency of the top drug combination in 5 different cellular models. By calculating the Bliss score, we were able to define the association as being highly synergistic. Thus, using high-throughput drug screening we uncovered therapeutic vulnerabilities that could be leveraged by combining ONC compounds with metabolic inhibitors, which could open novel therapeutic avenues for DIPG patients.

Keywords - diffuse intrinsic pontine glioma, drug screenings, therapeutic vulnerabilities, drug combinations

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Exploring new roles for XPF and SLX4 proteins in cancer cells

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DNA repair is crucial to maintain genomic integrity. In cancer cells, it is involved in the response to drugs interacting with DNA. Increased DNA repair activity or expression of associated proteins is related to decreased cell death upon exposure to such drugs. XPF and SLX4 are involved in various DNA repair mechanisms. However, the potential role of these proteins in different cellular processes, depending or not on DNA repair, is poorly described.

We developed cell models expressing or not XPF and/or SLX4 using lung (A549 and NCI-H1703) and gastrointestinal (HT-29) cancer cell lines and dual CRISPR/Cas9 technology. Validated models were compared for proliferation (CFSE), migration (wound healing), confluence, drug sensitivity, DNA integrity, reactive oxygen species, in vivo growth and by RNA sequencing.

Focusing results on the A549 model, we did not observe differences in proliferation or cell cycle distribution, whereas the ability to reach confluence was slightly higher in the S+/X+ and S-/X- and migration was decreased in the S+/X- and S-/X- models. An increased sensitivity to cisplatin was observed for all models, whereas this was less evident for S-/X- cells and mitomycine C. DNA double strand breaks in basic culture conditions, as determined by γH2AX staining, were more important in all modified models. S-/X- cells had a very high level of ROS both in control conditions and after exposure to 2 µM mitomycine C. Telomer maintenance was slightly modified in S-/X- cells as compared to other models.

When grown in scid mice after subcutaneous injection, S-/X+ cells had an increased growth whereas S+/X- and S-/X- cells had a slower growth than control cells. RNA sequencing identified approximately 6500 genes modified in S-/X- cells as compared to control cells, whereas this was around 1100 in the other models, including 688 genes modified in all three models. Preliminary analysis indicated modification in epithelial-to-mesenchymal transition state in the different models, and this was confirmed by Western blot analysis.

This work allowed the development and preliminary evaluation of original cell models suggesting an involvement in ROS metabolism, tumor growth and EMT. Future experiments include DNA repair capacities and validation of RNA sequencing data.

Keywords - DNA repair, cancer drugs, cell models, cell biology

Elevated Levels of Serum Thymidine Kinase 1 Predicts Poor Survival of Metastatic Prostate Cancer Patients Treated with Antiandrogens

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Introduction and Objectives - Prostate Specific Antigen (PSA) is a biomarker of pivotal importance for diagnosis and monitoring of prostate cancer (PCa). However, PSA is of limited value as a surrogate marker for overall survival. Thymidine kinase 1 (TK1) is an enzyme expressed by actively dividing cells. TK1 levels are elevated in the circulation of patients with various malignancies.

Patients and methods - sTK1 was examined in 261 patients divided in three retrospective cohorts with overall survival (OS) as the primary outcome. Cohort 1: 43 men diagnosed with de novo metastatic disease (mHSPC) managed with androgen-deprivation therapy (ADT); cohort 2: 102 patients with metastatic castration-resistant PCa (mCRPC) who received androgen receptor signaling inhibitors (ARSIs); cohort 3: 98 mCRPC patients treated with docetaxel (DOC) chemotherapy. sTK1 protein levels were determined by ELISA at prior to initiation of treatment.. sTK1 was correlated with clinicopathological parameters and clinical outcome using both univariable and multivariable Cox regression models.

Results - The median sTK1 value was 0.61 ug/L in the mHSPC patients (cohort 1). sTK1 levels above this value were associated with worse OS (age-adjusted hazard ratio (HR): 3.44 (1.64-7.19) $p > 0.0001$). The association remained significant in multivariate analyses including adjustment for Gleason score, PSA level, and clinical T-stage. Similarly, an association between elevated sTK1 levels and shorter OS was observed in mCRPC patients treated with androgen receptor signaling inhibitors (ARSIs) (cohort 2) (age-adjusted HR 2.53 (1.54-4.07), $p< 0.001$) using the same cut-off used in cohort 1. The association remained after adjustment for PSA levels and ECOG performance status. In the docetaxel-treated mCRPC group (cohort 3) the OS association did not reach significance (age-adjusted HR 1.62 (0.97-2.71) $p = 0.066$).

Conclusion - Serum TK1 predicted survival of both mHSPC and mCRPC patients, demonstrating additional predictive value over established clinical risk factors, including PSA. The strong association between sTK1 and disease progression in patients treated with antiandrogens raise the possibility that the proliferation-associated marker sTK1 can be used as a non-invasive test to identify patients that may benefit from treatment intensification.

Keywords - Prostate cancer, antiandrogens, docetaxel, prognostic marker

Towards precision dosing of oral anticancer drugs in clinical routine: Feasibility of a closed-loop Therapeutic Drug Monitoring process within the ON-TARGET study

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Introduction - Targeted oral anticancer drugs (tOADs) allow a selective tumour therapy, yet optimal treatment is often impaired by drug toxicity or lack of efficacy. Treatment could be substantially improved by dose individualisation. However, Therapeutic Drug Monitoring (TDM) strategies are still not widely used in oncology. Their successful implementation requires close interprofessional collaboration, infrastructure, and comprehensive TDM evaluation supporting clinical decision-making. The ON-TARGET study aimed to set up and explore the feasibility of a closed-loop TDM process in clinical practice in Germany.

Methods - The prospective, multi-centre, non-interventional study "ON-TARGET" evaluated TDM in patients with renal cell carcinoma treated with axitinib or cabozantinib. Venous and capillary blood samples were collected during routine check-up visits and drug concentrations analysed by validated bioanalytical LC-MS methods. Leveraging pharmacometric modelling, drug concentrations were evaluated with respect to a simulated expected individual concentration range and to published toxicity thresholds. A comprehensive risk assessment was reported back to the physician to support treatment decisions. Physicians were asked to rate the usefulness of the TDM evaluation choosing between "very helpful", "somewhat helpful", "not really helpful" and "not helpful at all".

Results - As of November 2023, a total of 323 venous and 174 capillary samples from 32 patients were collected in three pilot study centres. With 319 TDM evaluations reported back to physicians, 99% of the venous samples were evaluated. Approximately half of the evaluated axitinib (54%) and cabozantinib (56%) samples fell within the 90% prediction interval of the simulated expected individual concentration range, whereas 35% and 37% of samples were below, indicating further yet unknown impacting factors. Treating physicians perceived the TDM service positive: In 78% of the cases, the TDM was rated "very helpful"/"somewhat helpful".

Conclusion - The closed-loop TDM process was successfully implemented. First analysis of evaluated TDM concentrations revealed a high need for TDM of tOADs and high acceptance among participating physicians. To inform the design of the extension study, a comprehensive analysis of the real-world data will next be carried out, thus setting the foundation for model-informed precision dosing of tOADs in clinical routine. Interested centres are encouraged to contact the study group (www.fu-berlin.de/on-target).

Keywords - oral anticancer drugs, precision dosing, TDM

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An open source whole-body physiologically based pharmacokinetic and quantitative systems pharmacology approach supporting large molecule oncology drug development

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Many new oncology drug candidates are novel large molecule modalities (for example multispecific antibodies or antibody drug conjugates - ADCs) that provide numerous choices in their design. As the downside, the pharmacokinetic, pharmacodynamic properties and dose-effect relationships emerging from the convolution of multitude of mechanisms are hard to estimate in vivo. In fact, despite successful approvals, the clinical ADC landscape also features a number of failures1. Model-based approaches, especially the mechanistic ones - physiologically based pharmacokinetics (PBPK) and quantitative systems pharmacology (QSP) - could offer to predict factors leading to success and failure of these modalities early on during discovery and development.

