5th Annual Meeting | Coimbra | Portugal

ABSTRACT BOOK

STRATAGEM CA17104

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Coimbra evening landscape by José Luís Ribeiro

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New Diagnostic and Therapeutic Tools against Multidrug Resistant Tumours

UNIVERSITÀ DEGLI STUDI DI TORINO

Welcome to the 5th Annual Meeting Coimbra, 29th June – 1st July 2022

Dear Participants,

I wish you a very warm welcome to the 5th Annual Meeting of our COST Action STRATAGEM!

Four years have passed since our Action started. First of all, I would like to thank all of the Members of the Core Group, the Management Committee and all of the Working Group Participants, who have contributed, with extraordinary intellectual curiosity and deep commitment, to the success of this brilliant journey, which has been richly paved with initiatives, meetings, workshops, training schools and short-term scientific missions, despite the difficult times that we have all faced.

As with every year, the most important event is our Annual Meeting.

In this meeting, internationally recognized speakers will discuss the complexities inherent in discovering new biomarkers, in designing and producing new anti-cancer drugs and carriers, in validating their efficacy and safety *in vitro* and *in vivo*, and in facing unsolved clinical needs, with the final goal of identifying new predictive and prognostic biomarkers for patient stratification, and new therapeutic targets. Joining forces across different disciplines, as we have constantly strived to do in our Action, will lead to personalized diagnoses and treatments, and will improve patient outcomes.

Since Young Researchers and Investigators (YRIs) are the pillars of our Action, we are pleased that the majority of oral presentations and posters are by these scientists (please check) We will hear about the latest innovations and discoveries from enthusiastic and talented scientists with bright futures. As members of STRATAGEM, we find the high number of YRIs present most gratifying, and we encourage them to be an active part of the meeting. Establishing new networks of young, motivated scientists is the most precious resource that this meeting and this COST Action can provide for the research of tomorrow.

…. And do not forget!!! STRATAGEM will also continue its long tradition of special awards for the best oral communications and posters this year.

The Core Group and Management Committee will meet to plan the final activities of our Action and create the basis for future collaborations and projects. Last, but not least, the meeting will conclude with social activities so that we can continue our networking in a more informal environment.

Great THANKS go to Prof. Ana Bela Sarmento Ribeiro, Prof. Ana Cristina Gonçalves, Dr. Raquel Alves, and all of the Coimbra team for organizing this wonderful and fruitful meeting.

I look forward to meeting you in the coming days,

Chiara Riganti

Prof. Chiara Riganti
STRATAGEM Chair

Program at Glance

Day 1 – 29th June		Day 2 – 30th June		Day 3 – 1st July	
		9h	**Plenary session II** Nanomedicine in cancer approach	**9h**	**Plenary session IV** Impact of FC in MRD and Resistance
		WG2 Session		**WG4 Session**	
		10h	**WG2 Invited session** *Nanomedicines for tumour targeting*	**10h**	**WG4 Invited session** *Cardiooncology*
		10h45	*Coffee break*	**10h45**	*Coffee break*
		11h15	Oral Communications	**11h15**	Oral Communications
13h	Reception	**12h30**	Poster view	**12h30**	Poster view
14h	Opening Session	**13h**	*Lunch*	**13h**	*Lunch*
14h30	**Plenary session I** Advances in Cancer	**14h**	**Plenary session III** Immune system as therapy perspective	**14h**	MC meeting
WG1 Session		**WG3 Session**			
15h30	**WG1 Invited session** *Multi-omics in cell plasticity*	**15h**	**WG3 Invited session** *Glycosylation in cancer*		Social Events
16h15	*Coffee break*	**15h45**	*Coffee break*		
16h45	Oral Communications	**16h15**	Oral Communications		
18h30	CG meeting	**18h**	Poster view	**20h**	*Gala Dinner with Meeting awards*

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Day 1 – 29th June	
13h	**Registration**
14h	**Opening Session** **Ana Bela Sarmento Ribeiro** *(Local Organizer, Faculty of Medicine, University of Coimbra, Portugal)* **Chiara Riganti** *(Chair of STRATAGEM, School of Medicine, University of Torino, Italy)* **Cláudia Cavadas** *(Vice-dean of University of Coimbra, Portugal)* **Henrique Girão** *(Director of ICBR, Faculty of Medicine, University of Coimbra, Portugal)* **Isabel Marques Carreira** *(Director of CIMAGO, Faculty of Medicine, University of Coimbra, Portugal)*
14h30	**Plenary session I** **Rui Henrique** *(RISE@CI-IPOP, IPO-Porto, Porto Comprehensive Cancer Center & ICBAS, University of Porto, Portugal)* ***Advances in Cancer: towards Precision Medicine*** **Chair: Chiara Riganti** *(School of Medicine, University of Torino, Italy) &* **José Nascimento Costa** *(Faculty of Medicine, University of Coimbra, Portugal)*
	WG1 Session **Chair: Thomas Mohr** *(Medical University of Vienna & University of Vienna, Austria)*
15h30	**WG1 Invited session** **Christopher Gerner** *(Joint Metabolome Facility, University of Vienna, Austria)* ***Multi-omics characterization of functional cell plasticity supports drug resistance research***
16h15	*Coffee break*
16h45	**WG1 Oral Communications** **OC1 - Alexander Tolios** *(Austria)* Interpreting the Uninterpretable - How to Read the Algorithmic Mind **OC2 - Ana Cristina Gonçalves** *(Portugal)* DNA methylation is correlated with oxidative stress in myelodysplastic syndrome – Relevance as prognostic biomarkers **OC3 - Cesim Erten** *(Turkey)* Identification and Prioritization of Personalized Cancer Drivers **OC4 - Helena Branco** *(Portugal)* Identifying immunophenotypic protein markers in circulating Extracellular Vesicles from Acute Myeloid Leukemia patients: monitoring measurable residual disease **OC5 - Karolina Seborova** *(Czechia)* Methylome profile diversity in epithelial ovarian cancer patients with different therapy response
18h30	**Core Group Meeting**

Day 2 – 30th June	
9h	**Plenary session II** **João Nuno Moreira** *(Center for Neuroscience and Cell Biology & Faculty of Pharmacy, University of Coimbra, Portugal)* ***Nanomedicine: an approach in cancer management*** **Chair: Petra Heffeter** *(Institute of Cancer Research, Medical University of Vienna, Austria)* & **Constantinos Athanassopoulos** *(Department of Chemistry, University of Patras, Greece)*
WG2 Session **Chair: Catherine Passirani** *(University of Angers, France)*	
10h	**WG2 Invited session** **Bruno Sarmento** *(i3S, University of Porto & University Institute of Health Sciences-CESPU, Portugal)* ***Nanomedicines for local and intracellular gastrointestinal tumour modulation***
10h45	*Coffee break*
11h15	**WG2 Oral Communications** **OC6 - Elcin Cagatay** *(Turkey)* Targetting to the specific cancer cell with mAb bound surface functionalized exosome **OC7 - Milad Baroud** *(France)* Ferrocifen Nanoparticles: Targeting MDR tumors via the Thioredoxin Reductase Pathway **OC8 - Nanasaheb D. Thorat** *(UK)* Functional magnto-plasmonic nanohybrid platform for light and magnetic responsive cancer theranostics **OC9 - Shirley Sancha** *(Portugal)* Lycorine Derivatives for Reversing P-glycoprotein-mediated Multidrug Resistance in Human Colon Adenocarcinoma Cancer Cells **OC10 - Xi Liu** *(France)* Self-assembling dendrimer nanosystems to improve safety and bioavailability of the anticancer candidate ZZW-115
12h30	**Poster view**
13h	*Lunch*
14h	**Plenary session III** **Sergio Rutella** *(John van Geest Cancer Research Centre, Nottingham Trent University, UK)* **Immune system Friend or Foe? The role in and as therapeutic in cancer.** **Chair: Ana Bela Sarmento Ribeiro** *(iCBR/CIMAGO, Faculty of Medicine, University of Coimbra, Portugal)* **& Yordan Yordanov** *(Medical University of Sofia, Faculty of Pharmacy, Sofia, Bulgaria)*

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WG3 Session **Chair: Helena Vasconcelos** *(University of Porto, Portugal)*	
15h	**WG3 Invited session** **Celso Reis** *(i3S, University of Porto, Portugal)* **Glycosylation in cancer: dissecting the molecular mechanism and understanding the clinical implications**
15h45	*Coffee break*
16h15	**WG3 Oral Communications** **OC11 - Denis Collins** *(Ireland)* Pre-clinical HER2+ breast cancer models of targeted therapy resistance **OC12 - Maria Inês Costa** *(Portugal)* Zn in the modulation of the DNA damage response: preventive, genotoxic, and cytotoxic roles in acute myeloid leukemia **OC13 - Martina Godel** *(Italy)* C/EBP-β LAP regulates chemo-immuno-resistance in hypoxic non-small cell lung cancer **OC14 - Muriel Cuendet** *(Switzerland)* Developing tools to study the role of HDAC6 in multiple myeloma **OC15 - Valentin Van den Bossche** *(Belgium)* Microenvironment-mediated lipid metabolism reprogramming upon anti-EGFR therapy resistance in squamous cell carcinoma of the head and neck
18h	**Poster view**

Day 3 – 1st July	
9h	**Plenary session IV** **Alberto Orfão** *(Instituto Biosanitario de Salamanca (IBSAL), University of Salamanca, Spain)* **Impact of flow cytometry in measurable residual disease (MRD) and resistance in cancer** **Chair: Javier De Las Rivas** *(CiC-IBMCC, CSIC, University of Salamanca, Spain)* & **Artur Paiva** *(iCBR/CIMAGO, Faculty of Medicine, University of Coimbra, Portugal)*
	WG4 Session **Chair: Simona Saponara** *(University of Siena, Italy)*
10h	**WG4 Invited session** **Paulo Oliveira** *(Center for Neuroscience and Cell Biology, University of Coimbra, Portugal)* **Cardiooncology - a tale of old and new anti-cancer drugs**
10h45	*Coffee break*
11h15	**WG4 & WG1/2/3 Oral Communications** **OC16 - José M. Padrón** *(Spain)* Early Pharmacological Profiling by Live Cell Imaging **OC17 - Lana Nezic** *(Bosnia)* Overexpression of survivin and MRP1/ABCCC1 in R-CHOP resistant diffuse large B-cell lymphoma: prognostic and clinicopathological values **OC18 - Nihal Karakaş** *(Turkey)* Diagnostic and Therapeutic Autoantibody Discovery Studies for Brain Tumors **OC19 - Ryszard Ostaszewski** *(Poland)* Design, Synthesis and Anti-tumor Evaluation of Novel Peptidomimetic Hybrids of Ciprofloxacin against multidrug resistant tumors **OC20 - Hemma Schueffl** *(Austria)* Impact of albumin-targeting on the pharmacology, tissue distribution and anticancer activity of an oxaliplatin(IV) bismaleimide prodrug in vivo
12h30	**Poster view**
13h	*Lunch*
14h	**Management Committee meeting**
	Social Events
20h	**Gala Dinner with Meeting awards**

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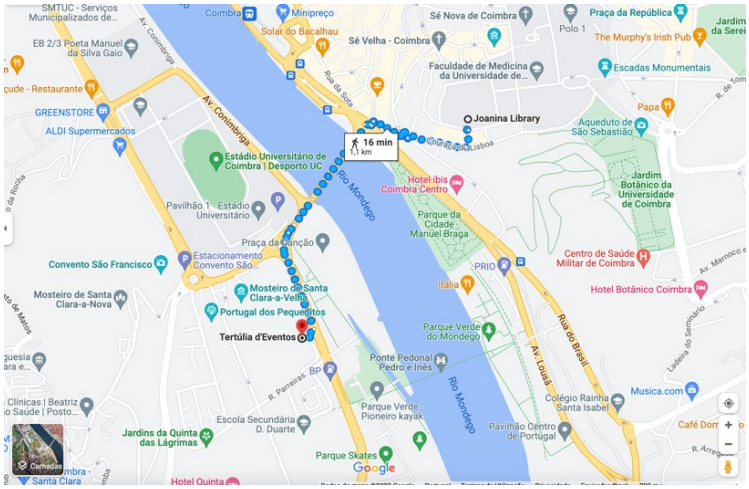
Social Event & Gala Dinner

The **social event** is a visit to University of Coimbra, conducted by university guides, that allow you to discover in more detail the Royal Palace, the Royal Chapel of S. Miguel and the Joanina Library. Aimed at all audiences, they provide a better understanding of the historical and cultural value of this heritage, classified as a World Heritage Site by UNESCO since 2013.

It will be two visit groups. The first group will leave conference venue around 2 pm and travel by bus to University of Coimbra Campus I. This visit will begin at 2h30 pm. Since some of us will have the MC meeting at 2 pm, a second group will leave the conference venue around 5 pm.

The **Gala dinner** will be held in "Tertúlia d'Eventos" restaurant near the Mondego River (Santa Clara riverside). The dinner will be service as a buffet from 8 pm to 10 pm and will be accompanied by Portuguese music (Fado) and a university choir "Estudantina". After 10 pm we can stay in dinner venue, but drinks need to be paid. From University of Coimbra Campus I to dinner venue is 16 min walking (you can also use Uber).



Plenary & Invited Sessions

Advances in Cancer: towards Precision Medicine

Rui Henrique [1,2]*

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Abstract:

Cancer will remain a foremost public health burden over the next decades, as population aging and exposure to carcinogens will continue to boost incidence. Although primary prevention may help decrease cancer burden, results will likely take decades to become apparent and require major changes in society's attitude towards factors that negatively impact on human health. Thus, early diagnosis coupled with appropriate therapy will continue to be the mainstays for effectively fighting cancer, reducing its associated mortality and morbidity. Nonetheless, the ever-increasing costs in healthcare, mostly related to innovative diagnostic technologies and therapeutics, allied to the growing awareness about quality of life, entail the need for development of more accurate strategies, targeting a specific tumor in a specific host. In this context, Precision Medicine applied to Cancer (i.e., Precision Oncology) holds the promise to deliver cancer- and patient-tailored diagnostics and therapeutics, reducing unwanted side effects, decreasing costs, and increasing patients' satisfaction. In this presentation, I will address some of the current promises, challenges, and caveats of Precision Oncology.

Multi-omics characterization of functional cell plasticity supports drug resistance research

Samuel Meier-Menches [1], Christopher Gerner [1,*]

[1] University of Vienna, Joint Metabolome Facility, Währinger Straße 38, 1090 Vienna, Austria
* Correspondence: christopher.gerner@univie.ac.at

Abstract:

Melanoma brain metastases are a powerful model to investigate drug resistance [1]. Here we aimed at the classification of metastasis-related cell properties associated with drug resistance by multi-omics profiling making use of cutaneous and brain-metastasizing variants from single melanomas sharing the same genetic ancestry. Previous experiments demonstrated that cultured cells derived from these xenografted variants maintain a stable phenotype associated with a differential metastatic behavior: The brain metastasizing variants produce more spontaneous micro-metastases than the corresponding cutaneous variants. Four corresponding pairs of cutaneous and metastatic cells were obtained from four individual patients, resulting in eight cell-lines presently investigated. Label free proteome profiling revealed significant differences between corresponding pairs of cutaneous and cerebellar metastases from the same patient. Indeed, each brain metastasizing variant expressed several apparently metastasis-associated proteomic alterations as compared with the corresponding cutaneous variant. Among the differentially expressed proteins we identified cell adhesion molecules, immune regulators, epithelial to mesenchymal transition markers, stem cell markers, redox regulators and cytokines. Similar results were observed regarding eicosanoids, considered relevant for metastasis, such as PGE2 and 12-HETE. The cell plasticity observed in melanoma cells was similarly investigated in case of acute myeloid leukaemia (AML) cell lines with different responsiveness to co-administration of arsenic trioxide (ATO) and all-trans retinoic acid (ATRA) [2]. Here, the cell differentiation state seemed to dictate cellular responses to drug treatment. We propose that multi-omics analyses elucidate the cytoprotective plasticity of cancer cells, which might serve as a general proxy to identify cell-specific resistance strategies and discover novel combination treatments in vitro.

References:

1. Neuditschko B, Janker L, Niederstaetter L, et al. The Challenge of Classifying Metastatic Cell Properties by Molecular Profiling Exemplified with Cutaneous Melanoma Cells and Their Cerebral Metastasis from Patient Derived Mouse Xenografts. Mol Cell Proteomics. 2020 Mar;19(3):478-489. doi: 10.1074/mcp.RA119.001886
2. S.M. Meier-Menches, Neuditschko B, Janker L, et al. A Proteomic Platform Enables to Test for AML Normalization In Vitro. Front Chem. 2022 Feb 1;10:826346. doi: 10.3389/fchem.2022.826346

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Nanomedicine: an approach in cancer management

João Nuno Moreira[1,2]*

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Abstract:

Cancer remains as stressful condition and a leading cause of death in the western world. Often, first line treatments of cancer disease rest as elusive alternatives, offering limited efficacy with extensive secondary effects as a result of severe cytotoxic effects in healthy tissues. The advent of nanomedicine brought the promise to change many fields including oncology, proposing advanced systems for cancer treatment. Drug delivery systems rest among the most successful examples of nanotechnology. Throughout time they have been able to evolve as a consequence of an increased understanding from cancer biology and the tumor microenvironment. This communication will provide an insightful vision on nanomedicine for cancer treatment, from a tumor biology perspective (1).

Acknowledgement: This work was supported by the following projects: QREN/FEDER MultiNanoMed (Ref: 23240). This work was also financed by the European Regional Development Fund (ERDF), through the Centro 2020 Regional Operational Program under project CENTRO-01-0247-FEDER-017646 (ODD4PEGASEMP), and through the COMPETE 2020 - Operational Program for Competitiveness and Internationalisation and Portuguese national funds *via* FCT – Fundação para a Ciência e a Tecnologia, I.P., under projects POCI-01-0145-FEDER-016390 (CancelStem), *Euronanomed* (FCT reference ENMed/0005/2015), CENTRO-01-0145- FEDER-000012-HealthyAging2020 and CIBB (FCT reference UIDB/ 04539/2020)

References:

1. 1. Fonseca, N. A.; Gregório, A. C.; Mendes, V. M.; Lopes, R.; Abreu, T.; Gonçalves, N.; Manadas, B.; Lacerda, M.; Figueiredo, P.; Pereira, M.; Gaspar, M.; Colelli, F.; Pesce, D.; Signorino, G.; Focareta, L.; Fucci, A.; Cardile, F.; Pisano, C.; Cruz, T.; Almeida, L.; Moura, V.; Simões, S.; Moreira, J. N. GMP-Grade Nanoparticle Targeted to Nucleolin Downregulates Tumor Molecular Signature, Blocking Growth and Invasion, at Low Systemic Exposure. Nano Today 2021, 37, 101095. https://doi.org/10.1016/j.nantod.2021.101095.

Nanomedicines for local and intracellular gastrointestinal tumour modulation

Bruno Sarmento[1]

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Abstract:

Our group is focused on the development of drug delivery systems, with special attention on nanotechnology, and their application to the pharmaceutical and biomedical fields. There is a particular interest in establish bioengineering targeted nanomedicines for oral delivery of anti-tumor drugs for gastrointestinal tumours as gastric and colorectal cancer. The group also developed and validated novel in vitro cell-based intestinal model to evaluate the permeability and performance of drugs and drug delivery systems and proposed an innovative multicellular 3D colorectal cancer spheroid model used to screen efficacy of anticancer nanomedicines.

In this presentation, our most recent achievements of the establishment of nanomedicines, with passive and active targeting features for cancer cells will be described. Our approach involves innovative nanomedicines, with deep and comprehensive physical-chemical, in vitro and in vivo evaluation. Particular attention will be given to chitosan-based micelles for oral delivery of poor-soluble anticancer drugs and CD44v6-targeting nanomedicines containing chemotherapeutic and anti-angiogenic active molecules.

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Immune system: friend or foe?
Its role in and as a therapeutic in cancer

Sergio Rutella[1]

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Abstract:

The immune system plays divergent roles in anti-tumour immunity. In most cancer types, immune infiltration has been correlated with control of tumour cell growth and with superior clinical outcomes.

Acute myeloid leukaemia (AML) is characterized by clonal expansion of poorly differentiated myeloid precursors, resulting in impaired haematopoiesis and often bone marrow (BM) failure. Most patients with AML are treated with cytotoxic chemotherapy, despite the recent approval of novel drugs. There is urgent need to identify immunogenomic biomarkers that support the prediction of response to immunotherapies that are currently being investigated in the clinic. The tumour microenvironment (TME) of AML is inherently immuno-suppressive and is equipped to hamper effector T-cell function and includes immune and inflammatory cells, soluble mediators such as interferon (IFN)-γ and extracellular matrix components.

We leveraged targeted gene expression profiling and spatial transcriptomic approaches to dissect the immune ecosystem of childhood and adult AML and how these molecular features correlate with therapeutic responses. We discovered immunologic subtypes of AML that are associated with chemotherapy resistance and with response to a novel CD123-targeting immunotherapy. The use of spatially resolved RNA/protein analysis allowed us to get crucial insight into the neighbourhood of T cell-infiltrated bone marrow specimens from patients with TP53-mutated AML, a disease subgroup with dismal prognosis, and to identify functional gene signature and pathways that predict poor clinical outcomes.

In conclusion, our studies advance our understanding of the immuno-biology of AML and could support the implementation of future immunotherapy clinical trials.

References:

1. Vadakekolathu J, Minden MD, Hood, T, et al. Immune landscapes predict chemotherapy resistance and immunotherapy response in acute myeloid leukemia. Science Translational Medicine 2020; 12 (546): eaaz0463. DOI: 10.1126/scitranslmed.aaz0463.

Glycosylation in cancer: dissecting the molecular mechanism and understanding the clinical implications

Celso A. Reis [1,2,3,4]

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[2] Ipatimup – Institute of Molecular Pathology and Immunology of the University of Porto, Portugal.
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Abstract:

Aberrant glycosylation is a common molecular alteration with major biological implications for cancer progression (1). Cancer is a heterogeneous disease that requires multidisciplinary treatment. Current targeted therapy depends on patient stratification based on molecular features of individual tumours. Recent results applying glycomic and glycoproteomic strategies in human cancer tissue samples and advanced glycoengineered cellular models have provided novel information with major clinical implications (1,2,3). This presentation will report on the alterations of glycosylation impact the activation of oncogenic receptors in tumour samples, glycoengineered cell models and clinical samples, including the recently described glycoproteomic map of the HER2 (ErbB2) in gastric cancer (3). Glycomic and glycoproteomic analysis of HER2 disclosed a site-specific glycosylation profile in gastric cancer cells. HER2-specific glycosylation affects receptor functionality and stability, and tunes the sensitivity of HER2-dependent gastric cancer cells to trastuzumab-induced cytotoxicity (3).

These results disclose novel functional aspects of glycosylation modifications occurring in cancer and highlights their potential for rational patient stratification, personalized medicine and for novel and improved therapeutic applications.

References:

1. Mereiter S, Balmaña M, Campos D, Gomes J, Reis CA. Glycosylation in the Era of Cancer-Targeted Therapy: Where Are We Heading? Cancer Cell. 2019 Jul 8;36(1):6-16. doi: 10.1016/j.ccell.2019.06.006.
2. Magalhães A, Duarte HO, Reis CA. The role of O-glycosylation in human disease. Mol Aspects Med. 2021 Jun;79:100964. doi: 10.1016/j.mam.2021.100964.
3. Duarte HO, Rodrigues JG, Gomes C, Hensbergen PJ, Ederveen ALH, de Ru AH, Mereiter S, Polónia A, Fernandes E, Ferreira JA, van Veelen PA, Santos LL, Wuhrer M, Gomes J, Reis CA. ST6Gal1 targets the ectodomain of ErbB2 in a site-specific manner and regulates gastric cancer cell sensitivity to trastuzumab. Oncogene. 2021 May;40(21):3719-3733. doi: 10.1038/s41388-021-01801-w.

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Impact of flow cytometry in measurable residual disease (MRD) and resistance in cancer

Alberto Orfão[1*]

Cancer Research Centre (IBMCC, CSIC-USAL), Department of Medicine and Cytometry Service (NUCLEUS), University of Salamanca; Institute for Biomedical Research of Salamanca (IBSAL) and CIBERONC, Salamanca, Spain.

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Abstract:

Along the 80's flow cytometric approaches for detection of minimal/measurable residual disease (MRD) by multicolour flow cytometry were initially develop for haematological malignancies, also providing a tool for isolation (FACS-purification) of treatment-resistant tumour cells for subsequent *omics* analyses. Since then, MRD monitoring by flow cytometry has proven to be of great clinical utility in most acute and chronic leukaemia patients, due to its increasingly high sensitivity in detecting treatment-resistant tumour cells that persist after starting therapy. Thus, MRD has become critical for in-depth assessment of the quality of conventional (complete) response to therapy, for risk stratification during and after therapy, and for the establishment of early decisions about the need for risk-adapted therapies in both MRD+ and MRD- patients in e.g., childhood and adult acute lymphoblastic leukaemia (ALL) and multiple myeloma (MM), among other haematological malignancies. Due to such important clinical utility, and progressive adoption of BM MRD testing in many centres worldwide, selection of the most adequate and robust MRD assay has become of utmost relevance. In recent years, next generation flow cytometry (NGF) techniques have become the preferred method for MRD monitoring based on the use of i) optimized and validated multi-colour (≥8-color) antibody panels, that include a set of markers for high-sensitive and specific detection of tumour cells, ii) acquisition of high numbers of BM cells (i.e., >10 million cells/sample), and iii) automated gating, data analysis and reporting for an improved reproducibility. The standardized and validated EuroFlow NGF panels and procedures are particularly suited for standardized MRD monitoring in e.g., ALL and MM. NGF methods also provide useful information about the composition of the tumour microenvironment (e.g., tumour stroma), including tumour-surrounding immune cells.

Despite all the above MRD monitoring in BM remains a suboptimal monitoring approach because of i) the invasive nature of BM aspiration procedures that cannot be repeated frequently, particularly in children and in elderly patients, ii) the variable and uncontrolled levels of haemodilution of BM aspirated samples, and iii) the potential lack of representativeness of the sample due a heterogeneous (patchy) pattern of BM infiltration by the tumour. In recent years, monitoring of circulating tumour cells (CTC) in blood has progressively become also feasible. Monitoring of CTC in blood can be more frequently performed, it provides absolute (in addition to relative) tumour cell counts, it more closely reflects tumour dissemination, and allows for simultaneous CTC and immune monitoring, particularly in the setting of patients treated with novel immunomodulatory and

immunotherapeutic agents. The increased sensitivity reached with the novel NGF approaches developed for BM MRD monitoring, has set the basis for investigation of their utility for CTC detection both at diagnosis and after therapy, particularly in patients with e.g., ALL, acute myeloblastic leukaemia (AML), and MM, among other haematological malignancies. Thus, early CTC studies performed in ALL, already showed a high degree of agreement between CTC levels in blood and BM MRD in T-ALL and AML, and to a less extent also in BCP-ALL and MM. In addition, recent studies in MM also proved that CTC are detectable by NGF at diagnosis in blood of virtually every MM patient with significant impact on disease outcome. In addition, it permits simultaneous monitoring of persistence of CTC in blood and disease response to distinct modalities of immunotherapy. As an example, protocols have been developed for simultaneous monitoring of up to hundreds of different subsets of CART cells and other immune cells, in addition to CTC, in patients who had received CART cell-based treatments. Based on such increased sensitivity of the new NGF approaches a realistic opportunity becomes feasible of applying these MRD monitoring approaches also in non-haematological cancers. In this regard, we have recently performed an in-depth characterization of tumour cell phenotypes in paediatric solid tumours, for identification of biomarkers for disease classification and subsequent monitoring of CTC, with promising results in neuroblastoma, among other paediatric cancer types.

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Cardiooncology - a tale of old and new anti-cancer drugs

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Abstract:

Oncocardiology primarily aims to identify mechanisms by which cancer and/or cancer therapy cause cardiovascular complications. The increasing number of cancer survivors is associated with a similar increase in individuals showing complications derived from different anti-cancer treatments. Ultimately, those complications may degenerate into congestive heart failure if undetected and treated. Anthracyclines are golden-standard chemotherapeutics that cause a dose-dependent and cumulative cardiotoxicity that is multifactorial, although involving progressive mitochondrionopathy. The mechanisms leading to the persistence of anthracycline cardiotoxicity over decades are not clearly defined but can involve a depression in mitochondrial DNA copy number, epigenetic alterations in the cardiac muscle, or progressive loss of cardiac cells via apoptosis. Although anthracyclines were introduced into the clinical practice decades ago, they stand as one of the most potent anti-cancer agents, and the full identification of their cardiotoxic mechanisms is critical to maximizing their efficacy. More recently, the introduction of trastuzumab and tyrosine kinase inhibitors revealed some problems associated to their cardiac toxicity, which need to be clarified. The present talk will mainly discuss the mechanisms associated with anthracycline cardiotoxicity, including cardiac mitochondrial disruption, epigenetic alterations, and alterations in circadian. We will then extend the information to newer agents and how they can cause cardiotoxicity. Strategies to decrease anti-cancer cardiotoxicity are here presented together with their limitations.

Oral Communications

Interpreting the Uninterpretable - How to Read the Algorithmic Mind

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Abstract:

The application of machine learning algorithms to life science projects allows us to find complex patterns in our data and to make non-obvious predictions. But this comes at the price of algorithmic interpretability. Especially in a medical setting, it is of utmost importance for a physician or scientist to be able to evaluate ones trust into a recommendation from an algorithm.

Explainable artificial intelligence (XAI) methods allow us to peek into the decision-making process of those algorithms. Different XAI methods are available, depending on the type of data used. Those analyses are even possible if the used algorithms are notoriously difficult to inspect (so-called "black-box" algorithms like e.g. neural networks).

This talk will present some of the most relevant XAI algorithms and talk about their advantages and disadvantages as well as when they can be used.

DNA methylation is correlated with oxidative stress in myelodysplastic syndrome – Relevance as prognostic biomarkers

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Abstract: Different cancer models suggest that high reactive oxygen species (ROS) levels contribute to cancer development and progression through genetic and epigenetic mechanisms. Oxidative stress (OS) and abnormal DNA methylation have been implicated in myelodysplastic syndromes (MDS). MDS is a heterogeneous group of clonal hematopoietic stem cell disorders characterized by dysplasia, impaired differentiation, and inefficient hematopoiesis, leading to blood cytopenias and progression to acute leukemia in 1/4 to 1/3 of cases. This fact leads us to investigate whether OS was correlated with localized and global DNA methylation (LINE-1) in peripheral blood of MDS patients to identify peripheral biomarkers of disease. Sixty-six MDS patients and 26 healthy individuals were analyzed. Several OS and macromolecule damage parameters were analyzed [peroxides and NO, antioxidant defences (uric acid, vitamin E, vitamin A, GSH, GSSG, TAS, as well as GPX and GR activities], and oxidative damage (8-OH-dG and MDA)]. Localized (*P15*, *P16*, *TP53*, *MGMT*, *DAPK*, and *KEAP1* gene promotors) and global DNA methylation (5-mC and 5-hmC levels; LINE-1 methylation) were assessed. MDS patients had lower levels of reduced glutathione and total antioxidant status (TAS) and higher levels of peroxides, nitric oxide, peroxides/TAS, and 8-hydroxy-2-deoxyguanosine, compared to controls. These patients had also higher 5-mC levels and lower 5-hmC/5-mC ratio and LINE-1 methylation and increased methylation frequency of at least one methylated gene. Peroxides levels and peroxides/TAS ratio were higher in patients with methylated genes than those without methylation and negatively correlated with LINE-1 methylation and positively with 5-mC levels. The 5-hmC/5-mC ratio was significantly associated with progression to acute leukemia (cut-off = 0.32, 109 ± 3 months vs. 87 ± 7 months; p=0.012) and peroxides/TAS ratio with overall survival (cut-off = 0.74, 59 ± 6 months vs. 87 ± 7 months p=0.002). This study points to a relationship between OS and DNA methylation, two common pathogenic mechanisms involved in MDS, and suggests the relevance of 5-hmC/5-mC and peroxides/TAS ratios as complementary prognostic biomarkers.

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Identification and Prioritization of Personalized Cancer Drivers

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Abstract:

A major challenge in cancer genomics is to distinguish the driver mutations that are causally linked to cancer from passenger mutations that do not contribute to cancer development. The majority of existing methods provide a single driver gene list for the entire cohort of patients. However, since mutation profiles of patients from the same cancer type show a high degree of heterogeneity, a more ideal approach is to identify *patient-specific* drivers. PersonaDrive is a novel method that integrates genomic data, biological pathways, and protein connectivity information for personalized identification of driver genes. The method is formulated on a personalized bipartite graph for each patient. It provides a personalized ranking of the mutated genes of a patient based on the sum of weighted pairwise pathway coverage scores across all the samples, where appropriate pairwise patient similarity scores are used as weights to normalize these coverage scores. It is compared against five state-of-the-art patient-specific cancer driver gene ranking methods. The comparisons are with respect to a novel evaluation method that takes into account the personalized nature of the problem. The proposed personalized driver ranking outperforms the existing alternatives for both the TCGA and the cell line data. Additionally, the KEGG/Reactome pathways enriched in PersonaDrive candidate drivers and those that are enriched in cell lines reference sets overlap significantly when compared to the overlaps achieved by the rankings of the alternative methods. Such findings can provide valuable information towards the development of personalized treatments and therapies.

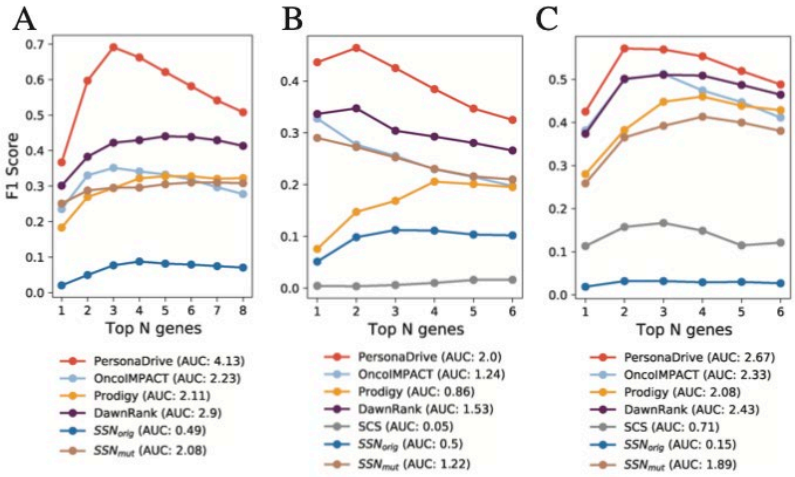

Figure 1. TCGA Data, STRING PPI network. A) COAD, B) LUAD, C) BRCA

Identifying immunophenotypic protein markers in circulating Extracellular Vesicles from Acute Myeloid Leukemia patients: monitoring measurable residual disease

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Abstract: Measurable Residual Disease (MRD) is currently monitored in acute myeloid leukemia (AML) patients through different leukemia associated immunophenotypic protein markers (LAIPs) in bone marrow aspirates. Alternative peripheral blood (PB) Extracellular Vesicles (EV)-based methods would be preferable as they are less invasive, real-time, and cost-effective for AML monitoring.
This work aimed to: i) verify the presence of LAIPs in the cargo of EVs shed by a human AML cell line; ii) select an appropriate method for EV isolation from the PB of AML patients; and iii) analyze LAIPs present in AML patient's plasmatic EVs and correlate their levels with disease progression.
EVs were first isolated from the OCI-AML3 cell line (by differential centrifugation) and characterized according to their size (by nanoparticle tracking analysis), morphology (by transmission electron microscopy) and protein cargo (by Western Blot). Results from proteomic analysis revealed that both OCI-AML3 cells and their EVs present CD14 and CD33 (LAIPs). Then, EVs were isolated from patients using three different techniques: Total Exosome Isolation Kit (Invitrogen, Thermo Fisher Scientific), Exo-spin™ Exosome Purification Kit (Cell Guidance Systems) and Size-Exclusion Chromatography (SEC) followed by Ultrafiltration (UF). The study was approved by the Ethical Committee of CHSJ, and patient's informed consent was obtained. SEC was selected as the most appropriate technique. Therefore, EVs from 12 AML patients' Poor Platelet Plasma were isolated by SEC and concentrated by UF. Patient EVs ranged from 50 nm to 300 nm and expressed EV-associated protein markers such as CD63, HSP70, Annexin XI, CD81, CD9 and Mitofilin. Several LAIPs were identified in paired samples at diagnosis and remission, with a differential expression throughout disease evolution, indicating that some of the EV-associated LAIPs may have potential as MRD biomarkers. Validation of results will be performed by increasing the number of patient samples.

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Methylome profile diversity in epithelial ovarian cancer patients with different therapy response

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Abstract:

Epithelial ovarian carcinoma (EOC) is associated with the highest mortality among gynecological carcinomas. High mortality is due to the diagnosis at advanced stages and development of resistance to anticancer therapy regimens based on taxanes and platinum derivatives. DNA methylation is recognized as an important regulatory mechanism in transcriptome profile modulation and its association with resistance and therapy response in EOC patients was investigated in this study.