To fill this gap, we present a PBPK (and QSP) modeling workflow for ADCs that is based on the open-source platform PK-Sim®/ MoBi® from the open systems pharmacology suite (OSP, open-systems-pharmacology.org). PK-Sim® already supports major mechanistic building blocks like antibody and payload ADME and tissue specific target expression patterns out of the box2,3. A layer of adaptation of that model in MoBi® allows to cover additional features of ADCs, including deconjugation pathways, tumor dynamics and “bystander effect” among others. With such a setup, key factors for clinical success, like target and antibody selection as well as payload and linker selection can be gauged in silico.

Thanks to the facile access (open source platform with graphical user interface) and the flexible implementation of extensions (multi-purpose QSP software) we propose that utilization of the OSP suite can foster widespread adoption of mechanistic modeling of ADC PK/PD and may pave the way towards holistic predictions of the therapeutic window of innovative ADC designs (see e.g., ref. 4).

Keywords - PBPK, QSP, OSP Suite, PK-Sim, MoBi, ADC, modeling

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Exploring a potential pro-metastatic biomarker in pancreatic cancer using the innovative organotypic liver model

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Introduction - Approximately 50-55% of pancreatic ductal adenocarcinoma (PDAC) patients present metastases at the time of diagnosis, with a disproportion for hepatic localization. One hypothesis for this high frequency centers around cell-cell communication, emphasizing the role of extracellular vesicles(EVs) in facilitating the transfer of information. We aim to assess whether EVs interaction with tumor cells change their invasive phenotype and to investigate new biomarkers and targeted therapies utilizing innovative organotypic-liver models. As biomarker of interest we chose the tyrosine-kinase-receptor AXL due to previous studies show its higher expression in blood and in tissue samples of PDAC-patients compared to those with chronic pancreatitis.

Materials and methods - Following the initial evaluation of the organotypic model using mouse liver, we proceeded to employ slides from human liver biopsies obtained through precision slicing with a vibratome.The relatively less aggressive/invasive primary cells PDAC1, were transduced with Firefly-luciferase, while EVs were obtained by ultracentrifugation from the most aggressive cells(PDAC3). Different conditions were defined by culturing liver slides with the more or less aggressive cells, with or without the PDAC3-derived EVs and with or without the addition of the AXL-inhibitor bemcetinib, to assess the influence of AXL on tumor growth. Cell growth was analyzed by bioluminescence assay(BLI). In addition, Mucin-1 was considered as a pancreatic marker, then used to quantify pancreatic-cell proliferation by RT-PCR and immunohistochemistry.

Results - We have achieved successful establishment of organotypic models using human liver biopsies and primary PDAC-cultures. Notably, RT-PCR analysis revealed a statistically significant increase in the expression of Mucin-1 when PDAC3-derived EVs were added($P<0.05$). This observation was corroborated by BLI. Moreover, RT-PCR analysis demonstrated a significantly higher expression of AXL when EVs were added, compared to those without EVs. Interestingly, the addition of bemcetinib resulted in a reduction of tumor areas.

Conclusion - Tumor-derived EVs increase the ability of tumor cells to proliferate in organotypic liver ex-vivo models, and AXL could be a potential 'pro-metastatic' biomarker in EVs, supporting future studies on AXL-inhibitors. These findings contribute to our understanding of the molecular dynamics influenced by PDAC-derived EVs and highlight the potential role of key-proteins in modulating tumor growth within this context.

Novel Imidazothiadiazoles Derivatives Targeting Focal Adhesion Kinase for PDAC Treatment: Synthesis, Characterization, and Mechanistic Insights

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Introduction - Pancreatic ductal adenocarcinoma (PDAC) has emerged as one of the most aggressive and resilient malignancies among various types of cancer.[1] Despite the efforts invested in developing more effective therapeutic chemotherapeutic regimens for PDAC, there is a clear need for new therapeutic strategies. Focal Adhesion Kinase (FAK), a pivotal non-receptor protein tyrosine kinase, is critical in oncogenic processes associated with cell adhesion, migration, proliferation, and survival. Its overexpression in PDAC underscores the potential for novel therapies targeting FAK, presenting a promising strategy for overcoming chemoresistance in PDAC.[2]

Materials and methods - This study introduces a novel series comprising twenty imidazo[2,1-b][1,3,4]thiadiazole derivatives. These compounds were systematically evaluated for their antiproliferative activity using the National Cancer Institute (NCI-60) panel and a comprehensive panel of PDAC models. The lead compound was subjected to detailed examination across various PDAC cell lines, including immortalized, primary, and gemcitabine-resistant clones. The assessment included IC50 determination, impact on cell migration, and spheroid shrinkage in vitro as well as in vivo studies. High-throughput kinase arrays were employed to decipher the molecular mechanism underlying the observed effects.

Results - Our compounds demonstrated potent antiproliferative activity with low micromolar IC50 values (1.04 – 6.90 μM) against a spectrum of PDAC cell lines. The most active compound significantly reduced cell migration and spheroid shrinkage. High-throughput kinase arrays unveiled substantial inhibition of the FAK signaling network, providing insights into cell cycle arrest at the G2/M phase, suppression of tumor cell invasion, and apoptosis induction. In vivo studies using PDAC xenograft mouse models confirmed its efficacy, markedly inhibiting tumor growth.

Conclusion - Our findings underscore the potent antitumor activity of imidazo[2,1-b][1,3,4]thiadiazole derivatives in both in vitro and in vivo PDAC models. These compounds show promise as primary candidates for developing FAK inhibitors, highlighting their potential as therapeutic candidates for PDAC treatment.

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Novel insights for DDB2 as a potent biomarker for the management of patients with PDAC

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Introduction - Pancreatic ductal adenocarcinoma (PDAC) is an aggressive disease with the lowest 5-year relative survival rate of 12 %. Despite the availability of various therapies, their effectiveness remains limited. Therefore, there is an urgent need to identify new effective biomarkers as well as new therapeutic targets. Damage specific DNA binding protein 2 (DDB2) is known to have dual roles in several cancers, acting as either an oncogene or a tumor suppressor gene depending on cancer localization. In this study we investigated the impact of DDB2 on epithelial-to-mesenchymal transition (EMT), migration, invasion and response to chemotherapy in PDAC cell models.

Materials and methods - DDB2 expression levels in PDAC patients were evaluated using publicly available data. The properties of DDB2 were investigated in two PDAC cell lines that were modified for DDB2 expression. DDB2 expression level was modified in DDB2-high T3M4 PDAC cells through a CRISPR/Cas9 knockdown (KO) (T3M4 DDB2-low) and in DDB2-low Capan-2 cells through a CRISPR activation (Capan-2 DDB2-high). The mRNA and protein levels of epithelial and mesenchymal markers were evaluated, as well as the mRNA levels of EMT transcription factors (EMT-TFs) SNAIL, ZEB1, and TWIST. Migration and invasive properties of PDAC cells were determined through scratch and transwell assays. The sensitivity to 5-fluorouracil, oxaliplatin, irinotecan and gemcitabine were also investigated.

Results - Clinical data analyses showed that PDAC patients had significantly decreased DDB2 expression levels. Furthermore, low DDB2 expression levels were associated with shorter disease-free survival. DDB2 expression level was also associated with the regulation of EMT. The downregulation of DDB2 in T3M4 DDB2-low cells increased the transcription of EMT-TFs, resulting in higher N-cadherin expression and lower E-cadherin levels. Migration and invasion properties were negatively correlated with DDB2 expression in both cell models. DDB2 sensitized Capan-2 DDB2-high cells to 5-FU, oxaliplatin and gemcitabine while it had no effect on T3M4 cells chemosensitivity.

Conclusion - Our study brings to light novel tumor suppressor effects of DDB2 in PDAC progression and response to chemotherapy. DDB2 may represent a promising biomarker to define prognosis and predict chemotherapy response in PDAC patients.

Keywords - Pancreatic cancer, DDB2, Chemotherapy, Biomarker

Evaluating the Effects of an Innovative Enzymatic Formulation on Pancreatic Cancer Cells Resistant to Gemcitabine

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The elevated mortality rate of pancreatic ductal adenocarcinoma (PDAC) is largely attributed to delayed diagnosis, early metastasis, and limited responsiveness to chemotherapy. Although chemotherapy remains a primary therapeutic approach against cancer, the prevalence of multiple resistance mechanisms poses a significant challenge, compromising clinical outcomes. Key contributors to chemoresistance include interactions among tumor cells, cancer stem cells (CSCs), and the fibrotic characteristics of the tumor microenvironment (TME), which hinder drug diffusion.