In total, 50 EOC patients with characterized treatment response (25 EOC resistant patients and 25 EOC sensitive patients) were selected. DNA methylation profile was measured by Infinium MethylationEPIC BeadChip (Illumina) arrays that allow us to analyze more than 850,000 methylation sites across the genome.

Differential methylation analysis showed, in total, 358 significantly differentially methylated sites between sensitive and resistant EOC patients. Differences in methylation profile were found membrane transporter genes playing role in multidrug resistance (*ABCC4, ABCB10, SLC1A7, SLC19A2, SLC50A1* and *ATP1A1*), DNA repair genes (*XPC*), transcription factors (*FOXO1*) and for lncRNAs (LIN00263, LINC00460 and NEAT1).

In conclusion, DNA methylation analysis showed potential candidates including genes and non-coding RNAs for further studies in the role of DNA methylation in EOC drug resistance development.

Acknowledgement: Study was supported by the Czech Ministry of Education, Youth and Sports INTER-COST project no. LTC19020, the Czech Science Foundation project no. 21-14082S, the Grant Agency of Charles University project no. GAUK 1074120.

Targetting to the specific cancer cell with mAb bound surface functionalized exosome

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Abstract:

Drug delivery nanotechnologies have recently gained a lot of interest because of their ability to improve medications solubility and targeting specificity. To accomplish targeted delivery, therapeutic drug carriers are currently modified with certain recognized ligands. Some aptamers, such as chemical agents, antibodies, and cell-targetting peptides can be modified to provide targeted therapeutic effects with high specificity, selectivity, and affinity (1). Because of their low antigenicity, exosomes have a lot of potential for delivering therapeutic agents for cancer therapy. Exosomes (50–150 nm) are extracellular nanovesicles released by all cells that play an important role in cell–cell communication by carrying endogenous molecules including RNA, siRNA, DNA, and proteins *in vivo (2)*. The main objective of our study to create surface functionalized, drug loaded exosome platform for targeted delivery of therapeutic drugs. A high yield exosome production culture were established from serum-free suspensive HEK 293 cells which used in non-GMP condition for exosome production (3). The purification of exosomes from this culture performed using multiple filtration and ultracentrifugation steps. The in vitro characterization showed the quality of the purified exosomes. According to the results, the size of the isolated exosomes was shown to be around 116 nm. To achieve cancer cell targeting, fully human monoclonal antibody (IgG1) that binds to high affinity to the extracellular domain of vascular endothelial growth factor receptor 2 (VEGFR2), were produced by suspension CHO cell lines. Furthermore, advanced surface modification and drug loading techniques will be developed to generate mAb-exosome complex. Finally, anti-cancer properties of the mAb-exosome complex will be validate in vivo and in vitro studies.

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Ferrocifen Nanoparticles: Targeting MDR tumors via the Thioredoxin Reductase Pathway

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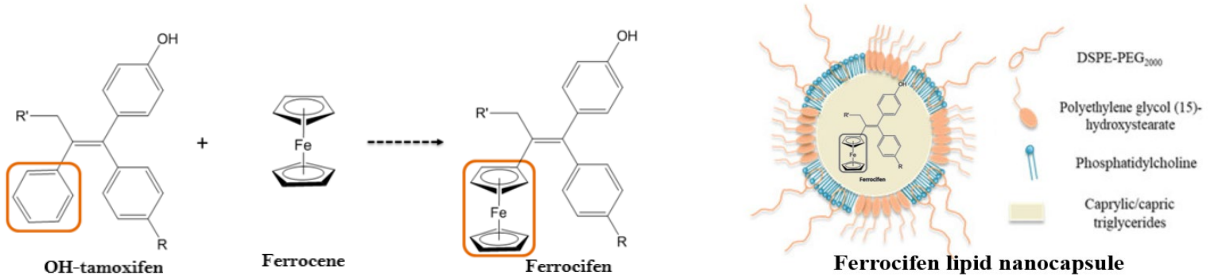
Abstract: Regardless of the leaps being achieved in the field of cancer treatment, 90% of the failures of chemotherapy arise from the multi drug resistance aspect gained by these tumors [1]. Aiming to overcome these limitations, ferrocifens a family of molecules obtained by combination of ferrocene with hydroxytamoxifen derivatives[2], encapsulated in lipid nano capsules, could offer an innovative approach to combat this resistance. Moreover, the functionalization of the obtained nanoparticles with polyethylene glycol would imbue them with stealth properties[3], increasing their half-life and circulation time, while utilizing enhanced internalization into cancer cells via the enhanced permeability and retention effect (EPR)[4].

Ferrocifen has been hypothesized to act via targeting the thioredoxin reductase (TrxR), a system responsible for thiol redox homeostasis and overexpressed in cancer cells leading to increased resistance[5,6]. This action might stem from TrxR displaying a selenocysteine residue at its C-terminal active site, which can be targeted by metal complexes.



This project, along-side producing ferrocifen nanoparticles, aims to verify and establish the mode of action of Ferrocifen on TrxR, using various genetic and molecular techniques. These studies will be performed on several resistant lung and liver cancer cell lines with varying TrxR levels. The initial cytotoxicity studies performed agree with the proposed hypothesis. Patient Derived Xenografts in vivo studies will complement these preliminary results.

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Functional magnto-plasmonic nanohybrid platform for light and magnetic responsive cancer theranostics

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Abstract:
New generation nanoscale systems that utilize remotely active as well as controlled drug release was established in order to enhance the cancer therapeutics. Although many existing nanodrug carriers have shown numerous advantages, their efficacy is largely constrained by their lack of the ability to achieve on efficient demand drug release. In light of the growing interest in the search for next generation effective solutions for cancer treatment, we designed novel magneto-thermally active nanocarrier in which the drug release can be activated on demand upon exposure to a magnetic field. Magnetically active graphene oxide/iron oxide nanocomposite that can be triggered using magnetic hyperthermia initiated from an external alternating magnetic (AC) magnetic field. The unique remotely-triggered functional nanocomposite with well-defined size and uniform distribution were designed. The synergetic effect of both the drug and magnetic hyperthermia is observed in the killing of the cancerous cells. The overall simplicity of action, durability and biocompatible nature of nanocomposite demonstrated herein are key for successful tumor cells targeted therapeutic systems for the kinds of cancer therapy being sought for modern personalized and precision medicine.

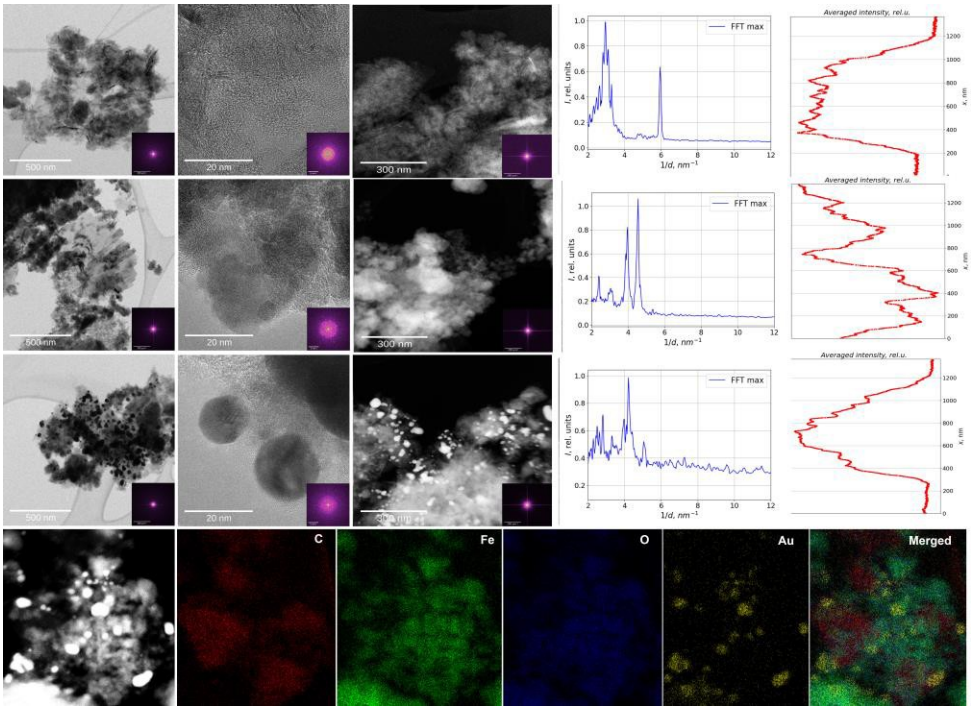

Figure 1. This is a figure representing electronic microscopic image of magneto-plasmonic nanohybrid.

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Lycorine Derivatives for Reversing P-glycoprotein-mediated Multidrug Resistance in Human Colon Adenocarcinoma Cancer Cells

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Abstract:

Multidrug resistance (MDR) to anticancer drugs has become a great challenge in cancer chemotherapy. One of the most important MDR mechanisms is due to the overexpression of ABC drug transporter proteins, namely P-glycoprotein (P-gp). Modulation of P-gp is among the most promising approaches for overcoming MDR.

Aiming at generating a small library of anticancer compounds for overcoming multidrug resistance, in the present study, lycorine, the major Amaryllidaceae-type alkaloid isolated from *Pancratium maritimum,* was derivatized. Thirty-two new derivatives were obtained by the chemical derivatization of hydroxyl groups of lycorine into carbamates. The structure of the compounds was established by NMR experiments, including 2D NMR (COSY, HMQC, and HMBC).

The compounds were evaluated as MDR reversers, through functional and chemosensitivity assays, in resistant human colon adenocarcinoma cancer cells (Colo 320), overexpressing P-gp. A significant inhibition of P-gp efflux activity was observed for some derivatives at non-cytotoxic concentrations. The effect on the ATPase activity of the strongest modulators showed that the compounds behaved as inhibitors. In drug combination assays, most of the compounds showed strong synergistic interactions with doxorubicin. Moreover, some derivatives showed a selective antiproliferative effect toward resistant cells, having a collateral sensitivity effect.

Keywords: *Pancratium maritimum*, Amaryllidaceae alkaloids, multidrug resistance, P-glycoprotein.

Acknowledgments: We thank the Fundação para a Ciência e Tecnologia for financial support in Portugal (Projects PTDC/MED-QUI/30591/2017 and Ph.D. grant SFRH/BD/130348/2017).

Self-assembling dendrimer nanosystems to improve safety and bioavailability of the anticancer candidate ZZW-115

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Abstract: Pancreatic cancer is a deadly cancer without efficacious treatment. We previously identified a drug candidate, ZZW-115, which exhibited an excellent anticancer effect on pancreatic cancer by targeting the nuclear protein 1, a stress-associated protein overexpressed in cancer tissues. However, ZZW-115 is not soluble in water, and binds to hERG channel, being poorly bioavailable yet with eventual cardiotoxicity. Nanotechnology-based drug delivery is widely expected to improve drug solubility, while at the same time, reducing drug toxicity and increasing drug efficacy via passive tumor targeting based on the Enhanced Permeability and Retention (EPR) effect of tumor microenvironment. Recently, we have explored self-assembling dendrimer nanosystems for drug delivery with remarkably high drug loading and small nanosize for tumor accumulation and penetration via EPR effect [1,2]. Here, we report that these self-assembling supramolecular dendrimer nanosystems effectively encapsulated ZZW-115 and formed small and uniform water-soluble nanomicelles. These nanomicelles accumulated preferentially in tumor via EPR effect, and showed rapid cell uptake, enhancing markedly anticancer activity while reducing the toxicity. This study highlights the promising potential of ZZW-115 formulated in dendrimer nanomicelle as a safe and effective anticancer candidate.

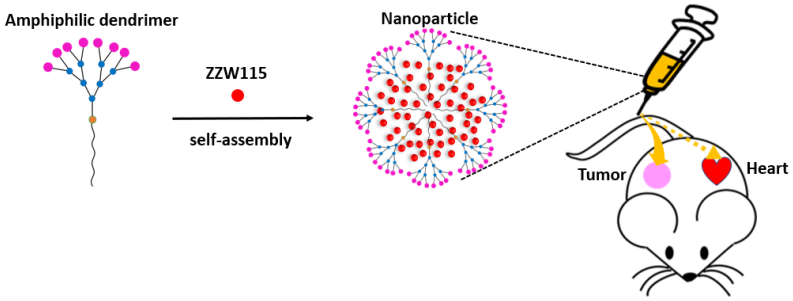

Figure 1. Cartoon illustration of supramolecular dendrimers for ZZW115 delivery

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Pre-clinical HER2+ breast cancer models of targeted therapy resistance

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Abstract:

Background: Targeted therapies have revolutionised the treatment of HER2+ breast (BC), but the emergence of resistance is a major clinical issue. The HER2-targetting monoclonal antibody (mAb) therapies trastuzumab and pertuzumab are approved for first line treatment of HER2+ BC, with the small molecule tyrosine kinase inhibitors (TKIs) lapatinib, tucatinib and neratinib approved in subsequent settings. Afatinib is an irreversible pan-HER TKI that is approved for the treatment of non-small cell lung cancer, but is not approved in HER2+ BC. Our lab has developed *in vitro* models of acquired mAb and TKI resistance in order to characterise resistance mechanisms and identify novel treatment strategies for targeted therapy refractory cancers.

Methods: Trastuzumab-resistant BT474-T and SKBR3-T were created by treating parental cell lines BT474 and SKBR3 with 10 μg/mL of trastuzumab continuously for 6 months. SKBR3 cells were treated with 150 nM afatinib twice-weekly for 6 months to create the SKBR3-A cell line. Growth response to drugs (trastuzumab, lapatinib, neratinib, afatinib, dasatinib) was assessed by 5-day acid phosphatase assay. Reverse phase protein array (RPPA) was used to determine alterations in key signaling pathways. Src, p-Src, EGFR, p-EGFR, ERK1/2, p-ERK 1/2 levels were examined by Western blotting.

Results: The BT474-T cell line was 2.4 fold, and the SKBR3-T cell line 1.8 fold, resistant to trastuzumab compared to their parental cell lines. Both the BT474-T and SKBR3-T cell lines displayed increased levels of EGFR compared to parental models, proving significant in the BT474-T cell line ($p<0.05$). SKBR3-A cells were 26-fold resistant to afatinib compared to the parental cells and were cross-resistant to lapatinib, neratinib and trastuzumab. EGFR levels were significantly increased in SKBR3-A compared to SKBR3. RPPA interrogation of the SKBR3-A cells showed significantly increased levels of p-Src (Y416). SKBR3-A cells are more sensitive to Src inhibition with dasatinib compared to SKBR3-Par cells. The combination of afatinib and dasatinib is highly synergistic in SKBR3-A cells (CI value = 0.09 ± 0.06), also proving effective in SKBR3-T. Afatinib and dasatinib inhibit EGFR and Src activation and downstream ERK 1/2 signalling in SKBR3-A cells.

Conclusion: HER2+ BC cells that are highly sensitive to HER2-targeted therapies can develop acquired in vitro resistance within six months, resulting in altered phenotypes. These models are capable of generating pre-clinical rationale for novel clinical approaches, such as utilising a Src inhibitor to overcome HER2-targeted therapy resistance.

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Zn in the modulation of the DNA damage response: preventive, genotoxic, and cytotoxic roles in acute myeloid leukemia

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Abstract:

The DNA damage response (DDR) is fundamental for the maintenance of genomic stability and zinc (Zn) is a cofactor of several DDR proteins. In acute myeloid leukemia (AML), plasmatic Zn levels are commonly decreased. Moreover, DDR defects have also been pointed in AML. However, the effects of Zn in the modulation of DNA repair capacity in AML are not yet understood. This work aimed to evaluate the role of Zn in the modulation of the DDR in AML and to address its therapeutic potential in combination with conventional therapy (cytarabine; AraC) and DDR inhibitors (olaparib; Ola). We used the AML cell lines HEL, NB-4, and K-562 and normal human lymphocytes (IMC). HEL and IMC cell lines were incubated in standard culture conditions, Zn depletion (Zn-depleted FBS), and Zn supplementation (40µM $ZnSO_4$). After 2, 7, and 15 days, cells were exposed to H_2O_2 and UV radiation. Chromosomal damage, cell death, and nuclear division indexes were evaluated by the cytokinesis-block micronucleus assay. DNA damage and repair kinetics were evaluated by γH2AX expression. Lastly, the AML cell lines were incubated for 72h with increasing concentrations of AraC and ola in monotherapy and combination with $ZnSO_4$ (IC_{25}). Cell density and viability were evaluated by trypan blue test. Cell death and cell cycle were analyzed by flow cytometry, using double-staining with annexin V/7-AAD and PI/RNAse, respectively. Results were statistically analyzed considering a significance level of 95% ($p<0.05$). In AML cells, Zn supplementation increased the genotoxicity of H_2O_2 and UV radiation, inducing cytotoxic and cytostatic effects and persistent activation of γH2AX. Repair kinetics was significantly compromised in Zn supplemented-AML cells ($p<0.01$). Oppositely, in normal lymphocytes, Zn improved DNA repair and prevented damage accumulation. The effects of AraC and ola were potentiated by $ZnSO_4$ in all AML cell lines ($p<0.05$). $ZnSO_4$ decreased 4.8-fold (HEL) and 19-fold (NB-4 and K-562) the IC_{50} of AraC at 48h, and 2.9-fold, 1.6-fold, and 7.5-fold the IC_{50} of ola in HEL, NB-4, and K-562, respectively. $ZnSO_4$ increased the percentage of cells in apoptosis comparatively to monotherapy ($p<0.05$). The association of $ZnSO_4$ with AraC increased the cytostatic effect in HEL and K-562, leading to G_0/G_1 arrest. The combination of ola with $ZnSO_4$ also induced G_0/G_1 arrest in HEL cells ($p<0.05$). In summary, Zn improved DDR in normal cells and the effects of AraC and ola that may result in more efficient and less toxic therapeutic regimens.

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C/EBP-β LAP regulates chemo-immuno-resistance in hypoxic non-small cell lung cancer

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Abstract:

Solid tumors subjected to intermittent hypoxia are characterized by resistance to chemotherapy and immune-killing by effector T-lymphocytes [1]. The aim of this work is to investigate the molecular circuitries determining this double resistance during intermittent hypoxia and to identify novel chemo-immuno-sensitizer approaches. From an initial screening on 28 human non-small cell cancer (NSCLC) cell lines, we focused on H2228 NSCLC cell line, that had the highest ratio between ABCB1-ABCB1 and ABCA1, to investigate the molecular mechanisms underlying the divergent expression of these transporters in intermittent hypoxia. Intermittent hypoxia induces a stronger chemo-immuno-resistance than continuous hypoxia in NSCLC cells, by up-regulating the drug efflux transporters ABCB1 and ABCC1, and down-regulating ABCA1, necessary for the anti-tumor activation of Vγ9Vδ2 T- lymphocytes. Cells exposed to intermittent hypoxia have impaired electron transport chain and high mitochondrial reactive oxygen species (ROS) that stabilizes the Hypoxia- Inducible Factor 1α (HIF-1α) and favors its binding to the mRNA of the CCAAT/Enhancer Binding Protein-β (C/EBP-β) transcription factor. This interaction increases the production of C/EBP-β LAP splicing variant that transcriptionally induces ABCB1 and ABCC1, favoring the efflux of cisplatin and docetaxel, and limiting their efficacy. LAP also decreases ABCA1, limiting the efflux of isopentenyl pyrophosphate (the endogenous activator of Vγ9Vδ2 T-cells), and favoring the escape to the immune-killing. The role of ROS/HIF-1α/LAP axis in chemo-immuno-resistance was validated by knock-out experiments and using pro-oxidant or antioxidant agents targeting mitochondrial metabolism, such as Elesclomol and MitoQ. This work demonstrated that the impairment of mitochondrial metabolism induced by intermittent hypoxia increases the ROS-dependent stabilization of HIF-1α/LAP axis that determines chemo-immuno-resistance. Mitochondrial ROS scavengers may be repurposed as new chemo-immuno-sensitizer agents in hypoxic solid tumors, resistant to conventional treatments.

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Developing tools to study the role of HDAC6 in multiple myeloma

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Abstract:

Multiple myeloma (MM) is a plasma cell malignancy that still represents an incurable disease despite the development of new therapeutic agents. The histone deacetylase 6 (HDAC6) is overexpressed in MM patients and plays a central role in the acquisition of resistance to conventional anti-proteasome treatments. HDAC6 contains three domains. The catalytic domains CD1 and CD2 are known to be involved in microtubule stability, but their individual role is not fully understood. The ZnF-UBP terminal domain recognizes polyubiquitinated motifs from misfolded protein aggregates. Therefore, HDAC6 is capable of activating the aggresome clearance pathway as a possible resistance mechanism. Using CRISPR-Cas9 technology, four edited MM cell lines (RPMI 8226) were generated through clonal selection: HDAC6 knockout, dysfunctional CD1 domain, dysfunctional CD2 domain and truncated ZnF-UBP domain. These cell lines are tools to get insight into the specific role of the different HDAC6 domains in MM. Besides, a library of ZnF-UBP inhibitor candidates was produced aiming at chemically blocking the aggresome pathway. The HDAC6-ubiquitin interaction was measured by a fluorescence polarization assay and several compounds were found to have an IC_{50} value in the low µM range. The development of these biological and chemical tools will contribute to elucidating the role of HDAC6 in MM pathogenesis, and to propose new therapeutic approaches for overcoming resistance in MM.

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Microenvironment-mediated lipid metabolism reprogramming upon anti-EGFR therapy resistance in squamous cell carcinoma of the head and neck

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Abstract:

Squamous cell carcinoma of the head and neck (SCCHN) is still associated with poor prognosis for patients with locally advanced or recurrent/metastatic disease. Indeed, clinical response to anti-EGFR therapy (cetuximab, CTX) in these patients is strongly limited by the occurrence of acquired resistance. While no genetic alteration is clearly associated with anti-EGFR therapy resistance, tumor microenvironment (TME) is thought to actively contribute to disease progression and clinical relapse in SCCHN. With the aim to characterize the molecular mechanisms supporting CTX resistance in SCCHN, we analyzed RNA-sequencing data from "in-house" CTX-sensitive and -resistant (CTX-R) patient-derived xenograft models. We identified deregulated lipid metabolism as a feature of acquired CTX resistance and gene set enrichment analysis (GSEA) indicated the *PPARA* gene signature to orchestrate the resistance phenotype. Acquired resistance to CTX was established in a panel of SCCHN cell lines, and they were compared to the parental cells for metabolic characterization. CTX-R cells showed higher uptake of fluorescently-labelled palmitate, enhanced oxygen consumption rate upon fatty acid treatment and increased expression of CD36, SLC27A5 and CPT1A (transporters of exogenous fatty acids and mitochondrial acyl-CoA, respectively). Neutral lipid staining with the BODIPY 493/503 dye also revealed lipid droplet accumulation in CTX-R cells. Cell viability assays highlighted the importance of extracellular fatty acids to support CTX resistance and revealed the specific toxicity of several drugs interfering with lipid metabolism in CTX-R cells. Importantly, by using either Transwell-based co-culture system or addition of conditioned medium, we also documented the protective role of cancer-associated fibroblasts (CAFs), but not healthy fibroblasts, towards CTX resistance in SCCHN cells. Flow cytometry experiments additionally indicated the active transfer of fatty acids from CAFs to SCCHN cells. Altogether, our data report a role for deregulated lipid metabolism in SCCHN cells to support acquired resistance to CTX and identify CAFs as key players to metabolically cooperate with SCCHN cells in order to support disease progression. Our work also paves the way for the use of lipid metabolism-interfering compounds as new therapeutic modalities to prevent and/or overcome anti-EGFR therapy resistance in SCCHN patients.

Early Pharmacological Profiling by Live Cell Imaging

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Abstract:

CX-A (Nanolive SA, CH) is a label-free live cell imaging automated microscope that allows the acquisition of holotomographic 3D image of cells. CX-A allows the assessment of drug responses in real time under label-free conditions. This enables a better characterization of drug effects throughout the exposure. In addition, CX-A works with 96-well plates, allowing the fast screening of several samples. The segmentation and analysis of the data which can deliver meaningful metrics with the highest biological relevance. We have envisioned the combination of CX-A experimental metrics and machine learning techniques in order to setup a methodology that could aid in the early pharmacological profiling of investigational small molecules. Our preliminary results on the scope of this novel approach will be presented.

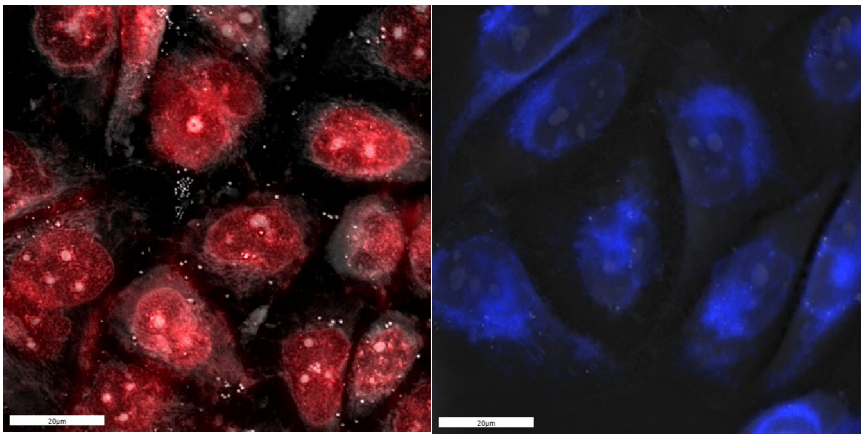

Figure 1. Differential localization of Dox (left) and Sdox (right) in SW1573 cells.

Acknowledgement: Funding from the Canary Islands Government (ProID2020010101 and EIS 2020 06_ULL, ACIISI/FEDER, UE). AP thanks the EU Social Fund (FSE) and the Canary Islands ACIISI for a predoctoral grant TESIS2020010055.

Overexpression of survivin and MRP1/ABCCC1 in R-CHOP resistant diffuse large B-cell lymphoma: prognostic and clinicopathological values

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Abstract:

Introduction: Diffuse large B-cell lymphoma (DLBCL), a highly heterogeneous lymphoma, is treated with the recommended regimen of rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone regimen (R-CHOP). However, approximately 40% of the patients experience treatment resistance and unsatisfactory clinical outcome. ATP binding cassette (ABC) transporters and survivin play role in multidrug resistance (MDR) in multiple tumour types. The study aimed to give molecular insight into the mechanisms of resistance to the current treatment options in DLBCL.

Methods: We analysed expression of Bcl-1, survivin and three ABC transporters: Pg glycoprotein/ABCB1, MRP1/ABCC1, and BCRP/ABCG2, using immunohistochemistry in 63 tumour specimens obtained from patients with DLBCL, classified as refractory, relapsed and in remission. All patients received first-line standard therapy with R-CHOP or equivalent regimen.

Results: ABCC1 and survivin intense expression were associated with refractory disease ($p = 0.01$) in 68% patients, and was marginally associated with poor failure-free survival ($p = 0.05$). After relapse, re-biopsy showed increased expression of both, ABCC1 and survivin in 42% patients. Standard Bcl-2 was in strong correlation ($r^2=0.82$, $p = 0.01$) with these biomarkers. In contrast, all tumors of patients in remission were negative for ABCC1 and survivin. Both ABCB1 and ABCG2 showed cytoplasmic expression in all DLBCL specimens without substantial difference in the intensity of expression across the groups. Moreover, a significant association were revealed between ABCC1 and survivin expression, respectively and advanced clinical stage (III + IV), higher International Prognosis Index score, elevated serum lactic dehydrogenase, and presence of bone marrow involvement together with reduced complete remission.

Conclusion: If DCLB patients harbour certain specific molecular signatures such as MRP1/ABCC1 and survivin along with advanced clinical stage that can predict resistance to R-CHOP, they must not be recommended standard first line therapy.

Diagnostic and Theraputic Autoantibody Discovery Studies for Brain Tumors

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Abstract:

Glioblastoma is one of the most lethal tumors with their therapy resistant phenotype and median survival of the patients is approximately 12-15 months. Drug resistant features and the high recurrence rate of glioblastoma makes it less curable tumor and novel biomarker studies and alternative therapy options are ultimately needed. In this study, we collected grade specific tumor and sera samples from glioma patients and those were subjected to proteomics, immunoprecipitation and immunoreactivity assays. Control samples were collected from non-tumor bearing brain tissue of epilepsy patients. Overall data showed that an autoantibody against one of the solute carrier proteins, SLC3a2; exist selectively in high grade glioma patient sera. The further *in vitro* and bioinformatical analysis of autoantibody-antigen interactions revealed that a subgroup of glioblastoma patients display a specific interaction pattern as it is compared to other high grade gliomas. In addition to that, autoantibody presence is correlated to increased survival rate of the patients. To sum up, our findings suggest that the autoantibody and the autoantigen are potential targets for glioma diagnosis and therapy. These outcomes can open up new avenues in the field and broaden our understanding of glioma biology which can then bring in novel insights and discoveries.

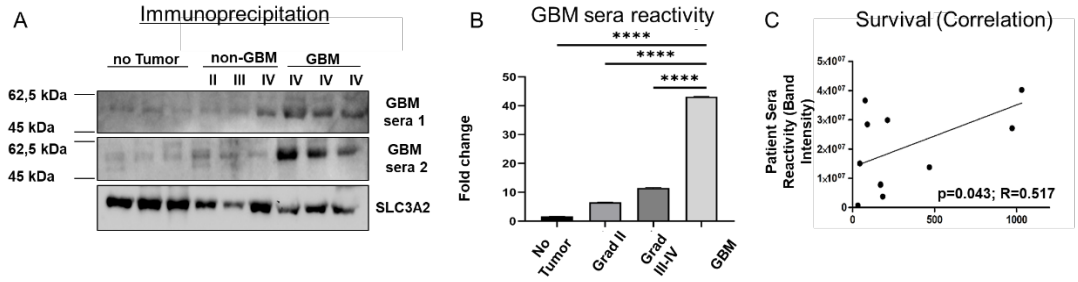

Figure 1. The autoantibody found present in GBM patients and correlated with prolonged survival.

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Design, Synthesis and Anti-tumor Evaluation of Novel Peptidomimetic Hybrids of Ciprofloxacin against multidrug resistant tumors

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Abstract:

Cancer became leading cause of death throughout the world. Due to this reason efforts on the development of anticancer agents were significantly exaggerated. The cytotoxic activity of ciprofloxacin on some cancerous cells has been recently confirmed. Considering features of peptidomimetics, such as biocompatibility and increased anticancer activity in comparison to natural peptides, we envisioned the design and synthesis of novel ciprofloxacin peptidomimetic hybrids to explore benefit of the both compounds' properties and therefore give better anticancer agents. The aim of this study was to elaborated a new and simple synthetic approach employing Passerini reaction to synthesize several new ciprofloxacin-peptidomimetic hybrids and to investigate the anticancer activity of the resultant compounds. The hybrids were prepared by Passerini reaction in reasonable yield and time. Anticancer activity of these compounds was examined on cancer cell lines and a compound 5e was found to be the most active one. General concept of the project will be show and discussed based on chemical biological data.

Acknowledgement: This work was supported by National Science Center, Poland, project OPUS no 2019/33/B/ST4/01118 and COST action CA17104

Impact of albumin-targeting on the pharmacology, tissue distribution and anticancer activity of an oxaliplatin(IV) bismaleimide prodrug in vivo

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Abstract:

The platinum(II) drug oxaliplatin is the state-of the-art therapy against advanced colorectal cancer. Nevertheless, like other platinum(II) drugs, treatment is often associated with severe side effects and drug resistance development due to lack of tumor specificity. With the aim to exploit the enhanced albumin consumption and accumulation in the malignant tissue, we have recently developed a new oxaliplatin-releasing and maleimide-containing prodrug KP2156. The maleimide moiety allows the drug to selectively bind the free thiol at cysteine 34 of endogenous albumin. In the presented study the fate and effects of the platinum(IV) drug in comparison to oxaliplatin was followed in tumor-bearing mice by several methods including cutting-edge analytical tools such as laser ablation-inductively coupled plasma-time of flight mass spectrometry (LA-ICP-ToFMS) and detection of newly synthesized isotope-labeled derivatives by nano-scale secondary ion mass spectrometry (NanoSIMS). These efforts show that the superior anticancer activity of KP2156 is based on a distinctly altered pharmacokinetic profile: A prolonged plasma half-life together with effective tumor accumulation and retention of the albumin-bound prodrug in the tumor tissue. There it is engulfed (and intracellularly activated) by the cancer cells via endocytosis. In conclusion, albumin targeting by linking maleimide to oxaliplatin is a promising strategy for tumor-specific drug delivery. Thus, KP2156 should be further developed towards clinical phase I testing.

Acknowledgement: HS was financed *via* the Austrian Science Fund (FWF) project AP32886 to PH.

References: Schueffl H, Theiner S, Hermann G, et al. Albumin-targeting of an oxaliplatin-releasing platinum(iv) prodrug results in pronounced anticancer activity due to endocytotic drug uptake in vivo. Chem Sci. 2021 Aug 26;12(38):12587-12599. doi: 10.1039/d1sc03311e.

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Posters

Characterization of DNA repair systems in acute myeloblastic leukemia

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Abstract:

Acute myeloblastic leukemia (AML) comprises a heterogeneous group of hematological malignancies that are characterized by the increased proliferation and accumulation of immature myeloid cells. The initiation and progression of malignant neoplasms arises from genomic instability. DNA damage and defects associated with its repair are the main drivers of this instability. Furthermore, altered DNA repair pathways can also induce resistance to standard chemotherapy. Thus, understanding the mechanisms of DNA damage and repair underlying AML is essential to comprehend its progression and response to therapy. The aim of this work is to evaluate the impact of DNA damage and DNA damage repair on AML. To achieve this goal, seven AML cell lines (HL-60, HEL, KG-1, K562, LAMA-84, THP-1, and NB-4) were used. Chromosome damage levels were evaluated by the cytokinesis-block micronucleus assay. To evaluate DNA repair kinetics, chromosome damage was determined by the CBMN assay immediately after exposure to hydrogen peroxide (H_2O_2), 4h and 24h after exposure. Double-strand breaks (DSBs) were evaluated by flow cytometry-based quantification of γ-H2AX. Data regarding gene mutations from each cell line was retrieved from the COSMIC database. Base excision repair function will be evaluated by ELISA-based quantification of 8-OHdG levels and with enzyme-modified fast-halo assay. Fanconi anemia repair function will be evaluated with the DEB assay. Homologous recombination repair function will be evaluated by flow cytometry-based quantification of γ-H2AX and RAD51.Results show that HEL cells have the lowest percentage of chromosome damage (3.6%) while LAMA-84 cells have the highest (8.8%). Additionally, LAMA-84 cells showed a higher frequency of nucleoplasmatic bridges, KG-1 cells showed a higher percentage of micronucleus, and the remaining cell lines presented a higher frequency of nuclear buds. After exposure to H_2O_2, cells were able to revert to their initial levels of chromosome damage after 24h, with exception of KG-1 and THP-1 cells. Moreover, sensitivity to H_2O_2 varied between the cell lines, with NB-4 and LAMA-84 being the most sensitive. Interestingly, they share a mutation in gene *PER1*, which activates the S-phase checkpoint, that is absent in the other cell lines. Levels of γ-H2AX indicated that LAMA-84 cells had the highest prevalence of DSBs, while THP-1 cells had the lowest. This results will help us to verify if DNA damage and repair could be biomarkers treatment response.

Examining the protein cargo of Extracellular Vesicles from Acute Myeloid Leukemia cell lines: searching for leukemia biomarkers – preliminary results

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Abstract:

Acute myeloid leukemia (AML) arises from the clonal expansion of undifferentiated myeloid precursors, resulting in impaired hematopoiesis and leading to bone marrow failure. The survival of undetected leukemic clones upon therapy leads to relapse, resulting in low patient survival rates. Residual leukemic cells are undetected by microscopic assessment, being assessed by flow cytometry and/or PCR techniques in the bone marrow. Additionally, AML is very heterogeneous and only a few subtype-specific AML biomarkers are known. Extracellular Vesicles (EVs) are cell-released particles isolated from several body fluids, whose content reflects the cell of origin, making them a potential source of peripheral biomarkers. This work aimed to confirm which AML biomarkers are present in the EVs shed by different subtypes of AML cell lines. For that, EVs were isolated by ultracentrifugation (UC), in triplicate, from six AML derived cell lines: KG-1a, HL-60, NB4 , ML-2 , THP-1 and MV4-11 . EVs size was analysed by nanoparticle tracking analysis (NTA) and their morphology by transmission electron microscopy (TEM). The proteins present in the cargo of these EVs were identified by mass spectrometry-based proteomics. Bioinformatic analysis is being performed and the most relevant proteins will be confirmed by Western Blot. Our results showed that UC allowed EVs´ isolation from different AML subtypes cell lines, with isolated EVs´ ranging from 100nm to 200nm (NTA), presenting a "cup-shape" morphology typical of EVs. Ongoing bioinformatics analysis of the proteomic study of EVs cargo will confirm which AML biomarkers are present in the EVs shed by different subtypes of AML cell lines, aiming to identify residual disease protein markers.