This project aims to identify novel adjuvant treatments that can alleviate chemoresistance. To achieve this, we generated resistant variants of PDAC cells in two laboratories if EORTC-PAMM members in Spain and in the Netherlands [1]. In these models we assessed the anti- tumor efficacy of a distinctive formulation comprising Trypsinogen and Chymotrypsinogen (PRP). This innovative treatment effectively suppressed cancer stem-like cells, hindering PDAC initiation and progression. In addition, a previous in vivo experiments confirmed PRP's ability to modulate the TME by reducing fibrotic content, a crucial characteristic in PDAC [2].

These studies revealed dose-dependent growth inhibition, reduced colony formation capacity, upregulated expression of tumor suppressor genes (e.g., E-cadherin), and decreased expression of epithelial-mesenchymal transition (EMT)-associated genes (e.g., Vimentin, Snail, and Slug). Additionally, PRP increased the expression of differentiation-associated markers, indicating a less malignant phenotype. It downregulated genes associated with gemcitabine metabolism and the TGF-beta signaling pathway. Furthermore, PRP enhanced both apoptotic and necrotic processes and increased gemcitabine susceptibility in chemoresistant cells.

Interestingly, previous studies suggested that this formulation's targets might impact the activation of cells responsible for immune response and anti-inflammatory effects [3]. In this context, our project also assessed the effect of pancreatic proenzymes on TME and cancer stem-like cells. Preliminary studies indicated a clear impact on cancer-activated fibroblasts, aligning with the previously reported anti-fibrosis effects [4].

Future studies will employ transcriptomics/proteomics approaches to identify biomarkers that hold potential for informing personalized treatment strategies for PDAC patients.

Keywords - Enzymatic Formulation, Pancreatic Cancer, Chemotherapy, Chemoresistance, Cancer Stem-like Cells

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PAMM
POSTERS

POSTER | N°1

Combined Inhibition of LDH-A and GLUT-1: A Promising Strategy for Treatment of Malignant Mesothelioma

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Objectives - Malignant mesothelioma (MM) is known for high expression of lactate dehydrogenase A (LDH-A) and glucose transporter type 1 (GLUT-1) which contributes to its aggressive nature. We used LDH-A (NHI-2) and GLUT-1 (PGL14) inhibitors and analyzed their effect on cell growth, nucleotide concentrations, apoptosis and migration.

Methods - The intracellular nucleotide concentrations were measured using RP-HPLC and migration was analyzed using a Wound-Healing assay in pleural (H2052, H2452) and peritoneal (STO, MESO-II) MM cell lines, after 24h treatment with NHI-2 and PGL14. Apoptosis was analyzed with Annexin V-FITC assay (48h incubation). The 3-dimensional (3D) spheroids were treated every two days, and evaluated by morphology and bioluminescence to estimate their size in proportion to the cell number.

Results - Changes in nucleotides balance after treatment were mainly observed in the H2052 cell line, with a decrease in ATP/ADP ratio from 21.2 to 6.8 and 9.1 and an increase in NADH/NAD+ ratio from 0.1 to 0.3 and 0.5, after PGL14 and NHI-2 treatment, respectively. PGL14 and NHI-2 decreased cell migration at 20h in the H2452 cell line to 67.9% and 88.6%, respectively and to 78.4% for the combination, as compared to the control. The apoptosis rate was increased by 4.5 in the H2052 cell line after incubation with PGL14 (2xIC50) and by 6.5 after incubation with PGL14/NHI-2 combination (2xIC50). In the MESO-II cell line, 2.3-fold induction of apoptosis was observed only after combination treatment (2xIC50). NHI-2 decreased the size of the STO spheroids to 56.7% after six days, while the number of spheroid-forming cells decreased from 1.3x105 in the control group to 9.7 x104 and 7.1x104 [RLU/s] after the NHI-2 and PGL14/NHI-2 treatment, respectively, with visible disintegration of spheroids in the PGL14 and combination groups.

Conclusions - Targeting LDH-A and GLUT-1 results in a disrupted nucleotide balance, impaired cell migration, stimulated apoptosis, and compromised 3D structure formation in malignant mesothelioma cells. Combined inhibition of these two targets, particularly in pleural mesothelioma increases their anti-cancer effect, making this approach highly promising for overcoming the drug resistance commonly associated with this disease.

Keywords - drug development, cancer metabolism, mesothelioma

POSTER | N°2

Extension of a bioanalytical method for the therapeutic drug monitoring of Axitinib in plasma and capillary blood to a generic method for oral oncology drugs

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Introduction - The number of prescriptions for oral oncological drugs has increased exponentially in recent years. The drug concentration in the blood depends on many factors. Therapeutic drug monitoring (TDM) can help to avoid toxicity or detect underdoses. TDM for oral oncological drugs is not established in Germany. One reason for this is the lack of infrastructure for standardised measurement and evaluation of drug concentrations. As part of the ON-TARGET study, a network for routine TDM of Axitinib and Cabozantinib has been established in three study centres throughout Germany since the beginning of 2021. The aim of this project was to extend the bioanalytical method used in the ON-TARGET study for the determination of Axitinib from capillary blood by volumetric absorptive microsampling (VAMS) to a generic bioanalytical method for the detection of various oral oncological drugs.

Material and methods - For this purpose, an LC-MS method with on-line solid phase extraction (SPE) was developed for the simultaneous detection of twelve tyrosine kinase inhibitors (Axitinib, Cabozantinib, Ruxolitinib, Dabrafenib, Nilotinib, Bosutinib, Osimertinib, Trametinib, Afatinib, Ibrutinib, Pazopanib and Neratinib), as well as for Tamoxifen and its three main metabolites (4-Hydroxytamoxifen, N-Desmethyltamoxifen and Z-Endoxifen) from capillary blood using VAMS technology.

Results - The newly developed method has been fully validated according to EMA and FDA guidelines for plasma and capillary blood. Cross-validation was performed for Axitinib and Cabozantinib using a routinely used method from the Pharmaceutical Institute in Würzburg, Germany (< 2% deviation for AXI/CAB). Furthermore, a correlation factor of 0.71 was determined for Axitinib between drug concentrations in plasma and capillary blood from 80 venous and capillary sample pairs collected over a period of 2.5 years. The limits of quantification of the 16 substances were between 0.5 and 2 µg/L. All tyrosine kinase inhibitors capillary blood samples are stable for 28 days at room temperature (RT), at +5 °C or at -21 °C, with the exception of Osimertinib which is stable for 14 days at RT.

Conclusions - A generic, reliable and resource-saving bioanalytical method using single quadrupole LC-MS has been developed for the TDM of various oral oncological drugs.

Keywords - Therapeutic drug monitoring (TDM), VAMS technology, tyrosine kinase inhibitors, Tamoxifen and metabolites, LC-MS

POSTER | N°3

Targeting the serotonergic signaling pathway to develop novel therapeutic options for medulloblastoma

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Medulloblastomas (MB) are embryonal tumors of the cerebellum and the most common childhood brain cancers.

Despite major advances in their therapeutic management - combining surgery, chemotherapy, and radiotherapy - 30% of children remain without effective treatment and survivors frequently have long-lasting side effects. To broaden the range of clinically effective and better-tolerated therapeutic options, our strategy is to apply drug repurposing, which consists in using already approved drugs in new indications.

There is increasing evidence that serotonin receptor (5-HTR) overexpression correlates with tumor progression in various cancers. To determine whether pharmacologically targeting 5-HTR could represent a novel therapeutic strategy for MB, we first screened a library of 5-HTR antagonists in human MB cell lines. A drug combination screen was then performed using a custom-made library of 110 approved drugs. The synergistic effects of the selected drug combinations were evaluated using 2D MB cell cultures, 3D tumor spheroids and ex vivo organotypic cerebellar model, based on the grafting of 3D MB spheroids into thick sections of healthy cerebellum.

Thanks to the monotherapy drug screening, we identified metergoline, an approved drug mainly used in the treatment of hyperprolactinemia-related disorders and migraines, which significantly reduces MB survival in 2D cultures and 3D spheroids. The combination screen revealed a highly synergistic interaction between metergoline and the antifungal agent Itraconazole. We next confirmed the benefits of this promising therapeutic association in an innovative ex vivo organotypic cerebellar model.