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Study of the impact of hypoxia and epigenetic modulation on mechanisms involved in chemoresistance of testicular tumors

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Abstract:

Testicular germ cell tumors (TGCTs) are among the most common malignancies in men aged 15-45 years. Successful treatment is primarily due to the high sensitivity of this solid tumor to cisplatin-based therapy (CDDP). However, 15%-20% of all patients are refractory to the treatment. There are currently no predictive biomarkers for identifying and predicting the survival of patients at high risk of failing conventional treatment. Our hypothesis is based on a set of preliminary data. miRNA microarrays identified miR-218 among significantly most upregulated in all resistant compared to all susceptible TGCT cell lines tested. Our previous omic analyzes identified both *PPP2R2A* and *PPP2R5A,* subunits of protein phosphatase 2A (PP2A), as direct targets of miR-218. Moreover, hypoxic areas with reduced oxygen levels are found in many solid tumors. Cellular adaptation to hypoxia is primarily mediated by the *HIF* family of transcriptional regulators. We suggest that upregulation of miR-218 by HIF reduces phosphatase expression and potentially contributes to aberrant modulation of homologous recombination, leading to resistance. The main goal of the project is to verify whether hypoxia and related epigenetic modulations are key regulators of signaling pathways that contribute to resistance and poor prognosis in patients with TGCTs.

We started by verifying whether increased expression of HIF isoforms is a common feature of cisplatin resistance compared to the susceptible cells, and we also evaluated phosphatase expression levels. RNA isolated from cell sediments was transcribed into cDNA using the reverse transcription method. Subsequently, we determined the relative expression of the selected genes using qPCR. In parallel, to verify the involvement of the studied regulatory axis in cisplatin resistance, miR-218 was inhibited and evoked expression changes were analyzed. Inhibition of miR-218 was achieved by gene silencing. The change in mRNA expression of selected genes was monitored by RT-qPCR.

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Generation of bipartite drug target networks derived from transcriptomic profiles to unravel anticancer drug targets and find interaction modules

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Abstract:

In clinical oncology today, a large collection of anticancer drugs are used for standard treatments and therapies. Most of these drugs are used because they target some specific proteins; but in many cases, we do not have enough knowledge about all the possible targets that each drug can affect, and it is quite relevant to explore alternative molecular mechanisms of action of these drugs. Based on pharmaco-genomic analysis, we developed a method to find out associations or interactions of anticancer drugs with specific targets (i.e., specific protein coding genes) [1,2]. We focused this analysis on a set of about one hundred FDA-approved chemical compounds used as anticancer drugs to treat different types of tumors. We calculated correlation and similarity between these drugs based on the putative molecular targets that they share. We constructed bipartite protein-drug networks derived from the significant associations found between anticancer drugs and human genes. We also provide a map of proximity or similarity between the drugs, that is based on profiles of common targets. The analyses provide new calibrated links between different anticancer drugs, rendering maps of associations between them and opening a new way to find possible common mechanims of action and possible candidates for drug-repurposing.

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Potential mechanisms of secondary resistance to hypomethylating agents in leukemic cell lines

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Abstract:

In the treatment of myelodysplastic syndromes and acute myeloid leukemia (AML), hypomethylating agents (HMAs) 5-azacytidine (AZA) and 5-aza-2'-deoxycytidine (DAC) are often used, mostly in elderly patients unsuitable for intensive chemotherapy and hematopoietic stem cell transplantation. However, many patients do not respond to the treatment due to primary resistance, while others eventually stop responding after the secondary resistance development.

In our laboratory, AZA- and DAC-resistant cell sublines were developed from AML cell lines MOLM-13 and SKM-1. We studied the expression of genes encoding proteins involved in HMA transport and metabolism in all our cell variants. At the mRNA level we did not observe differences in AZA-/DAC-resistant sublines compared to parental cell lines. However, differences were observed at the protein level. Deoxycytidine kinase (DCK), enzyme necessary for DAC activation, is downregulated in both DAC-resistant cell variants. These sublines are cross-resistant to two other antineoplastic drugs dependent on activation by DCK, but not to the second HMA, AZA, that is activated by different kinase. Unlike DAC-resistant sublines, our three AZA-resistant sublines differ significantly from each other. While one does not show any cross-resistance to DAC, the other is slightly less sensitive to this drug than the parental cell line, and the third shows strong cross-resistance to DAC. These cell variants also show differences in response to co-treatment with AZA and the inhibitor of *de novo* pyrimidine synthesis. These results suggest that there may be more than one mechanism of resistance towards AZA that can occur during long-term treatment with this drug.

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miR-30a-3p and miR-92a-3p as potential diagnostic biomarkers in parathyroid carcinoma

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Abstract:

Although one of the rarest known malignancies, with an estimated prevalence of 0.005% of all cancers, parathyroid carcinoma (PC) represents a clinical and therapeutic challenge. The identification of novel diagnostic biomarkers able to preoperatively distinguish among different parathyroid neoplastic types is of great clinical importance. However, there is a lack of both experimental and bioinformatics data in this field. In the present study, we examined the expression levels of two microRNAs frequently implicated in cancer, miR-30a-3p and miR-92a-3p, in 10 PC and 10 parathyroid adenoma (PA) tissues using reverse transcription quantitative (RT-qPCR). In addition, we analyzed their association with clinical and histopathological parameters. For statistical analysis we used non-parametric tests – Mann–Whitney U test and Spearman's correlation test. Furthermore, by using several online tools such as miRNet, miRror Suit, and DisGeNET we analyzed combinatorial target genes of the two miRs and investigated their potential roles in parathyroid carcinoma and adenoma. The expression levels of both miR-30a-3p and miR-92a-3p were upregulated in PC compared to PA ($p= 0.017$ and $p=0.0015$ respectively). A positive correlation between their expression levels was identified both in PC ($p=0.025$, $r=0.697$) and PA ($p=0.008$, $r=0745$), indicating their combined action. There were no significant associations between the expression levels of these two miRs and clinicopathological characteristics in the PC group. According to the in silico analysis, the two miRs share a number of target genes, predominantly included in gene expression, cell cycle regulation, and several signalling pathways such as WNT/β catenin, which was found to be frequently aberrant in PC. Although their role and diagnostic utility remain to be elucidated, the data here reported suggest that miR-30a-3p and miR-92a-3p might be involved in parathyroid cancer pathogenesis and that they might be candidates for differential diagnosis between parathyroid carcinoma and adenoma.

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Peripheral immune profiling of Soft Tissue Sarcoma: comparison between trabectedin and anthracycline-based chemotherapy

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Abstract:

Studies regarding the immune profiling of soft tissue sarcoma (STS) are sparse and the great majority do not consider the periphery nor the patient therapy.

Our work aimed to assess the effect of anthracycline and trabectedin-based chemotherapy on peripheral immune profiling overall survival of 31 STS patients. We used deep immunophenotyping, transcriptomics and soluble proteomics associated with immune response. We report here impacts on the immune cell composition and receptor repertoire, the expression of immune-related genes and the levels of soluble immune-related factors when the therapies and the number of trabectedin cycles were compared.

Our results suggest that trabectedin promote the activation of CD4 T cells and the increase of cytotoxic NK cells (CD56dim) with the upregulation of FCGR3A (CD16), and the decreased expression of the T cell-attractant chemokines CCL3 and CCL4. Trabectedin also stimulate suppressor memory regulatory T cells (Treg) and monocytic myeloid-derived suppressor cells (M-MDSC), and the expression of IL1B, SELL, and CXCL1. Higher soluble levels of the immune checkpoints PD-L2 and B7-H2 were found associated with long-term trabectedin therapy and were correlated with better overall survival.

Our results suggest that both the chemotherapeutic agent and the therapy duration impacts the immunological status of STS patients.

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The role of genetic variability and gene alterations associated with chemoresistance in testicular germ cell tumors

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Abstract:

During neoplastic progression, genetic changes in tumor cells play an important role in response to treatment of multiple human malignancies. Gene changes, whether germline or somatic mutations, alter gene function, which may contribute to the development or progression of malignancy as well as therapy resistance. Several risk loci have been identified in germ cell tumors (GCT), but genomic traits contributing to the onset and progression or phenotype of chemosensitivity / resistance remain incomplete. As for now, testicular germ cell tumours (TGCTs) do not show a significant number of mutations, but the development of advanced high-throughput sequencing technology has allowed profiling and successfully identifying new markers. More than 95% of human genes undergo alternative splicing (AS) as a normal physiological process to generate protein diversity. Moreover, accumulating evidence supports the significance of aberrant AS events in cancer; however, genome-wide profiling of progression-free survival (PFS)-related AS events in TGCT has not been reported.

To determine the potential impact of possible genetic changes on treatment response in TGCTs, we analyzed CDDP sensitive TGCT cell lines compare to CDDP resistant TGCT cell lines using the next generation sequencing (NGS) method and we focused first on the mitochondrial genome. In parallel, we focus on analysis of AS, we have started with analysis of excision repair cross-complementation group 1 (ERCC1) gene and its four isoforms resulting from AS. ERCC1 is an important factor of nucleotide excision repair (NER) limiting the rate of repair of DNA damage caused by cisplatin (CDDP). Among four isoforms generated by AS, only one ERCC1 isoform is expected to be functional in DNA repair. Our results can contribute to better understanding of cisplatin resistance, as accumulated mutations can have a significant impact on therapy response of patients and provide potential novel biomarkers of chemoresistance.

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Circulating microRNAs as drug resistance biomarkers in Multiple Myeloma patients

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Abstract:

Although new drugs have been approved and are widely used for the treatment of Multiple Myeloma (MM), a small percentage of patients who do not respond to initial therapy are considered to have primary refractory MM. The current treatment algorithm fails to identify these patients, and there are no consensual biomarkers. Interestingly, microRNAs are key regulators of several pathways, by targeting mRNAs which include oncogenes and tumour suppressor genes, thus impacting drug response in cancer - including in MM. Thus, microRNAs are promising candidates as MM biomarkers. Our study aims to identify microRNAs circulating freely in the plasma, with the potential to be used as non-invasive biomarkers of drug resistance in MM patients. Fourteen patients were included in this study, having newly diagnosed MM and who were treated upfront with immunomodulators, proteasome inhibitors, and steroids. Peripheral blood (PB) and bone marrow (BM) samples were collected simultaneously and before any treatment was started. The miRNAs were extracted using the miRCURY™ RNA Isolation Kit. The microRNA profiling was possible by Next Generation Sequencing (NGS) using the Ion Total RNA-Seq kit v2 protocol. The miRNAs found to be differentially expressed between PB and BM samples, and differentially expressed between samples from responders and non-responders were selected for confirmation of results by quantitative real-time PCR. Eight patients were identified as drug resistant, and six as drug responders to the upfront therapy. By NGS, we found different expressions of microRNA between PB and BM samples and between samples from responders and refractory patients. Five microRNAs (miR-145-5p, miR-186-5p, miR-143-3p, and miR-214-3p) were found more expressed in BM, and one microRNA (miR-203a-3p) was found less expressed in BM. Moreover, two microRNAs (miR-483-5p and miR-665) were more expressed in refractory patients than in responders. These results were confirmed (regarding the same trend in expression) by quantitative real-time PCR. These proof-of-concept results demonstrated that it is possible to isolate microRNAs from the plasma of patients with MM and suggested that the levels of specific circulating microRNAs may become biomarkers to identify patients with possible refractory disease and adjust their therapeutic strategy accordingly. Of the identified microRNAs, miR-483-5p is known to be upregulated in MM and associated with neoplastic plasma cell proliferation and escape from apoptosis [1]. Likewise, miR-665 targets BLC2, MAPK3 and STAT3 mRNAs, which is associated with resistance to immunomodulators and proteasome inhibitors [2]. Future work will validate these results in a larger cohort of MM patients and investigate the role of these differentially expressed microRNAs in drug resistance.

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Identification of MDR Related miRNAs in Calorie Restriction Applied Ageing Mice Brain

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Abstract:

Small noncoding miRNAs may play an essential role in chemotherapy effectiveness since they are epigenetic regulators of cancer progression, metastasis, and tumor microenvironment According to recent research, multiple drug resistance (MDR) genes are repeatedly increased in chemotherapy resistant cancer cells, leading to increased chemotherapeutic drug efflux. Likewise, studies have shown that miRNAs regulate such processes in cancerous cells by targeting MDR genes. Calorie restriction (CR) is an important intervention that prevents age-related diseases including various cancer types. Nevertheless, there have been few studies on the effects of CR on miRNAs that are linked to MDR genes. Hence, the current study sought to determine the effects of two different types of calorie restriction on miRNAs associated with MDR genes. In this study, female C57BL/6 mice were applied to three different dietary regiments; ad libitum (AL), chronic calorie restriction (CCR) and Intermittent calorie restriction (ICR) feedings. Brain samples were removed at 10, 49/50, and 81/82 weeks of mouse age. RNA samples were isolated, and an Affymetrix GeneChip miRNA 4.1 microarray was conducted to assess changes in miRNA expression levels in the brain in response to various types of CR application. The relationship between miRNA and MDR gene target was built by combining several computational public target prediction databases for any significantly regulated miRNA. As a result of differential miRNA analysis comparing calorie restricted groups (both CCR and ICR) to AL group, we found that a total of nine miRNAs targeted six different MDR genes namely ABCB11, ABCG2, ABCG4, ABCB10, and ABCB7, at week 49/50, and a total of five miRNAs targeted five different MDR genes namely ABCC5, ABCC4, ABCB7, ABCC6, and ABCC12 at week 81/82. Compared to the AL group at week 49/50, the expression levels of miR-670-5p, and miR-10a-5p were downregulated while at week 81/82, miR-7069-3p were upregulated in the CCR group which had less mammary tumor incidence rate. These results indicate that miR-7069-3p may play an important role in the regulation of MDR in cancer and suggest that it could be used to predict drug resistance. Another finding is that the manner in which calories are consumed may play an important role in the regulation of MDR genes in a mouse cancer model.

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Prognostic Significance of SERPINB1 Expression in Gliomas

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Abstract:

Glioblastoma (GB) is the most prevalent brain tumor with high morbidity. Determination of tumor initiative and prognostic factors are ultimately needed for therapy options. The effects of Serine Protease Inhibitor (SERPIN) B1 on tumor progression have been reported in several cancer types. However, the relation between SERPINB1 expression levels and glioma progression is still to be elucidated. In this study, we aimed to determine the SERPINB1 expression levels in glioma patients and investigate its prognostic effect on patient survival. We first examined the expression of SERPINB1 in glioma patients (n=37) with different grades (according to WHO classification) by western blotting and immunohistochemistry. Expression of SERPINB1 in tissue lysates was significantly higher in glioblastoma samples than in low grade glioma (p=0,0056). Additionally, SERPINB1 overexpression was associated with high glioma grades in the overall pattern. In addition, cisplatin treatment was applied to both wild type and SerpinB1 overexpressed GBM cell lines showed less therapy response. Survival analysis by using TCGA (French) Glioma cohort data (303 patients) showed that the mRNA expression of SERPINB1 correlates with decreased survivals in both GBM and non-GBM patient groups (p< 0.0001). Our results showed that the SERPINB1 expression is significantly high in glioblastoma patients correlated with poor prognosis. Here, we suggest that SERPINB1 may be a promising biomarker for gliomas and may further investigations will provide more knowledge about its function and mechanism of action.

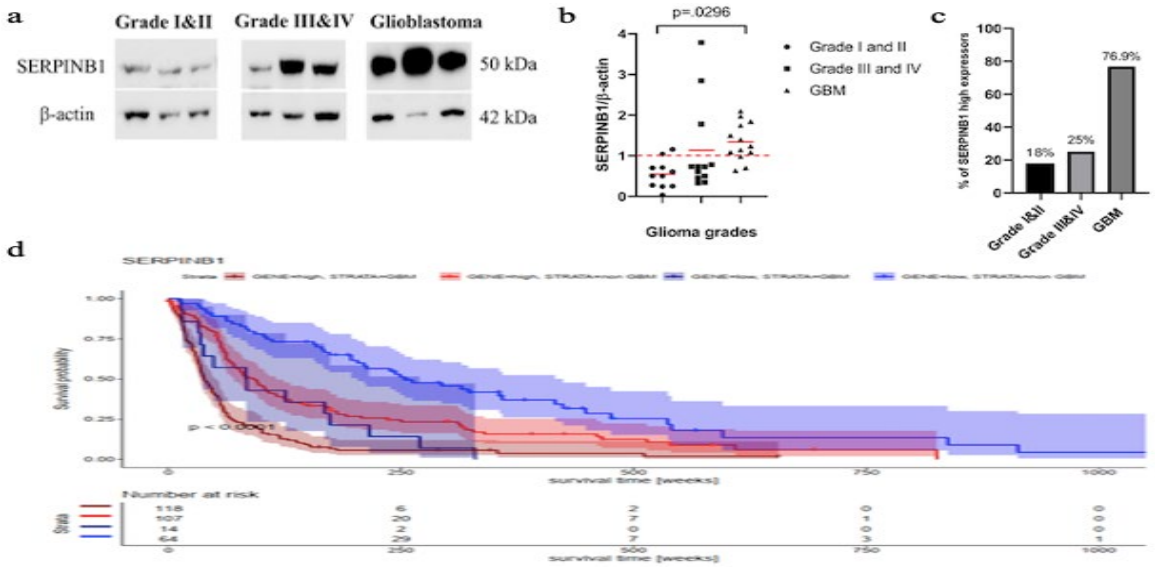

Figure 1. SERPINB1 expression differs in all glioma grades and SERPINB1 high expression in high grade gliomas is linked to poor prognosis.

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RNASeq as an instrument for ovarian cancer research

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Abstract:

Epithelian ovarian cancer (EOC) is one of the most common type of gynecological cancer among women. Due to late diagnosis and development of drug resistance, the overall suvival in advanced stages is very low. The aim of this study was to develop and optimize the next generation sequencing technology for analysis of transcriptome in primary and interval debulking surgery collected tumor EOC samples.

As the best option for preparation of RNA seq libraries was selected in our laboratory Lexogen QuantSeq 3' mRNASeq Library Prep Kit FWD for Illumina. According to the amount of RNA standard or low input protocol was used. The first strand of cDNA was synthesised followed by RNA removal and second strand synthesis. Purification of synthetisized dsDNA was based on magnetic beads. Before library amplification qPCR was used for calculation of the right number of PCR cycles. Low input samples are usually very different from each other, thus the calculation of the optimal cycle number for endpoint PCR is a necessity. Endpoint PCR for amplification of RNASeq libraries also allows to mark each sample with specific primer. The last step in library preparation was once again purification on magnetic beads.