Future experiments will investigate 1) the combination between metergoline and chemotherapy and radiotherapy protocols currently used to treat MB patients 2) the safety and efficacy of the new treatments in PDX models in vivo, and 3) the protein partners and molecular pathways involved in the mechanism of action of metergoline, alone and in combination, in MB cells using chemoproteomics.

Keywords - Medulloblastoma, Oncopharmacology, Drug repurposing.

POSTER | N°4

Longitudinal follow-up of circulating cell-free DNA in patients operated for a pancreatic ductal adenocarcinoma and enrolled in the PANCREAS-CGE cohort.

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Prognosis of pancreatic cancer is one of the worst of all solid tumors. Survival is drastically worsened due to the presence of a micrometastastic disease present in the majority of patients who undergo surgery. Recently, the role of circulating cell free (cfDNA) in disease monitoring, response to treatment and prognosis has been demonstrated.

However, no biomarker has yet been validated in pancreatic cancer. Using the PANCREAS-CGE cohort of patients with pancreatic ductal adenocarcinoma who underwent surgery, the aim is to characterize the tumors of the first 15 patients and then evaluate interest of cfDNA monitoring.

Genomic DNA from 15 FFPE samples from the PANCREAS-CGE cohort were extracted using the AllPrep® DNA/RNA FFPE kit (Qiagen). The cfDNA from 60 plasmas from these 15 patients, collected at 4 different times, was extracted using the QIAmp Circulating Nucleic Acid Kit (Qiagen). Extraction yield and quality were assessed using the Qubit fluorometer (ThermoFisher Scientific) and Fragment Analyzer (Agilent). The 679-genes SureSelect Cancer CGP panel (Agilent) was used to sequence DNA extracted from tissues on the NextSeq 2000® (Illumina).

All samples were analyzed. Overall, 67% of patients had a KRAS mutation, with the G12D mutation found in 27% of cases. A pathogenic BRCA2 mutation was detected in one of the 15 patients. Time-dependent follow-up of cfDNA concentrations showed that a cfDNA concentration greater than 1.05 ng/µL ($p = 0.008$; $HR = 0.09$) after surgery was associated with a poor prognosis. Conversely, a low cfDNA concentration at diagnosis and after adjuvant chemotherapy indicates a low risk of recurrence.

In conclusion, the profiles of the 15 selected patients are similar to those described in the literature. No rare mutations were identified. The cfDNA monitoring mirrors fluctuations associated with events such as surgery or diseases progression, confirming the relevance of longitudinal monitoring and residual disease detection using cfDNA

Keywords - Pancreatic cancer, liquid biopsy, cfDNA

POSTER | N°5

Inhibition of Phosphatyidlinositol-4 Kinase B (PI4KB) as a potential treatment for Multiple Myeloma

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Introduction - Despite recent therapeutic advances, Multiple Myeloma (MM) poses significant challenges due to high relapse rates and limited options for refractory cases. This study identifies Phosphatidylinositol 4-Kinase Beta (PI4KB) as a potential therapeutic target for MM. Given its crucial role in regulating Golgi protein trafficking, PI4KB becomes particularly relevant for malignancies such as MM characterized by excessive protein synthesis and secretion pathways.

Materials & Methods - We evaluated PI4KB sensitivity using a panel of 10 MM cell lines and a library of small PI4KB inhibitors. IC50 values were determined through MTT assays, and cell viability was assessed using AO/PI staining and cell counting with Nexcelom K2. Additionally, an in vivo zebrafish embryo model of MM was employed to gauge in vivo activity.

Results - Out of the MM cells tested, a subset (3/10) exhibited sensitivity to PI4KB inhibitors with IC50 values below 50 nM, while the remaining cell lines showed no sensitivity within the tested range. Inhibition of PI4KB induced proteotoxic stress and cell death in the sensitive cells as confirmed by a time dependent accumulation of polyubiquitin and HSP70 expression and decreased AO/PI viability staining. In the in vivo zebrafish MM model, PI4KB inhibition significantly reduced tumor burden and cell dissemination. Interestingly, sensitive cell lines were characterized by relatively high expression levels of PI4KB as determined by western blotting, potentially serving as a sensitivity marker.

Conclusion - PI4KB inhibitors show promise for future investigations, providing insights into potential therapeutic strategies for MM, particularly those centered on disrupting protein synthesis and secretion pathways. Our study underscores the potential of targeting PI4KB in MM, prompting further exploration of the molecular mechanisms governing sensitivity.

Keywords - PI4KB, Multiple Myeloma

POSTER | N°6

High Dose Methotrexate in Hematologic Malignancies: covariates of interest to prevent toxicites

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Introduction and Objectives - Methotrexate (MTX) has various indications, particularly at high doses (>500mg/m²) in haematological malignancies (HM). However, acute kidney injury (AKI) induced by high-dose MTX is a known issue, with an incidence ranging from 2% to 10% of high-dose MTX courses. Despite its long-standing use, there is insufficient evidence to predict this toxicity. The primary objective of this study is to identify covariates associated with delayed elimination. We will investigate whether delayed elimination is associated with hematological toxicities and its impact on patient survival. Additionally, we will examine covariates that affect the time to onset of aplasia and overall survival, as well as those that affect patient survival.

Materials and Methods - Data were collected on 69 patients who received MTX-HD for HM between 2013 and 2020. Clinical, paraclinical, pharmacokinetic, therapeutic, and iatrogenic covariates were collected using APHM software. Survival data were collected up to 2022. Statistical analysis was performed using R software.

Results - 14 cases of delayed elimination were observed, with a mean methotrexate level of 2.4 ±2.8µM at 48 hours. Of the covariates tested, 4 had a statistically significant effect on the occurrence of delayed elimination. When focusing on delayed elimination during the first course of treatment, 5 covariates had a significant effect on its occurrence. Additionally, 6 covariates had a significant effect on the time to onset of aplasia. Median overall survival was not reached in our cohort. No statistically significant association was found between delayed elimination and short- or long-term mortality, consistent with literature. Although patients with a dose reduction showed a trend towards higher mortality, this association was not statistically significant.

Discussion / Conclusion - A low rate of delayed elimination was observed, which may be attributed to the strict usage recommendations. Covariates that significantly impacted the occurrence of delayed elimination and aplasia were related to pharmaceutical analysis, highlighting the importance of the pharmacist's role in the care team. A multicenter study could validate the covariates isolated in this research, standardize MTX-HD administration practices, and enhance the safety of managing patients treated with MTX-HD.

Keywords - methotrexate, clinical oncology, toxicity

POSTER | N°7

The role of circulating tumor cell-expressed PSA mRNA and PSMA mRNA in metastatic hormone-sensitive prostate cancer

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Introduction and Objectives - Metastatic hormone-sensitive prostate cancer (mHSPC) ultimately progress to metastatic castration-resistant prostate cancer (mCRPC). There is no marker yet to predict progress. We investigate the role of circulating tumor cell (CTC)-based PSA mRNA or PSMA mRNA in mHSPC.

Materials & Methods - 44 patients with mHSPC were prospectively enrolled for CTC collection by microfluidic magnetophoresis. The expression of six gene (PSMA, PSA, AR, AR-V7, EpCAM, KRT 19) from CTCs were measured by droplet digital PCR. The association between the six gene expression levels and time to progression to mCRPC (mCRPC-progression free survival rate) was investigated using Kaplan-Meier and cox-analysis.

Results - CTC detection rates were 95.3% for mHSPC and samples yielded an average of 11.2 CTCs/mL. mCRPC progression occurred in 29.5% (13/44). The median follow-up was 11.5 (2.0–19.0) months. Both PSMA and PSA expressions were higher in patients with progression to mCRPC. The detection rates for PSMA and PSA expression levels were also higher in patients with mHSPC who progressed to mCRPC than those who did not (100% (13/13) vs. 70.9% (22/31) and 76.9% (10/13) vs. 67.7% (21/31)). Kaplan–Meier analysis further confirmed the difference in 12-month mCRPC-PFS between patients with low and high PSMA mRNA expression (66.7% vs. 16.0%, p = 0.019). Similar findings were found for PSA mRNA (58.8% for those with low expression vs. 18.6% with high expression, p = 0.026). However, the expression of other transcripts, namely, AR, AR-V7, KRT19, and EpCAM, and the CTC count showed no significant correlation with mCRPC-PFS. The Cox model also showed a poorer mCRPC-PFS rate for patients with high expressions of PSMA mRNA and PSA mRNA than for patients with low expression (PSMA mRNA, HR: 3.83, 95% CI: 2.21–12.97, p = 0.031; PSA mRNA, HR: 3.47, 95% CI: 1.07–11.23, p = 0.038).