For quality control of prepared libraries High Sensitivity DNA kit from Agilent was used. The last step before the prepared samples sequencing on NextSeq platforma was pooling of libraries. EdgeR package and R tools were used for analysis of RNASeq data. Analysis of RNA seq data revealed potential novel significant prognostic and predictive biomarkers of ovarian carcinoma, i.e. *MYH11, JAK2, SETDB2, SUCNR1, IRAG2, CCN5, C1orf21, FOXP2, GCNT3, KCNN3, LGI4, EGFLAM* or *HPGDS.*

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Targeting cancer multidrug resistance in lung cancer cells with Ru cyclopentadienyl compounds: a structure-activity study

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Abstract:

The effectiveness of drugs in cancer therapy is a clinical problem and is mainly due to the development of multidrug resistance (MDR). One of the main mechanisms of MDR is the overexpression of ATP Binding Cassette (ABC) efflux transporter proteins. As a solution to overcome this limitation, our research group has been devoted to developing prospective "Ru-cyclopentadienyl" ("RuCp") metallodrugs. Several have been already emphasized as promising transporter proteins inhibitors [1-3]. In a recent structure activity study, fourteen compounds with general formula $[Ru(\eta^5\text{-}C_5H_4R)(bipy)(PPh_3)]^+$ (R = H, $-CH_3$, CHO, CH_2OH or CH_2Biotin and bipy = 2,2' bipyridine functionalized ligands) were tested against four types of non-small cell lung cancers (NSCLC) with different expression levels of P-gp and MRP1 transporters namely, A549, NCI H228, Calu-3 and NCI-H1975. Among them, six compounds presented remarkable activity towards cisplatin resistant NSCLC cells. Focused on the mechanism of action of these compounds, we found that, when administered at non-cytotoxic doses, they were able to increase cisplatin cytotoxicity in resistant cells by targeting P-gp and MRP-1 transporters, thus inducing collateral sensitivity. In addition, some of them significantly inhibited the migration of endothelial EA.hy926 cells, however, without increasing the number of dead cells. Molecular docking calculations with a set of "RuCp" drugs helped to identify the best binding pockets in P-gp and correlate their binding affinities with the experimental data. As a result, by using *in silico* and cell-based studies, we will show how small modifications in the compounds' structure drastically affect the anticancer activity against NSCLC.

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Synthesis of Therapeutic Antibody-Drug Conjugates

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Abstract:

The selective delivery of highly effective drugs by antibody drug conjugates (ADC) is a promising approach for the treatment of cancer. ADCs consist of monoclonal antibodies (mAbs) conjugated to the cytotoxic agent via a linker, thus combining the potent action of cytotoxic drugs with the specific therapeutic power that mAbs alone provide on the target. The payloads conjugated to monoclonal antibodies are activated after it is taken into cancer cells so that the side effects and multiple drug resistance (MDR) of drugs have been minimized. For these reasons, ADCs emerge as more effective and safer drug candidates compared to current chemical drugs used in cancer treatments.

In the study, mAbs highly expressed by ovarian cancer cells have been homogeneously conjugated to the cytotoxic drug using click chemistry (Figure 1). In addition to using the high selectivity target antibody, using a polyethylene glycol (PEG) group to impart a hydrophilic structure to the linker aimed to overcome the MDR of the ADC to be synthesized.

Until now, endoglycosidase and mutant enzyme have been produced and their activities were demonstrated by SDS-PAGE and LC-MS. These enzymes were used to hydrolyze the glycans on the mAb, and to transfer the azido-labeled glycans produced to the same place again, respectively. The produced azido-labeled glycans were characterized using RP-HPLC and NMR. After synthesis and full characterization of ADCs, their efficacy will be demonstrated by in vitro studies in ovarian cancer cell lines.

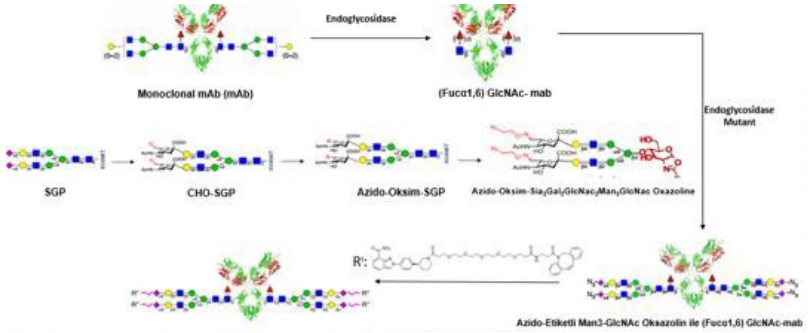

Figure 1. The study outline.

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New protoflavone hybrids overcome cancer resistance connected to mutant p53

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Abstract:

Breast cancer is the most common type of cancer affecting over 2 million women each year. Triple-negative breast cancer is the most difficult subtype to treat as current treatment options are limited and need chemotherapeutic agents. New treatment options are essential. Protoflavones (natural flavonoids with a para-quinol B ring) were shown to induce apoptosis and inhibit the ataxia-telangiectasia and Rad3-related protein (ATR)-dependent signalling and so the activation of checkpoint kinase 1, an important step in DNA damage response [1].

In most cancer cells the p53 protein is inactivated or mutated and so cannot function as a tumor suppressor. Some tryptophanol-derived oxazoloisoindolinones are able to restore wild-type-like function to mutant p53 [2]. ATR inhibitors, on the other hand, are selectively toxic to cancer cells expressing mutant p53 [3].

In this study we aimed to prepare new hybrid compounds by combining the above mentioned two types of compounds with different antitumor mechanism to achieve increased antitumor activity. Utilizing modified sidechains and copper(I) catalysed alkyne-azide cycloaaditional reactions (click-reaction), we synthesized 6 new hybrid compounds. On MTT antitumor tests one of the best compounds showed 0.7 ± 1.1 and 0.5 ± 1.0 μM IC50 values on MCF-7 and MDA-MB-231 cell lines. Tests on HCT116 cell lines have shown that even p53(-/-) cells are not resistant to the new hybrids.

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Andrographolide Derivatives for Targeting Multidrug Resistant Cancer Cells

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Abstract:

Cancer is the second most common cause of mortality and morbidity worldwide. Despite the advances in cancer treatment, drug resistance remains a challenging task for medicinal chemists. Aiming at developing new compounds for overcoming drug resistance, a set of derivatives of andrographolide, a major constituent of *Andrographis* species (Acanthaceae), was prepared. Twenty-one new triazoles were obtained by introducing an azide group into the acetylated derivative of andrographolide and subsequent reaction with alkynes. The compounds were elucidated mainly by NMR, including bidimensional (COSY, HMBC, HSQC) experiments. The cytotoxicity of some of these compounds was evaluated against a panel of human cancer cell lines (Panc-1, MCF7, MDA-MB-468, and IGROV-1) and were found to be cytotoxic, displaying IC_{50} values < 10 μM. They were also evaluated against a non-malignant cell line HFF-1 and some of the derivatives were found to be slight selective.

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Modulation of Multidrug Resistance by Flavonoids

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Abstract:

Multidrug resistance (MDR) to chemotherapeutic agents and antibiotics is a hot topic in drug development, and there is strong evidence that flavonoids and flavonolignans may modulate the transporters involved in MDR.[1,2] Our objective was to investigate MDR modulation by selected flavonoids, flavonolignans and their derivatives. The silymarin components silybins and 2,3-dehydrosilybins, silychristin A, anhydrosilychristin, isosilychristin and 2,3-dehydrosilychristin A inhibited P-glycoprotein (P-gp, ABCB1). In doxorubicin-resistant ovarian carcinoma cells overproducing P-gp, the sensitization was observed mainly for 2,3-dehydrosilybin A, silychristin A, and 2,3-dehydrosilychristin A. Anhydrosilychristin and isosilychristin affected the expression of P-gp and ABCG2 genes. Silybin B acted directly on P-gp and down-regulated the expression of P-gp, MRP-1 and BCRP.[3,4] In MDR *S. aureus*, silybin A, 2,3-dehydrosilybin B, and 2,3-dehydrosilybin AB completely reversed antibiotic resistance at ≤ 20 μM. 2,3-Dehydrosilybin B and AB decreased gene expression of efflux pumps from the major facilitator (MFS), multidrug and toxic compound extrusion (MATE), and ATP-binding cassette (ABC) families. 2,3-Dehydrosilybin B also inhibited NorA and MdeA-mediated ethidium bromide accumulation and antibiotic-induced efflux.[5] Finally, brominated derivatives of silybin and 2,3-dehydrosilybin[6] reversed the resistance of *S. aureus* to gentamicin and of *P. aeruginosa* to colistin.[7] Therefore, flavonoids and flavonolignans are promising agents for modulating bacterial resistance to antibiotics in particular.

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Compounds from Argentinian native plants with the ability to reverse multidrug resistance mediated by P-gp

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Abstract:

Permeability glycoprotein (P-gp) is an important ATP-dependent transmembrane transporter whose overexpression leads to low efficiency of numerous chemotherapeutic agents against cancer cells. To date, several substances have been tested as inhibitors of this pump, but none of these have passed phase III clinical trials. This is one of the reasons why natural products and their derivatives have received increasing attention in recent years.

New molecules with the ability to reverse multidrug resistance (MDR), mediated by P-gp, have been obtained from native plants from Argentina. The lignan pinoresinol (**1**), isolated from *Melia azedarach* and the triterpene betulin (**2**), isolated from *Ligaria cuneifolia*, showed by themselves inhibitory properties on P-gp function[1,2]. In order to identify derivatives with improved activity with respect to these compounds, molecular modelling studies were performed and according to the information obtained, a panel of analogues were synthetized. These compounds were tested against K562 human myelogenous leukemic cells and its overexpressing P-gp counterpart, Lucena 1.

The results obtained showed that 8-acetoxypinoresinol, was 64 times more effective than compound **1** according to the minimum effective concentrations (MECs) obtained by the reversal assay (0.11 and 7 μM, respectively) and the doxorubicin (Dox) accumulation assay (0.87 and 56 μM, respectively). On the other hand, Bet-38 and Bet-42 were 4- and 8-times, respectively more active than compound **2** (MECs 0.39 and 0.19 and 1.56 μM, respectively) for increasing the intracellular rhodamine 123 accumulation in Lucena 1. In addition, Bet-38 and Bet-42 were shown to be 2 and 16 times, respectively more effective than the lead molecule (MEC 0.39 μM) in reversing Dox resistance.

It should be noted that none of the mentioned compounds enhanced the effect of Dox in the K562 cell line and they even showed the same and/or better level of activity ($p > 0.05$) than that observed with the reference molecule, verapamil.

In conclusion, this work demonstrates the importance of natural products as a source of bioactive molecules and repositions these entities as leading compounds for obtaining derivatives capable of reversing MDR phenotype, mediated by the P-gp protein.

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Using computational methods to evaluate the role of TME acidity in MDR

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Abstract:

Targeted cancer therapeutics remain a central goal of cancer research. The tumor microenvironment (TME) is an important component of tumor development that influences several key processes such as tumor cell phenotype, proliferation, immune evasion, and drug resistance [1]. An important feature of the TME is the increased acidity of the extracellular milieu (pH 6.0-6.8), generated by enhanced anaerobic glycolysis coupled with higher levels of proton extrusion via upregulated proton pumps. This process creates a pH gradient between the extracellular and intracellular environments, potentially creating a barrier for hydrophobic Lewis base drugs to enter the cells. The high pK_a values of these compounds (7.5-9.5) including for example some tyrosine kinase inhibitors, like sunitinib and nintedanib, require them to first undergo deprotonation before passively diffusing through the plasma membrane into the cells, which may become more difficult in acidic microenvironments, like the TME.

This study aims at investigating the pH-dependent membrane insertion mechanism and quantifying the impact of the TME on the membrane permeability of two well-known chemotherapeutics, sunitinib and nintedanib. We propose a new protocol based on Constant-pH Molecular Dynamics [2] coupled with an Umbrella Sampling scheme (US-CpHMD) [3] and applied it to the Lewis-base drugs interacting with a POPC lipid bilayer. The membrane permeability coefficients were calculated using the inhomogeneous-solubility diffusion model [4]. The calculations were performed at different pH values, namely 7.5 to mimic a healthy cell, 6.0 to model the TME acidity, and 4.5 to capture the strong acidity of the lumen of the lysosome. The latter can provide some insights into the lysosomal sequestration phenomenon, which has been proposed as a drug resistance mechanism [1]. We have calculated the impact of acidity on the bioavailability of both sunitinib and nintedanib, which will help us design a new compound as a proof of concept that can circumvent these limitations.

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Cell Cycle and Apoptotic Effect of Doxorubicin in Melanoma Cells

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Abstract:

Melanoma, the most aggressive type of skin cancer, has an increasingly higher incidence in the population, and when detected in advanced stages has a poor prognosis with conventional treatments (Tang et al., 2017). Upconversion nanoparticles (UCNPs) have unique properties allowing their use in several biomedical applications. One of these applications is targeted drug delivery, where UCNPs are loaded with anticancer drugs, such as DOX, improving tumour specificity and sustained released. This work aimed to evaluate the cell cycle and apoptotic effects of free doxorubicin (DOX), a chemotherapeutic agent for melanoma treatment, on four melanoma cell lines (A375, SK-MEL-28, MNT-1 and B16-F10). Our results showed that DOX have the ability to induce damage in different melanoma cell lines. It also demonstrates its potential to be used in upconversion systems with the capability to be used as drug delivery systems to melanoma therapy.

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Cytotoxic Activity of diterpenes from *Plectranthus* spp. for MDR cancer therapy

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Abstract:

Plectranthus genus (Lamiaceae family) is widely used in traditional medicine, and the presence of pharmacologically active compounds, specifically diterpenes, is well reported. The cytotoxic diterpene royleanones 7α-acetoxy-6β-hydroxyroyleanone (**Roy**) and 6,7-dehydroroyleanone (**DeRoy**) are the major compounds of *P. grandidentatus* Gürke (acetonic extract) and *P. madagascariensis* (Pers.) Benth. (essential oil), respectively. In this work, **Roy** and **DeRoy** were investigated as potential antitumor agents through the activation of protein kinase C (PKC) isoforms (α, βI, δ, ε and ζ) and inhibition of the efflux pump, P-glycoprotein (P-gp). Additionally, the reactivity of **Roy** and **DeRoy** was explored to synthesize a library of new derivatives to be also evaluated as cytotoxic agents. PKC-α, βI, δ, ε, and ζ activation was tested on a yeast-based screening assay. Interestingly, one benzoylated derivative showed selective PKC-δ activation, while **DeRoy** exhibited enhanced PKC activity in all tested isoforms, compared to the positive control. Moreover, inhibition of P-gp activity was evaluated in human non-small cell lung carcinoma NCI-H460 and its MDR counterpart NCI-H460/R. It was possible to identify an analogue with P-gp inhibitory activity higher than the natural diterpenes **Roy** and **DeRoy**, and comparable to Dexverapamil (positive control). Several other semi-synthetic products are currently under investigation as potential chemotherapeutic agents.

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Anticancer Activity of RS4690, a New Dishevelled 1 Inhibitor

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Abstract

Wingless/integrase-11 (WNT)/β-catenin pathway is a crucial upstream regulator of a huge array of cellular functions. Its dysregulation is correlated to neoplastic cellular transition and cancer proliferation. Members of the Dishevelled (DVL) family of proteins play an important role in the transduction of WNT signaling by contacting its cognate receptor, Frizzled, via a shared PDZ domain. Thus, negative modulators of DVL1 are able to impair the binding to Frizzled receptors, turning off the aberrant activation of the WNT pathway and leading to anti-cancer activity.

Through structure-based virtual screening studies, we identified racemic compound RS4690 (1), which showed a promising selective DVL1 binding inhibition with an EC_{50} of 0.74 ± 0.08 μM. Molecular dynamic simulations suggested a different binding mode for the enantiomers.

In the in vitro assays, enantiomer *(S)*-1 showed better inhibition of DVL1 with an EC_{50} of 0.49 ± 0.11 μM compared to the (*R*)-enantiomer. Compound *(S)*-1 inhibited the growth of HCT116 cells expressing wild-type APC with an EC_{50} of 7.1 ± 0.6 μM and caused a high level of ROS production. These results highlight *(S)*-1 as a lead ompound for the development of new therapeutic agents against WNT-dependent colon cancer (Table 1 and table 2).

Table 1. DVL binding and WNT Pathway Inhibition by Compounds **1**, (*S*)-**1** and (*R*)-**1**

	EC_{50} ± SD (μM)[a]	EC_{50} ± SD (μM)[b]
Compd	DVL1 binding Inhibition	WNT Pathway Inhibition
1	0.74 ± 0.08	3.46 ± 0.07
(*S*)-**1**	0.49 ± 0.11	3.09 ± 0.05
(*R*)-**1**	29.5 ± 0.9	19.49 ± 0.06

[a]Inhibition of the DVL1 binding. [b]Inhibition of the WNT pathway.

Table 2. Growth Inhibition of SW680, SW620 and HCT116 Hman Colon Carcinoma Cell Lines by Compounds **1**, (*S*)-**1** and (*R*)-**1**

	EC_{50} (μM)/cell lines		
Compd	SW480[a]	SW620[a]	HCT116[b]
1	39.17 ± 1.58	38.54 ± 1.6	15.2 ± 1.1
(*S*)-**1**	54.95 ± 2.2	45.9 ± 2.0	7.1 ± 0.6
(*R*)-**1**	59.47 ± 2.1	54.75 ± 1.9	28.3 ± 1.2

[a]Incubation time was 72 h. [b]Incubation time was 48 h.

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Investigation about non-canonical G-quadruplex stabilization as an alternative approach to PARP1 inhibition

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Abstract:

PARP1 is a nuclear enzyme involved in DNA repair processes. Since its inhibition causes sensitization to DNA damaging chemotherapy (the so-called "synthetic lethality"), several inhibitors have been recently developed and exploited for clinical use. However, the emergence of resistance to PARP1 inhibitors increased the interest towards alternative approaches able to interfere with PARP1 activity. In particular, the promoter region of PARP1 gene was mapped and a characteristic, non-canonical G-quadruplex-forming sequence was identified. A strong correlation between G-quadruplex stabilization in gene promoters and transcriptional regulations has been proposed for several oncogenes. Since no PARP promoter modulators have been identified so far, we were intrigued by the possibility that appropriately designed molecules could allow PARP1 inhibition by a different mechanism. In fact, stabilization of G4 of the promoter region could cause an upstream down-regulation of PARP1 itself and a consequent, less efficient answer to DNA damage in cancer cells. Given the peculiar characteristics of PARP1 G4, its interaction with a small collection of G4 binders was investigated, taking into account that a variegated structural diversity could be helpful in the rationalization of the key molecular features required for an effective binding. Six compounds, extensively studied and known for showing great affinity towards canonical G4, were selected and NMR, CD, and fluorescence titration studies were carried out. The obtained results demonstrated that widely functionalized flat molecules with flexible chains were not able to perturbate nor stabilize the structure; whereas planar, more compact, and less extended systems demonstrated to establish specific interactions with the external G-tetrads. These results confirm that the structural requirements for an optimal interaction between the ligand and this peculiar G4 portion are quite strict. Nonetheless, this could be considered as a key starting for the identification and characterization of ligands able to selectively interact with the PARP promoter G4.