Conclusion - The quantified CTC- expressed CTC- based PSMA mRNA and PSA mRNA level can serve as a highly useful biomarker for predicting rapid progression of mHSPC to mCPRC.

Keywords - Prostate cancer, Circulating tumor cells, Prostate specific antigen, Prostate specific membrane antigen

POSTER | N°8

Strong antiproliferative potential of copper(II) complexes with paullone ligands in colorectal tumor cells

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Introduction - There is a growing interest in the rational design of multi-targeted metal-based drugs as potential alternative to platinum anticancer chemotherapeutics, in the efforts to improve drug efficacy and diminish resistance and toxicity. Organometallic complexes of ruthenium (II) and copper(II) are extensively investigated due to their ability to exert multi-targeted mode of action and to carry/deliver bioactive ligands.

Here we report the biological analysis of several ruthenium (II) and copper (II) complexes with ligands of structural formula 5,6,7,9-tetrahydro-8H-indolo[3,2-e]benzazocin-8-one (HL), that incorporate an 8-membered azocine ring and belong to paullone family of compounds as potent inhibitors of cyclin-dependent kinases (CDKs).

Materials and Methods - Antiproliferative activity was investigated in human tumor cell lines (LS-174, HCT 116, MDA-MB-361, A549 and PC-3) and non-tumor cells MRC-5, by MTT assay and Flow cytometry studies.

Results - MTT assay yielded IC50 values in low micromolar range for copper (II) complex and deriving ligands and suggested selective sensitivity of colon cancer cells HCT116 and LS-174 to their action, comparing to cisplatin (IC50 =13.6 μM). Particularly complex 5, [CuIICl(HL5)]+, and its HL5 ligand (carrying substituents R1 = Br, R2 = CH3) were the most cytotoxic in HCT116 (IC50 = 0.8 ± 0.1; 0.9 ± 0.2 μM respectively) and showed strong impact on cell cycle progression, apoptosis induction and as well notable inhibitory potential in vitro against several kinases including Cdk2 and Cdk5 (IC50 = 1.4 - 6.1 μM).

Prominent cytotoxicity of copper complexes encouraged us to develop novel series of compounds [CuIICl(HL)]+ by structural optimisations. MTT analysis of novel compounds revealed copper complex 8 of structural formula [CuIICl(HL8)]+, and its ligand HL8, (carrying substituents R1 = H; R2 = CH3) with cytoselective activity in HCT116 cells (IC50 = 0.9±0.2; 0.76±0.01 respectively). Flow cytometry showed potency of 8 and HL8 to arrest cell cycle in G1 in time and concentration dependent manner (up to 68.73 % comparing to control 51.68 %) and induce sub-G1.

Conclusion - Results all together suggested that complexation of indolo[3,2-e]benzazocines ligands to copper may result in enhanced (synergistic) and multitargeted mode of action.

Keywords - copper (II)complexes, HCT116, paullone ligands, MTT, cell cycle, CDK inhibitor

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POSTER | N°9

Analysis of response to nutlin-3a of KRAS mutant non-small cell lung cancer xenografts model through spatial transcriptomic profiling

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Introduction and Objectives - KRAS mutation (MT) in non-small cell lung cancer (NSCLC) serves aggressiveness risk in tumor. Until 2021, targeting KRAS directly or indirectly was challenging, and it was considered undruggable. The approval of sotorasib (Lumakras) as the first KRAS-G12C inhibitor marked a breakthrough. However, its limited efficacy for non-G12C KRAS mutations and the inevitable development of resistance emphasize the need for alternative therapeutic strategies. Spatial transcriptomics, which is the method for exploring the spatial distribution of mRNA molecules, enables us to reveal the tumor heterogeneity of differential drug response in a tumor lesion. A previously conducted RNA-sequencing analysis compared control and nutlin-3a treated samples in KRAS MT and WT NSCLC cells. We identified the HBP related metabolic pathway as significantly enrichment only in KRAS MT/p53 WT NSCLC cells. To expand on these findings, we investigated the cellular composition and interaction between spatial gene expression and the cancer microenvironment response to nutlin-3a treatment using a spatial transcriptomics platform (GeoMX) in the KRAS MT NSCLC xenograft model.

Materials & Methods - The GeoMx platform was used to analyze spatial transcriptomic information on cells. KRAS MT NSCLC cell-derived xenograft models were compared before and after nutlin-3a treatment as well as immune-rich and immune-poor regions after nutlin-3a treatment. Integration and comparison of RNA sequencing data and spatial gene expression data were performed. Enrichment of Gene ontology (GO): molecular functions (MF), GO: Biological processes (BP), GO: Cellular Components (CC), as well as KEGG pathway enrichment analysis, were conducted.

Results and Discussion - GO and KEGG analysis showed enrichment in the metabolic pathway, extracellular matrix (ECM), and inflammatory response in KRAS MT/p53 WT NSCLC cells upon nutlin-3a treatment. These findings suggest that nutlin-3a induced an interaction between metabolic changes and the ECM, accompanied by an inflammatory response. Further analysis is in pending.

Conclusion - This study can investigate the difference of heterogeneity and interaction of cancer microenvironment in KRAS MT NSCLC cells response to nutlin-3a by spatial transcriptomics.

Keywords - Spatial Sequencing, NSCLC, KRAS

POSTER | N°10

MiR-195-5p-enriched tumor-derived extracellular vesicles as an adjuvant strategy for melanoma treatment in 3D model

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Introduction - Melanoma is a highly aggressive and resistant form of skin cancer. To date, advanced-stage patients often relapse to standard-of-care approaches, prompting the search for innovative therapies. Recently, Extracellular Vesicles (EVs) have emerged as promising drug carriers due to their innate cargo delivery capability.

Particularly, modulation of tumor derived EVs cargo, with enrichment of antitumoral molecules, holds great promise when used as adjuvants. We recently showed that melanoma-derived EVs carrying the tumor suppressor miRNA, miR-195-5p, restrain cells proliferation and increase targeted therapy response, through miR-195-5p/BCL2-L1 axis. As target prediction analysis showed that this miRNA may also regulate DNA damage response (DDR)-related genes, we aimed to analyze the effect of miR-195-5p-loaded EVs in 3D tumor growth and response to radiotherapy (RT), which is often used in unresectable cases and as palliative care in melanoma patients.

Methods - EVs were isolated from the conditioned media of SKMel-28, UACC-62 and WM-1366 cells using differential ultracentrifugation followed by size exclusion chromatography and characterized according to MISEV2018 guidelines. Nanoparticle Tracking Analysis was used for EVs quantification, and the presence of common markers was analyzed by western blot. MiR-195-5p was loaded into isolated vesicles by electroporation, which was confirmed by RT-qPCR post RNAse A treatment. 3D tumor spheroids were observed 72 hours after plating 1.000 cells/well in 96w plates coated with 1% agarose layer. Three days-old spheroids were submitted to fractionated RT for 5 days (5 x 3Gy) and EVs were added 1h prior to the last three doses.

Results - RT-qPCR showed an increased expression of genes related to DDR, stemness and RT resistance after the fractionated treatment, including ALDH1, ATM, OCT4, SOX2 and PDL-1, as well as the lncRNAs LINC00473 and LINC00511. Although melanoma spheroids presented three times lower EVs uptake capacity compared to cells in monolayers, treatment with miR-195-loaded vesicles suppressed the up-regulation of these RT-induced genes, and reduced spheroids proliferation and clonogenic capacity, resulting in 15% to 50% reduction in the number of viable cells and 20% smaller spheroids diameter upon re-growth challenge.

Conclusion - mir-195-EVs can enhance the efficacy of RT when used as an adjuvant in melanoma treatment. Funding: FAPESP (2021/13681-2).

Keywords - Melanoma, Extracellular Vesicles, MiR-195-5p r

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POSTER | N°11

Relevance of neoadjuvant chemotherapy in patients with locally advanced cervical cancer: A retrospective cohort study at the Joliot Curie Institute of Dakar

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Background - Cervical cancer is a public health problem. Worldwide, it is the second most common cancer in women. GLOBOCAN reported an annual incidence of 604 127 new cases and 341 831 deaths per year worldwide due to cervical cancer. In Senegal, it is the most common malignancy among women, responsible for a burden of 1 937 new cases and 1 312 deaths in 2020. In Senegal, as in other all low-income countries, availability of radiation therapy constitutes a real impediment for oncologists and cervical cancer patients.

Patients & Methods - This study is a retrospective cohort study conducted at the Joliot Curie Institute of Dakar over a three-year period from 2017 to 2020. Study included 383 patients with cervical cancer and aimed to describe epidemiological, clinical and therapeutic patterns patterns of cervical cancer and furthermore to assess clinical and histological responses to neoadjuvant chemotherapy as well as chemotherapy-related toxicity.

Results - The patients were aged 27 to 87 years with a mean age of 53.69 years; the 50–59 age group was the most represented. Clinically, metrorrhagia represented the major circumstance of discovery (84% of cases) and tumor size was in most cases greater than 4 cm. Stages III and IVA were predominant (76%). The most used chemotherapy protocol was carboplatin and paclitaxel in 97% of cases. An objective response was described in 64% of patients who underwent neoadjuvant chemotherapy and 55% of patients who underwent concurrent chemoradiotherapy. Overall survival was 77%. O and KEGG analysis showed enrichment in the metabolic pathway, extracellular matrix (ECM), and inflammatory response in KRAS MT/p53 WT NSCLC cells upon nutlin-3a treatment. These findings suggest that nutlin-3a induced an interaction between metabolic changes and the ECM, accompanied by an inflammatory response. Further analysis is in pending.

Conclusion - The absence or scarcity of radiation therapy in our low-income countries means that neoadjuvant chemotherapy can constitute a relevant therapeutic substitute. Multicenter prospective studies should be further carried out in order to better investigate this therapeutic strategy.

Keywords - Neoadjuvant chemotherapy, Cervical cancer, Radiochemotherapy, Surgery

References - Globocan 2020, Metaanalyse cochrane 2005, cancero.net 2023 santor Edition

POSTER | N°12

Unlocking New Therapeutic Synergystic Strategies: Hsp90 Inhibitor 17-AAG and TRAIL in Non-Small Cell Lung Cancer

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Investigate the role of 17-AAG in enhancing the apoptosis-inducing effect of TRAIL in NSCLC cells relatively sensitive and resistant to TRAIL (i.e., H460 and A549)

Keywords - TRAIL, 17-AAG, HSP90, NSCLC, synergy, apoptosis

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POSTER | N°13

Cellular response of pleuric mesothelioma cells to paclitaxel and testing of extracellular vesicles as potential drug delivery tools

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Introduction - Pleural mesothelioma (PM) is a rare aggressive neoplastic disease with poor patient survival due to the lack of early symptoms and to the absence of effective therapeutic strategies. PM is heterogeneous and lacks actionable alterations. Thus, the aim of this study was to examine cellular response of PM cells to paclitaxel and to examine the drug delivery capability of extracellular vesicles (EVs) derived from mesenchymal stromal cells.

Materials & Methods - Cell sensitivity to paclitaxel of MSTO-211H and H28 cell lines was assessed by cell growth inhibition assays, protein levels by antibody arrays, western blotting and cytofluorimetric analysis. Concentration/size/morphology and identity of EVs prepared by ultracentrifugation were defined by confocal microscopy/TEM/nanosight and flow cytometry.

Results - Compared to H28, MSTO-211H cells resulted more sensitive to paclitaxel. Paclitaxel was found to modulate the levels of apoptotic proteins. Antibody array analyses indicated a marked modulation of pro-apoptotic proteins. At earlier time points (i.e., 24 h exposure), an increase of factors such as Bax, TRAIL-R1/DR4, TRAIL-R2/DR5 and TNFRI as well as the adaptor protein FADD and p21/CIP1 was found. At later time points (i.e., 48 h drug exposure), increased levels of CD95/FAS were observed. The modulation of death receptors was validated by flow cytometry and western blot that confirmed modulation of CD95/FAS and of Bax, FADD, and p21/CIP1. Cell-cycle perturbation analyses indicated paclitaxel-induced G2/M accumulation of cells and decreased G1 cell levels 48 h after drug exposure. Annexin V-binding assays indicated that apoptosis was concentration- and exposure time-dependent. EVs showed a size distribution of 171,46±32,93 nm, high expression of both the typical EV markers CD9, CD81 and CD63 and mesenchymal markers CD29, CD44, CD146 and CD105. Confocal microscopy assay showed that fluorescent-labeled EVs accumulated in the perinuclear area of MSTO-211H cells.

Conclusion - Taken together, our results indicate that paclitaxel induces a marked apoptotic effect in MSTO-211H cells, and that EVs can accumulate in pleural mesothelioma cells. These findings support the relevance of preclinical models to develop new therapeutic strategies and suggest that EVs may be exploited to deliver chemotherapeutic agents to tumor cells.

Keywords - Pleural mesothelioma; Drug delivery; Paclitaxel; Apoptosis; Extracellular vesicles

POSTER | N°14

Understanding the role of MYC regulated LncRNA, MYRCL, in Lung cancer

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Lung cancer constitutes approximately 40% of all lung cancer cases, with a significant portion of patients receiving diagnoses at advanced stages, leading to treatments involving surgery and chemotherapy. The identification of biomarkers and therapeutic targets is crucial for improving outcomes, and Long non-coding RNAs (LncRNAs) present themselves as promising candidates due to their high specificity for cancer.

In our research, we delved into RNA-sequencing data to assess the MYC activity in lung cancer patients, encompassing both Non-Small Cell Lung Cancer (NSCLC) and Small Cell Lung Cancer (SCLC). Employing the MYC score, we unveiled a novel LncRNA, termed MYRCL, as a direct target of MYC. Rigorous validation through RNA quantification and Chromatin Immunoprecipitation (ChIP) experiments solidified the evidence of MYC-mediated regulation of the MYRCL gene. Functional analyses demonstrated the indispensability of MYRCL for cell proliferation. Knocking down MYRCL resulted in inhibited cell growth, induced senescence, and led to the accumulation of DNA damage, emphasizing the role of MYRCL in maintaining the integrity of the DNA damage repair machinery. Further investigations revealed that MYRCL interacts with HNRNPL, enhancing its stability.

Depletion of MYRCL compromised HNRNPL stability, consequently impairing the DNA repair pathway and causing an accumulation of DNA damages. Intriguingly, the adverse effects of MYRCL depletion were rescued by the overexpression of HNRNPL, highlighting the intricate interplay between these molecular components. In a clinical context, our study leveraged multiple datasets to establish MYRCL as a valuable prognostic and diagnostic marker for lung cancer.

The comprehensive understanding of MYRCL's involvement in cellular processes and its clinical relevance underscores its potential as a target for therapeutic interventions and emphasizes the importance of exploring LncRNAs in the intricate landscape of lung cancer biology.

Keywords - Lung cancer, LncRNA, MYC

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POSTER | N°15

Follow-up of endocrine therapy resistance mutations using liquid biopsy and FFPE samples in advanced breast cancer patients: CICLADES study

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Endocrine therapy (ET) is considered as the mainstay in hormonal breast cancer (BC) management. They can be combined with cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) but are subject to resistance. The ESR1 gene is one of the main actors of ET resistance, other genes such as PIK3CA and AKT1 are involved in CDK4/6i resistance. The aim of the CICLADES study is to associate baseline levels of these mutations in Formalin-Fixed Paraffin-Embedded (FFPE) tissue samples with a liquid biopsy follow-up in advanced or metastatic BC patients treated with aromatase inhibitors and CDK4/6i. This will allow us to identify resistance-associated genomic signatures and monitor clonal evolution.