Changed nucleotide metabolism in cancer cells with acquired Triapine resistance

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Abstract:

The α-*N*-heterocyclic thiosemicarbazone Triapine is a clinically investigated anticancer metal chelator and well known as a potent inhibitor of the ribonucleotide reductase (RNR). In clinical trials, Triapine showed promising activity against haematological malignancies, but not against solid cancers, possibly due to resistance mechanisms. Previous studies already demonstrated the loss of phosphodiesterase 4D (PDE4D) as an important genetic alteration in resistance development against Triapine [1]. As PDE4D affects several cellular metabolic pathways, the aim of this study was to investigate whether Triapine-resistant cells support growth and proliferation by reprogramming their metabolism. Interestingly, mRNA expression arrays revealed that Triapine-resistant cancer cells harbour an altered glucose metabolism compared to parental sensitive cells. Subsequent flow cytometry experiments confirmed an increased glucose uptake in the resistant cells. In line with RNR being the main target of Triapine, we observed not only an upregulation of RNR on mRNA as well as protein level, but also a significant increase of the dCTP pools in resistant compared to parental cells. Inhibition of different metabolic pathways with pharmacological inhibitors indicated an altered nucleotide metabolism in particular in the *de novo* pyrimidine and purine synthesis pathway. Finally, changes in levels of intracellular metabolites between sensitive and resistant cells were further confirmed by analysing metabolites in a targeted LC-MS metabolomics workflow. Consequently, our results indicate that development of acquired resistance towards the clinically investigated Triapine results in an altered cellular metabolism that could be exploited for specific combination treatment strategies.

Acknowledgement: Alessia Stefanelli is financed by an DOC Funds fellowship by the Austrian Science Fonds (FWF).

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1- Miklos, et al. (2014). Loss of phosphodiesterase 4D mediates acquired triapine resistance via Epac-Rap1-Integrin signaling. Oncotarget. 7. 10.18632/oncotarget.11821.

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In vitro and *in vivo* efficacy of novel Stony Brook taxane derivatives on the model of ovarian carcinoma

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Abstract:

Taxanes are key players in the treatment of various types of cancer. The development of multidrug resistance remains a serious problem in therapy. Therefore, novel taxane derivatives are being developed to overcome the resistance. This study aimed to analyse *in vitro* and *in vivo* efficacy of second and third generation of Stony Brook taxanes (SB-Ts) in ovarian carcinoma models.

We used three ovarian carcinoma cell lines – the multidrug resistance NCI/ADR-RES model, sensitive model SKOV-3 and paclitaxel-resistant model SKOV-3/RES. All cell lines were incubated with different SB-Ts and the viability of cells was measured with CellTiter-Blue Cell Viability Assay. Based on IC_{50} results, SB-T-121605 and SB-T-121606, were chosen for *in vivo* experiments, where all three cell lines were used for the establishment of CDX tumours in immunodeficient nude (nu/nu) mice model. All tumour-bearing mice were treated with 10 mg/kg of paclitaxel (PTX) or combination of PTX and SB-T derivative (9 + 1 mg/kg, 7 + 3 mg/kg, or 5 + 5 mg/kg of PTX + SB-T) twice a week. The efficacy of the treatment was analysed weekly by measuring tumour volume.

Both SB-Ts in combination with PTX, but not PTX alone, slowed down the tumour growth or even reduced the tumour volume at lower doses (<3mg/kg) *in vivo*.

In conclusion, SB-T-121605 and SB-T-121606 are very promising candidates for further studies, that could potentially lead to the development of novel cancer therapeutics effective in the therapy of taxane-resistant tumours.

Acknowledgement: This study was supported by the Czech Ministry of Education, Youth and Sports programs INTER-COST, project no. LTC19020 (COST Action CA17104 STRATAGEM), and INTER-ACTION, project no. LTAUSA19032, the Czech Science Foundation, project no. 21-14082SS, and the National Institutes of Health (NIH) U.S.A. grant R01 no. CA103314.

The impact of glutaminolysis inhibition in monotherapy and in combination with epigenetic modulators in chronic lymphocytic leukemia – Preliminary results

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Abstract:

Chronic lymphocytic leukemia is a lymphoproliferative disorder of clonal B lymphocytes and the most common leukemia in western countries. Glutaminolysis is a metabolic pathway that is augmented in multiple tumors, since it allows the synthesis *de novo* of crucial molecules for tumor progression. Glutamine has demonstrated a role in the formation of 2-hydroxiglutarate, in cells overexpressing glutaminase. 2-HG is an oncometabolite that inhibits dioxygenases that mediate epigenetic events, including DNA demethylation and histone acetylation. In CLL, glutaminolysis and epigenetic alterations are involved, constituting new therapeutic targets in this type of leukemia. This work investigated the therapeutic potential of Telaglenastat (CB-839), a glutaminase inhibitor, in a CLL cell line, in monotherapy and in therapeutic association with epigenetic modulators, the hypomethylating agents, azacytidine (AZA) anddecitabine (DAC), and the histone deacetylates inhibitors, vorinostat (SAHA), and panobinostat (PANO)]. The HG3 cell line was incubated in the absence and presence of a range of increasing concentrations of CB-839 for 72h. Every 24h, metabolic activity was evaluated using the resazurin assay. Afterwards, CB-839 was combined, at the IC_{25} dose, with epigenetic modulators (2.5µM AZA + 5nM CB-839, 0.5µM DAC + 5nM CB-839, 0.5µM SAHA + 5nM CB-839 and 5nM PANO + 5nM CB-839). The type of cell death was assessed by flow cytometry (CF), using Annexin V/7AAD staining and by optic microscopy (May-Grünwald-Giemsa staining). Cell cycle distribution was assessed by CF with propidium iodide/RNase staining. Mitochondrial membrane potential was evaluated by CF, using the JC-1 probe. Statistical analysis was performed, considering a significance level of 95% ($p<0.05$). CB-839 reduced the metabolic activity of HG3 cells in a dose and time-dependent manner (IC_{25}: 5nM, IC_{50}: 25nM, 48h), inducing cell death by apoptosis and a cell cycle arrest in G_0/G_1. Combinations of this glutaminase inhibitor with epigenetic modulators also reduced the metabolic activity ($p<0.001$), being synergistic effect of the drug combination more significant with hypomethylating agents (AZA and DAC). CB-839 In summary, the combination of glutaminolysis inhibitors and epigenetic modulators may constitute a future therapeutic alternative in CLL.

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The therapeutic potential of niraparib in lymphoid malignancies – Preliminary results

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Abstract: DNA damage is a major source of genomic instability, a cancer hallmark. However, cells developed mechanisms to protect genome integrity and repair damaged DNA, the DNA damage response (DDR), where PARP1/2 are key players. Defects or upregulation of proteins involved in DDR and repair are common in lymphoid malignancies, thus B-cell malignancies, such as multiple myeloma (MM), chronic lymphocytic leukemia (CLL) and acute lymphoblastic leukemia (ALL). This work aims to characterize DNA damage and repair signature in lymphoid neoplasias, namely MM, CLL and ALL, and explore the therapeutic potential of niraparib, a PARP1/2 inhibitor, in these malignancies. To this end, three cell lines were used: U-266 (MM cell line), HG-3 (CLL cell line), and 697 (ALL cell line). DNA damage and repair activity characterization was assessed by cytokinesis-blocked micronucleus assay, without and with exposure to H_2O_2. Further, the cells were incubated in the absence and presence of increasing concentrations of niraparib and the antiproliferation and cytotoxic effects of this DDR inhibitor were assessed using trypan blue assay for 72h. Cell cycle distribution was assessed by flow cytometry (FC) using propidium iodide/RNAse assay and cell death by annexin-V/7-AAD by FC as well as by optic microscopy (May-Grünwald-Giemsa staining). Results were statistical analyzed, considering a significance level of 95%. Niraparib reduced cell proliferation and viability in a dose-, time- and cell line-dependent manner. U-266 was the most resistant cell line (IC_{50} 79 μM), followed by HG-3 (IC_{50} 48 μM) and 697 was the most sensitive (IC_{50} 2 μM). The predominant damage biomarker found was the presence of micronucleus. Basal chromosomal damage was more present in HG-3 cell line, while 697 cell line had the lowest. Additionally, this cell line presented a del(13)(q12q32) and a *PARP1* mutation, which may be related with the observed higher sensitivity to niraparib. B-cell malignancies cell lines have different DNA damage profiles and sensitivity to niraparib which may dependent on the genetic characteristics of the cancer cells. A further characterization of DDR pathways in these cell lines and in patient samples may help to select the patients who will benefit more from DDR inhibitors improving patients' outcomes.

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Rilmenidine binds to and inhibits the activity of MDR pumps in pancreatic ductal adenocarcinoma

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Abstract:

Pancreatic ductal adenocarcinoma (PDAC) is the sixth leading cause of death worldwide and the fourth in Europe with a 5-year survival rate. The common cause of treatment failure in PDAC patients is multidrug resistance (MDR) due to the increased expression of plasma membrane efflux pumps that limit the intracellular uptake and retention of numerous xeno- and endobiotics. As the 93.3% of pancreatic carcinomas expressed P-glycoprotein (P-gp-MDR1/ABCB1) and 31% co-expressed multidrug resistance protein 1 (MRP1/ABCC1) with MDR1 P-gp, the inhibition of these pumps may be the target for novel anticancer drugs.

We used the FRED 3.2.0.2 software to predict the affinity of I1-imidazoline receptor ligand rilmenidine within the binding site of P-gp-MDR1/ABCB1 and MRP1/ABCC1, and flow cytometry to evaluate the effect of rilmenidine phosphate and rilmenidine fumarate on the efflux pumps in PDAC cells in vitro.

The results of the molecular docking studies indicate that rilmenidine has the binding affinity for both P-gp-MDR1/ABCB1 and MRP1/ABCC1 efflux pumps. While, *in vitro* studies show that rilmenidine fumarate has better potential to inhibit Calcein AM efflux than rilmenidine phosphate, and it did so in a dose-dependent manner.

Our results indicate that rilmenidine has the affinity to bind to MDR efflux pumps and to inhibit their activity. This potential of rilmenidine to overcome multidrug resistance in PDAC should be further investigated in order to develop more effective PDAC therapy.

Keywords: pancreatic ductal adenocarcinoma; multidrug resistance; molecular docking; rilmenidine

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Hybrids of sclareol and 1,2,4-triazolo[1,5-a]pyrimidine inhibit P-glycoprotein function in glioblastoma cells

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Abstract:

Background: Natural products exhibit a wide range of biological activities and they are starting point in the drug discovery process. Sclareol (SCL) naturally occurring labdane diterpene isolated from Clary sage (*Salvia sclarea* L.) shows diverse biological properties such as antioxidative, antimicrobial, anti-inflammatory, and anticancer activities. It is well established that fusing two pharmacophores can lead to significant improvement in the biological potential of the molecule by modifying its physicochemical properties. We envisioned that chimeric molecules synthesized by linking triazolo[1,5-*a*]pyrimidine pharmacophore to SCL would have more potent anticancer activity than their parental compound – SCL. Therefore, the cytotoxic potential of a series of SCL derivatives was compared with SCL. We have also studied their potential to increase the accumulation of substrates of membrane transporter which causes resistance of cancer cells – P-glycoprotein (P-gp). **Methods:** Cytotoxic potential, selectivity towards cancer cells, and resistance profile of SCL and its derivatives were examined by MTT assay after 72 h exposure in human glioblastoma cells (sensitive U87 and multidrug-resistant U87-TxR) and rat microglial cells (BV-2). We also investigated the effect of SCL and its derivatives on P-gp activity in U87-TxR resistant cells by determining the level of accumulated P-gp substrates (rhodamine 123 and doxorubicin) by flow cytometry. **Results:** More than half of the tested SCL derivatives considerably reduced glioblastoma cell viability with a concentration of 5 μM. Tested compounds evaded the resistance of glioblastoma cells showing similar or better activity against U87-TxR cells in comparison with U87 cells. All compounds significantly increased the accumulation of rhodamine 123 pointing to the inhibition of P-gp. However, only three of them increased the accumulation of doxorubicin likewise tariquidar, a well-known third-generation P-gp inhibitor, implying that these three SCL derivatives can be valuable as chemo-sensitizing agents. **Conclusion:** Our results showed that SCL derivatives can be considered as modulators of P-gp activity especially pointing to several lead compounds whose detailed molecular mechanism of anticancer action should be studied.

Presence of compensatory mechanism after silencing of CBP-induced TUBB3 regulates ovarian cancer cells drug resistance

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Abstract:

Resistance to platinum- and taxane-based chemotherapy represents a major obstacle to long-term survival in ovarian cancer patients. Here, we studied the interplay between acquired carboplatin (CBP) resistance in two ovarian cancer cell models, MES-OV CBP and SK-OV-3 CBP, and non-P-glycoprotein-mediated cross-resistance to paclitaxel (TAX) in MES-OV CBP cells. Decreased platination, mesenchymal-like phenotype and increased expression of α-, β- and γ-tubulin were observed in both drug resistant variants compared to parental cells. In particular, both variants revealed increased protein expression and differences in the class III β-tubulin (TUBB3) localization and nucleus morphology. Transient silencing of TUBB3 sensitized MES-OV CBP cells to TAX as well as to CBP. This was not observed in SK-OV-3 CBP variant due to compensation by other tubulin isotypes in response to gene silencing. Reduced TUBB3 levels in MES-OV CBP cells decreased trafficking of DNA repair proteins and increased whole cell platination level leading to CBP sensitization. In summary, TUBB3 silencing augments therapeutic efficiency in ovarian cancer cells lacking tubulin β subtypes compensation.

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Bioprospection of well-known European herbs

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Abstract:

Many plant species growing in Europe are known and used to treat various diseases, we have chosen some of them and screen mainly for immunomodulatory and antibiotic resistance modulation activities. Plant ethanolic extracts, made from 54 European herbs, were tested in vitro for the ability to reduce the effects of inflammation through nitrate oxide, TNFα and IL 6 production. The extracts were tested for their antioxidant activity (oxygen radical absorbance capacity assay), modulation of bacterial virulence (microdilution method, efflux pump inhibition assay and antibiofilm properties) and cytotoxic activity (resazurin assay) as well.

Although activity on tissue cultures was not observed, some extracts were able to absorb reactive oxygen species. Antimicrobial activity was observed mainly on Gram-negative bacterial species. In addition, at non-toxic concentrations, selected extracts were able to inhibit bacterial communication (quorum sensing) and bacterial adhesion. Moreover we have found that these samples potentially inhibit bacterial efflux pumps. Several samples were able to sensitize multidrug resistant bacterial strains. Overall, our data suggest that we have found plant extracts suitable for adjuvant therapies mainly for antibiotics.

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Modulation of DNA damage repair in the treatment of acute myeloblastic leukemia - *in vitro* study

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Abstract:

DNA integrity is threatened by endogenous and exogenous sources that can induce DNA lesions. Hence, cells have developed mechanisms to protect genome integrity and repair damaged DNA, the DNA damage response (DDR). Key DDR elements include sensor proteins (e.g., PARP1/2), mediators and signaling transducers, and effector molecules (e.g., CHK1). Increased DNA damage and altered DDR are critical features of genetic instability presumably implicated in the pathogenesis of acute myeloblastic leukemia (AML). AML is a clonal malignant disease of hematopoietic stem and/or progenitor cells characterized by a block in myeloid differentiation and increased proliferation. Our goal was to assess the potential of DDR as a therapeutic target in AML, using *in vitro* models, in order to identify new therapeutic approaches for this acute leukemia.

Six cell lines of different AML subtypes were used (HEL, HL-60, K-562, KG-1, LAMA-84, and NB-4 cells). Cells were incubated in the absence or presence of increasing concentrations of two DDR inhibitors, CCT245737 (CHK1 inhibitor) and niraparib (PARP1/2 inhibitor). Cell density and viability were assessed, for 72 hours, by trypan blue assay. The cell cycle was evaluated using propidium iodide/RNAse by flow cytometry (FC). Cell death was assessed by FC (Annexin V/7-AAD double staining) and by optic microscopy (May-Grünwald-Giemsa staining). The results were statistically analyzed, considering a significance level of 95%.

CCT245737 and niraparib reduced cell proliferation and viability in a dose-, time- and cell line-dependent manner. KG-1was the most sensitive cell line to CCT245737, with an IC_{50} at 48 hours of 48 μM, and NB-4 was the most sensitive cell line to Niraparib, with an IC_{50} at 48 hours of 25 μM. LAMA-84 was the most resistant cell line to both inhibitors with an IC_{50} at 48 hours of 203 μM and 66 μM, respectively. Both inhibitors led to an increase in the percentage of cells in late apoptosis and cell cycle arrest in the S phase.

In conclusion, AML cell lines have different sensibilities to CCT245737 and niraparib, with KG-1 and NB-4 cell lines appearing to be the most sensitive to DDR inhibition (CHK1 and PARP1/2 inhibition, respectively), whereas LAMA-84 appears to be the most resistant. Hence, this work may help to identify new therapeutic approaches that could eventually improve AML patients' outcomes.

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Pirfenidone sensitizes non-small cell lung cancer cell lines to paclitaxel and to a combination of paclitaxel plus carboplatin

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Abstract:

Non-small cell lung cancer (NSCLC) is the most common type of lung cancer, accounting for 85% of the cases. Despite recent advances in NSCLC treatment, a large proportion of patients have platinum-based chemotherapy as the main therapeutic approach available, and thus new therapeutic strategies are required. Interestingly, pirfenidone (PF), an antifibrotic drug clinically approved for the treatment of idiopathic pulmonary fibrosis, was recently shown to sensitize distinct cancer models to some anticancer agents. The aim of this work was to verify whether PF increases the sensitivity of NSCLC cells to paclitaxel (PAC) or to a combination of PAC plus carboplatin (CBP). Our results (1) revealed that PF sensitizes NCI-H460 cells to PAC treatment (verified using the sulforhodamine B assay). The combined drug treatment consisting of PF with PAC significantly reduced NCI-H460 cell viability (analyzed by the trypan blue exclusion assay) and proliferation (verified using the BrdU incorporation assay), induced major alterations in the cell cycle profile (shown by flow cytometry following PI staining) and increased the % of cell death (verified by flow cytometry following Annexin V-FITC/PI staining). Furthermore, the combination of PF with PAC did not increase cytotoxicity of non-tumorigenic cell lines (MCF-10A and MCF-12A), when compared with treatment with each drug individually. Importantly, PF also sensitized NCI-H460 cells to the combined treatment of PAC plus CBP. Together, our pre-clinical work supports the benefit of using PF in combination with PAC or with PAC plus CBP, thus contributing to the long-term goal of verifying the possibility of repurposing PF as a perioperative measure for the treatment of NSCLC.