Twenty FFPE samples selected in the CICLADES cohort were qualified, selected, macrodissected and DNA was extracted with the AllPrep® DNA/RNA FFPE kit (Qiagen). Sixteen plasma samples were selected in the same cohort and cell-free DNA (cfDNA) was extracted. Sequencing was performed by Hybridization Capture-based Target Enrichment with a 516-gene panel and a 33-gene bespoken panel for FFPE and plasma samples respectively. The bioinformatic analysis detected single nucleotide variants, indels and copy number variations. Highly mutated genes and genomic signatures as described by Alexandrov et al, 2020 were identified in FFPE samples and specific mutations in genes of interest were detected in both types of samples.

Nineteen DNA from FFPE samples reached the quality criteria to be sequenced. Several genes involved in the PI3-Kinase pathway showed mutations in some samples, however no other relevant mutations were found at baseline. Three genomic signatures were detected, corresponding to validated COSMIC (Catalogue of Somatic Mutations in Cancer) signatures 5, 6 and 30, associated with DNA damage repair and deficiency of the base excision repair system. Sequencing of cfDNA showed several mutations of interest, notably in the PIK3CA and CCND2 genes.

The signatures detected in our cohort matched with those already described in BC. The mutations in the PI3-K pathway found at baseline in FFPE samples seem to appear in cfDNA as well, but further analysis is required. With the knowledge gathered here we are provided with a clear baseline mutational landscape for the profiling of the rest of the CICLADES cohort.

Keywords - Breast cancer, liquid biopsy, cell-free DNA, genomic signatures

POSTER | N°16

Synergistic activity of ezurpimtrostat, a palmitoyl-protein thioesterase 1 (PPT1) inhibitor, in combination with MEKi in cholangiocarcinoma in vitro models

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Background - MEK inhibitors (MEKi) exhibit limited activity in patients with KRAS-mutated cholangiocarcinoma (CCA). Resistance has been associated with increased autophagy, suggesting that MEKi combination with an anti-autophagy drug could be a potentially effective therapeutic strategy to improve the survival of patients. Ezurpimtrostat is a late-stage autophagy inhibitor that presents potent anti-tumoral activity in various CCA preclinical models and a favorable safety profile with preliminary signals of activity (NCT03316222). The current study aimed to evaluate the anti-tumoral potential of the combination of ezurpimtrostat plus MEKi in a panel of intrahepatic CCA (iCCA) cell lines.

Methods - The effect of ezurpimtrostat, first alone and then in combination with MEKi (trametinib, cobimetinib), on cell viability was evaluated by treating HuCCT-1, RBE, and OZ KRAS-mutated cells for 72 hours to obtain the half-maximal inhibitory concentration (IC50) values. Synergy scoring was analyzed with CrownSyn, MacSynergy, or SynergyFinder using Bliss model. All conditions were tested in triplicate and three independent experiments at least were performed.

Results - The IC50 of ezurpimtrostat was 2.04 ± 0.07 µM (mean ± SD), 1.31 ± 0.18 µM, and 0.93 ± 0.16 µM in the HuCCT-1, RBE, and OZ cells, respectively, demonstrating a potent anti-tumoral effect. We next assessed the combination of ezurpimtrostat with MEKi, and showed synergy in all cell lines except OZ, which exhibited additivity (CrownSyn). Focusing on HuCCT-1, we observed a synergy score of 62.78 ± 23.90 (trametinib) and of 90.22 ± 8.40 (cobimetinib), considered to be a significant moderate synergy score using MacSynergy software. The best synergistic scores were found at an ezurpimtrostat concentration of 1.77 ± 0.82 µM (with trametinib) and 1.63 ± 0.36 µM (with cobimetinib), similar to the individual compound IC50 value.

Conclusion - Our study highlights that ezurpimtrostat in combination with MEKi presents a synergistic activity on the reduction of iCCA cell viability. These promising results support the ongoing phase 1b/2a study that aims to evaluate the safety and efficacy of ezurpimtrostat in combination with trametinib in patients with advanced KRAS-mutated CCA after failure of standard-of-care first line therapy (NCT05874414).

POSTER | N°17

Development of fast and sensitive isolation of PDAC-specific extracellular vesicles media

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Introduction - Extracellular vesicles (EVs) are small membranous vesicles released into the extracellular environment by all cell types. EVs contain molecules (proteins, nucleic acids, and lipids) from their progenitor cells, and thus their molecular cargo can reflect their physiological and pathological state. For this reason, EVs have emerged as a promising medium for liquid biopsies, disease diagnosis, prognosis, and personalized therapy/risk assessment.

The isolation of EVs specific to pancreatic cancer cells would greatly increase the potential of EV-based liquid biopsies. However, the vast majority of EVs present in plasma originate from platelets and monocytes. Moreover, the size-based methods typically used to isolate EVs (centrifugation, size exclusion chromatography) do not separate EVs from circulating lipoprotein particles. Here we report the development of a multiplex sample preparation method for the sensitive isolation of EVs and PDAC-specific EVs.

Methods - PDAC-patient plasma and PANC-1 cell line were used to develop and optimized the method. That method combines precipitation of EVs to eliminate most soluble proteins and size exclusion chromatography to separate EVs from co-precipitated soluble protein. When plasma was used, EV preparations were filtrated through an ion exchange resin to deplete lipoproteins. The resulting EV-containing eluates were then characterized for particle size, morphology, protein concentration, and used for omics analysis, or subject to immuno-affinity enrichment to target PDAC-specific EVs.

Results - Here we demonstrate the utility of the approach to analyse EV proteins from PDAC cell lines and PDAC-patient plasma and using the PDAC biomarker AXL for PDAC-specific EVs.

Conclusion - These results should prompt the widespread application of this methodology in investigations employing EVs within liquid biopsies, with the aim of discerning biomarkers crucial for early detection, prognostication, and the assessment of drug efficacy.

Keywords - pancreatic cancer, extracellular vesicles, proteomics

POSTER | N°18

Inhibition of USP14 as an approach to improve sensitivity to cisplatin

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Introduction - Deubiquitinases (DUBs) are critical regulators of tumor cell survival through various mechanisms including modulation of apoptosis and DNA repair. Since cells that acquire resistance to antitumor agents, particularly to cisplatin have alterations in these processes, DUBs may represent targets for modulating cisplatin efficacy.

The proteasome-associated ubiquitin-specific protease-14 (USP14) has been proposed as a valuable therapeutic target in anticancer therapy. Since USP14 may be implicated in survival of drug-resistant cells, the aim of the present study was to target USP14 in cisplatin-sensitive and resistant cells by molecular and pharmacological tools.

Materials and methods - We examined the expression levels of USP14 in different ovarian carcinoma cell lines by quantitative Real time PCR and we used ovarian carcinoma cells, including cisplatin-resistant variants with increased survival features to evaluate the efficacy of molecular and pharmacological targeting of USP14 as a strategy to overcome drug resistance.

Results - An analysis of USP14 mRNA levels indicated a variable level of expression across different cell lines including pairs of cisplatin-sensitive and -resistant cells. Molecular targeting of USP14 was carried out in IGROV-1/Pt1 cells by transfection of siRNAs. With this approach we were able to show that knockdown of USP14 results in impairment of various aggressive features of resistant cells such as proliferative, migratory and invasive abilities.

Regarding drug sensitivity, a mild sensitization to cisplatin was evident by colony forming assays in plastics in IGROV-1/Pt1 but not in IGROV-1 cells, suggesting that USP14 acts on resistance-relevant substrates in drug-resistant cells. We also examined the preclinical efficacy of VLX1570, an inhibitor of USP14 with also affinity for UCHL5, in cisplatin-sensitive and –resistant cells. An analysis of the effects of the drug combination in vitro indicated the occurrence of a synergistic interaction in cisplatin-resistant cells when cells were pretreated with the USP14 inhibitor. In cisplatin-sensitive IGROV-1 cells, a favorable drug interaction could be found upon simultaneous cell exposure to the combination of cisplatin and VLX1570.

Conclusion - Taken together, our results support the interest of USP14 as a target for modulation of cisplatin efficacy in ovarian carcinoma models.

Keywords - Deubiquitanase; USP14; Cancer

POSTER | N°19

Safety and clinical efficacy of IOA-289, a novel autotaxin inhibitor, plus gemcitabine and nab-paclitaxel (GnP) in patients with metastatic pancreatic ductal adenocarcinoma (mPDAC)

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Background - Autotaxin (ATX) plays a critical role in inflammation and resistance mechanisms in a wide range of malignancies. IOA-289 is a novel inhibitor of ATX, with anti-fibrotic, immune-enhancing and anti-tumour activity. This first-in-human trial evaluated safety, and preliminary antitumor activity of IOA-289 with GnP in patients with mPDAC.

Methods - IOA-289 was investigated in a dose escalation study evaluating the continuous twice daily dosing (BID). IOA-289 was initially administered for 7 days as a monotherapy (Cycle 0) before adding GnP at standard doses in 3 weekly schedule followed by 1 week pause (Cycle 1 onwards). Primary objective: Safety and tolerability. Secondary objectives: PK; PD (e.g., serum CA19-9); radiographic responses (RECIST 1.1.); PFS and OS.

Results - Preliminary data from the first 3 cohorts are included: Cohort 1 assessed the safety at 100 mg BID (n=4), Cohort 2 at 200 mg (n=4), and Cohort 3 at 400 mg (n=5).
Safety: No treatment-emergent adverse events (TEAE) led to study drug discontinuation, or a Dose Limiting Toxicity (DLT). Most toxicities were consistent with the known toxicity profile of GnP.

Median time on treatment for Cohort 1 was 4.8 months, for Cohort 2 and Cohort 3 patients are still under treatment and clinical outcomes are not established yet.

No responses were observed in Cohort 1. In Cohort 2, 2 out of 4 patients (50%) achieved a Partial Response. No mature data is available yet for ongoing later Cohorts.

Exposure to the compound resulted in reductions in LPA C18:2, with a protocol-defined inhibition of >50% for over 24 hrs.

Consistent reductions in levels of CA19-9 have been observed since cohort 2 (200 mg BID), with more than 50% reduction already from Cycle 2, with durable reductions lasting longer than 4 months. No mature data is available for ongoing later Cohorts.

Conclusion - IOA-289 is well tolerated at all tested dose levels in combination with standard GnP. Patients in the higher dose Cohorts are experiencing robust CA19-9 reductions consistent with ORR.

POSTER | N°20

Pegylated liposome encapsulating docetaxel using microfluidic mixing technique: process optimization and results in breast cancer models.

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Purpose - The development of nanoparticles could help to improve the efficacy/toxicity balance of drugs. This project aimed to develop liposomes and immunoliposomes using microfluidic mixing technology.

Methods - Various formulation tests were carried out to obtain liposomes that met the established specifications. The liposomes were then characterized in terms of size, PDI, docetaxel encapsulation rate and lamellarity. Antiproliferative activity was tested in human breast cancer models ranging from near-negative (MDA-MB-231), positive (MDA-MB-453) to HER2 positive. Pharmacokinetic studies were performed in C57BL/6 mice.

Results - We synthesized many batches of liposomes with identical molar ratios and by modifying the microfluidic parameters TFR, FRR and buffer. All synthesized liposomes have a size < 200 nm, but only Lipo-1, Lipo-6, Lipo-7, Lipo-8 have a PDI < 0.2, which meets our initial requirements. The size of the liposomes was correlated with the total FRR, for a 1:1 FRR the size is 122.2 ± 12.3 nm, whereas for a 1:3 FRR the size obtained is 163.4 ± 34.0 nm (p=0.019). We obtained three batches of liposomes with high docetaxel encapsulation rates > 80%. Furthermore, in vitro studies on breast cancer cell lines demonstrated the efficacy of liposomes obtained by microfluidic mixing technique. These liposomes also showed improved pharmacokinetics compared to free docetaxel, with a longer half-life and higher AUC (3-fold and 3.5-fold increase for the immunoliposome, respectively).

Conclusion - This suggests that switching to the microfluidic process will produce batches of liposomes with the same characteristics in terms of in vitro properties and efficacy, as well as the ability to release the encapsulated drug over time in vivo. This time-efficiency of the microfluidic technique is critical, especially in the early stages of development.

Keywords - liposomes, biopharmaceutical development, microfluidic mixing technique, breast cancer, docetaxel, pharmacokinetics

POSTER | N°21

Comparison of quality of life of patients with HER2-negative breast cancer put on taxane-based regimens

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Breast cancer is a major health problem. In Algeria, it ranks first among women with more than 12,536 new cases and 4,116 estimated deaths in 2020. It manifests itself as a nodule or abnormal appearance of the breast and it is the clinical, radiological, biological, and pathological diagnosis that confirms the presence of cancer.

Most treatments for cancer in general, and breast cancer in particular, are aggressive to say the least and responsible for physical, psychological, and social consequences. Prolonging survival is no longer done at any cost. The benefits linked to the increase in the (sometimes uncertain) chances of survival of patients cannot reasonably be envisaged without taking into account the quality of this life.

The quality of life of patients with breast cancer can be affected by many factors, including treatments, side effects, pain, anxiety, depression...

The general objective of this research is to compare the quality of life of patients with early non-metastatic HER2-negative breast cancer under a taxane-based protocol, namely Paclitaxel and Docetaxel.

To clearly define our objective, we evaluated the quality of life of these patients through the EORTC QLQ-BR45 questionnaire. This is a retrospective, single-center descriptive study of 40 patients with non-metastatic HER2-CS. The duration of the study is 6 months (between December 2022 and May 2023). This study was carried out at the level of the Medical Oncology Department of the Oran Hospital and University Establishment (EHUO).

According to the EORTC-QLQ-BR45 questionnaire on 40 patients under two protocols based on taxanes, the quality of life was less favorable in the majority of patients, and who declared the presence of post-chemotherapy adverse effects.

Our study may be insufficient in its substance, but it nevertheless allows us to have an idea of the quality of life of patients on taxane and the total cost of a patient with breast cancer HER2- in Algeria.

Keywords - Quality of life, Breast cancer HER2-, non metastatic, Paclitaxel, Docetaxel

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Methylmalonic acid - novel oncometabolite modulates tumor energy metabolism and progression.

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Objectives - One of the recently discovered oncometabolites is methylmalonic acid (MMA), a by-product of propionate metabolism. MMA treatment induces a complete pro-aggressive epithelial-to-mesenchymal transition-like phenotype. MMA may serve as a mitochondrial toxin that can disrupt redox homeostasis by inhibiting electron transport complex II. However, the precise impact of MMA on energy metabolic pathways in cancer cells has not been fully elucidated. Thus, this study aims to evaluate the effect of MMA on metabolic activity in a breast cancer (BC) cell line. Moreover, to translate the obtained results to clinical relevance, we have analyzed MMA levels in BC patients' serum and correlated them with cancer progression parameters.

Methods - The 4T1 mouse BC cell line was treated with 1 and 5mM MMA for 24,48, and 72 hours. Subsequently, cell metabolic activity was assessed using an MTT assay. The energetic demand of the cells was analyzed with the Seahorse Bioanalyzer, while intracellular nucleotide concentrations were measured using RP-HPLC. Moreover, the MMA level in the serum of BC patients was analyzed using an ELISA test.

Results - We observed a significant decrease in metabolic activity in the MTT assay, in 4T1 cells treated with 5mM MMA, but no effect was observed after 1mM MMA treatment. The intracellular ATP/ADP ratio increased after 48 h of 1mM MMA treatment (from 9.3±0.1 to 11.5±0.1, $p<0.0001$). Seahorse analysis indicated that after 48 hours of treatment with 1mM MMA, basal respiration and ATP-linked respiration increased (from 6293±555.8 to 12224±1300 pmol O2/min/mg protein, $p<0.001$, and from 5354±489.6 to 10060±1174 pmol O2/min/mg protein, $p<0.01$, respectively). MMA serum levels were increased in BC patients with higher Ki-67, a proliferation marker (6.9±1.1 vs. 182.2±63.8 ng/ml, $p<0.01$), and in patients with the presence of metastasis in lymph nodes (26.9±35.08 vs. 125.6± 6.7 ng/ml, $p<0.01$).

Conclusion - We hypothesize that MMA may modulate tumor energy metabolism, potentially translating into more invasive and metastatic phenotypes. Interestingly, the link between MMA and cancer progression was confirmed by the increased MMA serum concentration in BC patients characterized by more aggressive cancer parameters. Nevertheless, more in vitro and in vivo studies are needed to investigate the potential usefulness of regulating MMA concentration in BC therapy.

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