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Immunomodulatory activity of yeast glucan particles as carriers for silymarin and propolis

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Abstract:

Natural substances are a rich source of biologically active compounds. Several of them, namely silymarin extracted from the *Silybum marianum* (milk thistle), and propolis, a complex mixture of vegetable waxes and enzymes, have previously been published for their ability to modulate drug resistance in tumours, and the immune system response. The immune system is one of the most important mechanisms of homeostasis regulation, protecting organs from exogenous pathogens as well as destroying the apoptotic or malignant cells. Regulation of the inflammatory response is critical in many serious diseases, including malignancies, where pro-inflammatory cytokines alter the expression of drug resistance determinants.

However, the bioavailability of natural substances is usually low due to their poor solubility in water. To increase the bioavailability of natural substances, micro or nanocarriers can be used, which improves their physical properties and streamline their targeting. One such approach is the use of yeast glucan particles (porous hollow shells of *Saccharomyces cerevisiae*) and the encapsulation of natural substances within these particles. Glucan particles can potentially improve the bioavailability of an active compound by two mechanisms: a) better dissolution kinetics *via* an amorphization of an active compound and b) activation of an immune response, and thus mediate transport of the active compound.

The aim of this project is to monitor the anti-inflammatory activity of selected natural substances in different solvents and to compare their activity with the activity of prepared glucan particles with an encapsulated substance. Immunomodulatory activity was monitored as ability to reduce production of nitric oxide (NO), tumour necrosis factor (TNF-α) and interleukin (IL-6) by bacterial lipopolysaccharide-induced macrophages (RAW 264.7). The results showed a significant inhibition of the release of all monitored signalling molecules, indicating that the encapsulation of biologically active substances into the glucan carrier particles increases both its solubility and bioactivity.

Acknowledgement: This research was funded by the project from the Czech Science Foundation, project number 21-00551S. This article is based upon work from COST Action 17104 <STRATAGEM>, supported by COST (European Cooperation in Science and Technology, http://www.cost.eu).

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Protonation-dependent lysosomotropism and crystallization as determinants of Nintedanib anticancer activity

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Abstract:

Nintedanib (BIBF112) is a clinically-approved small-molecule FGFR inhibitor, used for treatment of FGFR-driven cancer cells (i.e., small and non-small lung cancer cells). In a previous publication, we reported the intrinsic fluorescence properties of this drug. Nintedanib shows bright blue fluorescence in cell-free conditions and within the cellular cytoplasm (405 nm excitation laser, 420 nm emission filter). However, we also detected an unexpected signal in the green emission range (488 nm excitation laser, 556-558 emission filters) at subcellular structures. Further analyses revealed that nintedanib sequestration into lysosomes, acidic cellular compartments, is selectively associated with the green fluorescence behavior. Nintedanib lysosomal accumulation was strictly dependent on the acidic pH, as alteration of acidity within the lysosomal compartment (through alkalizing agents, such as Bafilomycin A1) prevented the respective green fluorescence signal in lysosomes. The molecular mechanisms underlying this unique fluorescence signal are unclear and have been addressed in this project. In order to identify the structural/chemical factors involved in the yield of nintedanib green fluorescence activity, we conducted cell-free and in vitro experiments using an inverted-fluorescence microscope and spinning-disk confocal microscope. Furthermore, we performed flow cytometry (FACS) and RAMAN spectroscopy to investigate the role of drug protonation behind the drug green fluorescence acquisition. For the isolation of green nintedanib crystals in vivo, cryosections of subcutaneous mice xenografts were generated. In this paper we show that nintedanib gets protonated once diffused in the lysosomes. Obviously, this protonation leads to accumulation of nintedanib in lysosomes to high concentrations leading to crystallization events. Accordingly, crystalline structures exhibiting intense green fluorescence were observable within the lysosomal lumen by high resolution spinning disk microscopy. Also, fluctuations of lysosomal acidity (ergo quantity of hydrogen ions available in the organelle compartment) affect the intensity of nintedanib green fluorescence signal. The synthesis of non-protonable nintedanib derivatives allowed us to prove that protonation is an unavoidable cause of pH-dependent fluorescence activity and drug resistance development due to the irreversibility of lysosomal trapping after nintedanib crystal formation. In summary, we uncover that cancer cells experience nintedanib crystals formation after drug lysosomal trapping due to protonation. Drug resistance, as a consequence of intra lysosomal deposition in form of nintedanib crystal accumulation, can be overcome by avoiding drug protonation.

Reversal of multidrug resistance by selenocompounds in bacteria and tumor cells

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Abstract

In previous studies, selenoesters have been shown to be effective derivatives with excellent biological activity. Based on these results, new selenoesters were synthesized and the antibacterial and antitumor effects of these compounds were studied *in vitro*.

The antibacterial activity of selenoesters was determined on sensitive and resistant *Staphylococcus aureus* strains, and on *Salmonella enterica* subsp. *enterica* serovar Typhimurium strains. The eruption of mature biofilm and the anti-biofilm activity were tested on biofilms produced by *Pseudomonas aeruginosa* and *S. aureus*. For the evaluation of the anti-efflux pump activity, *S.* Typhimurium and *S. aureus* strains were applied. Sensitive and resistant human colon adenocarcinoma and human embryonal lung fibroblast cell lines were used in the cytotoxicity assays. The ABCB1 inhibiting and apoptosis-inducing effects of the derivatives were further investigated in the resistant colon adenocarcinoma cell line.

The ketone-selenoesters showed to be more effective than the cyano-selenoesters. All derivatives possessed anti-biofilm activity; the activity of efflux pumps was successfully inhibited in *S. aureus* MRSA strain. All ketone-selenoesters inhibited effectively the ABCB1 pump and one ketone-selenoester was also able to induce early apoptosis.

These results suggest that ketone- and cyano-selenoesters could be effective compounds reducing the multidrug resistance in bacteria and in tumor cells. Selenium-containing compounds could provide alternative and effective scaffolds to overcome multidrug resistance.

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Hyperthermia and doxorubicin combined effects in melanoma cells

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Abstract:

Melanoma is the most aggressive skin cancer and very difficult to treat in advanced stages.[1] The increasing incidence in the last few decades has creating a need for novel treatment approaches. Thus, in the present study we evaluated the effects of doxorubicin (DOX) and hyperthermia in combination on A375 and MNT-1 human melanoma cell lines. Cells were exposed to DOX IC10 and IC20 and then to hyperthermia at 43 °C for 30, 60, and 120 min. Effects on cell viability were analyzed, as well as interference on cell cycle dynamics, reactive oxygen species (ROS) production and apoptosis. Combined treatment significantly decreased cell viability, but not in all tested conditions, suggesting that the effect depends on the drug concentration and heat treatment duration. Furthermore, treatment combination mediated a G2/M phase arrest in both cell lines, as well as increasing ROS levels and it induced early apoptosis in MNT-1 cells. These findings demonstrate that hyperthermia enhances DOX effect through cell cycle arrest, oxidative stress, and apoptotic cell death.

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Therapeutic inhibition of Heat sock protein 90 in chronic myeloid leukemia – A new therapeutic approach to circumvent imatinib resistance

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Abstract:

Heat shock protein 90 (HSP90) facilitates the maturation, stability, activity and intracellular folding of more than 200 proteins, called 'client proteins'. In cancer cells, HSP90 helps to overcome multiple environmental stresses, such as genomic instability/aneuploidy, proteotoxic stress, increased nutrient demands, and reduced oxygen levels. One of these "client proteins" is BCR-ABL1, the oncoprotein responsible for chronic myeloid leukemia (CML). Cancer cells that depend on this oncoprotein for survival are sensitive to HSP90 inhibition. Hsp90 inhibitors target the "client proteins" to proteasome degradation by interfering with HSP90 chaperone activity. Alvespimycin (17-DMAG) is an HSP90 inhibitor that has better pharmacokinetic properties and fewer side effects compared to others benzoquinone ansamycins.This work aimed to study the effect of 17-DMAG in CML cell lines (sensitive and resistant to imatinib) and to explore the role of HSP family in the sensitivity to imatinib.We used 3 CML cells lines: K562 (sensitive to imatinib), and the K562-RC and K562-RD (resistant to imatinib). Cells were incubated in the absence and presence of increasing concentrations of 17-DMAG (1 to 1000 nM) for 72h. Metabolic activity was measured by resazurin assay. Cell death was determined by microscopy (May-Grunwald Giemsa staining) and by flow cytometry (FC), using annexin V and propidium iodide (PI) double staining. The caspase expression and mitochondrial membrane potential were evaluated by FC using the Apostat and JC-1 probes, respectively. The PI/RNase assay were used to analyses cell cycle by FC.The protein expression levels of HSP family were analyzed by western blot. Our results showed that 17-DMAG decrease cell viability, in a dose, time and cell type dependent manner, with an IC_{50}, at 48h, of 50nM for K562 and K562-RD cells and lower than 50nM for the K562-RC. 17-DMAG induces cell death mainly by apoptosis, confirmed by morphological analysis, FC and by the increase of JC-1 monomers/aggregates ratio. Furthermore, 17-DMAG induces G_0/G_1 arrest in K562 cells. The HSP protein analysis showed that K562-RC have slightly increased in HSP90 expression comparing with K562 cells. In conclusion, our results suggest that inhibition of HSP90 by alvespimycin could be used as a new potential approach in the treatment of CML, even in case of imatinib resistance.

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Therapeutic potential of buparlisib in chronic myeloid leukemia - Preliminary results

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Abstract:

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm characterized by the presence of the BCR-ABL oncoprotein, which has deregulated tyrosine kinase activity. The 1st line treatment of CML is the imatinib (IMA), a tyrosine kinase inhibitor. However, some patients develop IMA resistance, so the identification of new therapeutic targets that may constitute alternatives treatments for these patients is extremely important. Thus, this work aimed to evaluate the therapeutic potential of Buparlisib, a PI3K inhibitor, in *in vitro* models of CML sensitive and resistant to IMA. The study was carried out in an IMA-sensitive CML cell line, K-562 cells, and two IMA-resistant cell lines, the K-562 RC and K-562 RD cells. Cell lines were incubated in the absence and presence of Buparlisib in a single dose and in a daily fractionated administration. The metabolic activity was evaluated by the resazurin assay. Cell death was evaluated by flow cytometry (FC) using annexin V and 7-AAD staining and by cell morphology using light microscopy (Giemsa staining). Cell cycle was analyzed by FC using propidium iodide (PI)/RNase. Genes involved in cell signaling and the cell cycle were analyzed through qPCR. The results were statistically analyzed and were considered significant when $p<0.05$. Buparlisib reduced the metabolic activity in a time and dose-dependent manner, with the IC_{50}, after 72h, of 1.1 µM in K-562 cells, 1.2 µM in K-562 RC and 1.0 µM on the K-562 RD. Daily fractionated administration showed no benefit over single administration. The buparlisib induced apoptosis and cell cycle arrest in the G2/M phase. In conclusion, our preliminary results suggest that buparlisib could be a new potential approach in the treatment of CML, even in case of IMA resistance.

Chemotherapeutic potential of the natural drug lead Royleanone against glioblastoma cells

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Abstract:

Glioblastoma (GBM), the most malignant glioma of the Central Nervous System, has reduced survival rate after diagnosis, around 14-18 months, due to the lack of efficient therapeutic approaches and poor prognosis. Regarding this, new treatment options based on drug leads from natural sources are an appealing initial point to improve patients' survival and well-being. Therefore, in this work, we studied the antitumoral activity of Royleanone (Roy), a drug lead from a natural source: *Plectranthus hadiensis* (Forssk.) Schweinf. ex Sprenger. Roy was isolated from the acetonic extract of *P. hadiensis* and its antitumoral activity was assessed in a panel of 5-glioma cell lines (A172, U87, H4, U118 and U373). Briefly, cell viability was evaluated by Alamar Blue assay, cell death and cell cycle regulation were analyzed by flow cytometry and measurement of apoptosis-related genes expression was performed by qPCR. This work showed that this natural drug lead inhibited proliferation, induced cell cycle arrest at G2/M phase and caspase-dependent and -independent apoptosis in the tumor cell panel. Furthermore, the necessary dose of Roy (23.19 µg/mL) to induce inhibition of proliferation by 50% was substantially lower than the temozolomide (TMZ) (first-line treatment) dose (72.07 µg/mL) required to promote the same effect (Table 1). Thus, the data from this study shows the potential to reduce the multidrug resistance associated with conventional therapeutics and suggests Roy as a potential drug lead for future chemotherapeutic treatment in GBM.

Table 1. Cytotoxic effect of Roy and TMZ: IC_{50} values for U87 cell line, 48 hours after treatment.

Compound	IC50 (µg/mL)
Roy	23.19
TMZ	72.07

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Novel collateral sensitizers of multidrug-resistant tumor cells

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Abstract:

Reversing multidrug resistance (MDR) has become one of the main challenges in cancer research. The development of collateral sensitizers, i.e. compounds capable of exploiting the upgraded defense mechanisms of MDR cells as weaknesses, has been proposed as a strategy to overcome MDR. The aim of our work was to investigate the potential of two novel compounds as collateral sensitizers. The compounds, which are of related structure, were synthesised in three steps from commercially available starting materials in 28-32% overall yield. The activity of these compounds was tested in pairs of drug-sensitive and MDR non-small cell lung cancer (NSCLC, NCI-H460 and RH460) and colon cancer (DLD1 and RDLD1) cell lines, kindly provided by Dr. Milica Pešić [1,2]. The effect of both compounds on cell growth was investigated in both pairs of cell lines. The effect on cell viability, cell proliferation and cell cycle profile was evaluated in the NSCLC pair of cell lines and the cytotoxicity was studied in the non-tumorigenic cells, MCF10A. To understand the mechanisms behind the collateral sensitivity effect, the following was evaluated on the NSCLC pair of cell lines: drug efflux pumps activity (Rhodamine-123 efflux assay), ROS production (CM-H2DCFDA staining), disruption of the GSH/GSSG balance (GSH/GSSG-Glo™ Assay) and expression of key proteins associated with metabolism and redox balance (which were previously found to be differentially expressed between the NCI-H460 and RH460 cells) [3]. Results showed that both compounds presented a lower GI_{50} concentration in the MDR cell lines, when compared to the sensitive counterpart cell lines. One of the compounds caused an increase in ROS production, a disruption of the GSH balance and an alteration in the expression of proteins associated with protection against oxidative stress, particularly in the MDR cell line. Future studies will focus on uncovering the mechanisms behind the collateral sensitivity effect of the other compound.

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Functional diagnostics as a new concept for the improvement of personalized targeted therapy

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Abstract:

Although advances in sequencing technology and target identification enabled the implementation of a personalized therapy approach, unfortunately, only 3-9% of cancer patients who receive the targeted therapy show an adequate response. On the other side, there are exceptional responders to targeted therapy among cancer patients without common genetic alterations. Therefore, current patient classifications relying only on sequencing are not sufficient to determine optimal treatment. Our intention is to start in the opposite direction to conventional diagnostics by performing pharmacological screening on patient-derived cancer cells *ex vivo* because testing of multiple drugs is not possible in clinical trials. An incomplete understanding of how tumour genotype reflects on tumour phenotype limits the efficacy of DNA and mRNA sequencing for personalized therapy. Functional diagnostics using patient-derived cancer cells is recently implicated to overcome this limitation and it is clinically available for haematological malignancies. We plan to perform the immunofluorescence-based drug-screening assay to determine non-small cell lung carcinoma (NSCLC) patients' cancer cells' response to targeted therapeutics, particularly tyrosine kinase inhibitors (TKIs) within the time frame necessary to influence patient care. The usage of the functional diagnostics approach should be an addition to clinical trials and complement DNA and mRNA sequencing.

In contrast to similar research efforts [1], we will shorten the cultivation of NSCLC patient-derived cells to 1-2 weeks because we intend to test drugs on a mixture of cancer and stromal cells (fibroblasts). It is well-known that the sensitivity of cancer cells depends on their interaction with the microenvironment including neighbouring cells. In addition, we will examine the changes in the expression level of ATP Binding Cassette transporters (ABCB1, ABCC1, and ABCG2) in both cancer and stromal cells that may occur during TKIs and chemotherapy treatment. In such way, we will gain knowledge about (i) which TKI or chemotherapeutic induces multidrug-resistant (MDR) phenotype in our NSCLC patients' cohort, (ii) whether the induction of MDR depends on the ratio between cancer and stromal cells, (iii) whether the induction of MDR is prevalent in cancer cells, and (iv) whether MDR induction depends on individual patient's characteristics (comparison with Whole Exome Sequencing results).

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Role of sensory neurons, vagus nerve and neuroimmune pathways in breast cancer metastasis

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Abstract:

Recent studies document the importance of neuronal dysfunction in cancer development and metastasis. We previously reported that both depletion of neuropeptides in capsaicin-sensitive sensory nerve endings as well as vagotomy increases metastasis of triple negative breast carcinoma. Similarly, activation of sensory nerve fibres with low dose capsaicin decreases the number of lung metastasis. Using a high throughput screening assay, we also found that inactivating sensory nerve fibres decreases the expression of possible tumour suppressive genes (1,2, 3). These findings suggested that neuropeptides released from sensory nerve fibres may inhibit tumor growth. Our further studied demonstrated that, of these neuropeptides, Substance P has anti-tumoral effects (3). Specifically, continuous exposure to low doses of Substance P in combination with radiotherapy inhibited the metastasis of brain metastatic cells of breast carcinoma (4TBM) cells in which have a cancer stem cell phenotype and induce extensive visceral metastasis after orthotopic inoculation into the mammary pad (4, 5). Mechanistically Substance P-induced inhibition of metastasis seems to be involved in activation of anti-tumoral immunity such that SP treatment decreases the number of tumor-infiltrating myeloid-derived suppressor cells as well as the TNF-a release while increasing IFN-g secretion.

These studies suggest that mimicking sensory nerve activity by means of treatment with low dose neuropeptides or activating the vagus nerve provides an effective adjuvant therapy for metastatic breast cancer (6).Do not change the page and margin formatting.

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Investigations of potential parameters influencing the albumin homeostasis in the murine melanoma model B16

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Abstract:

Despite their potent anticancer activities, current cancer therapeutics are often limited by rapid development of drug resistance as well as severe side effects due to their insufficient tumor specificity. To circumvent those adverse effects, tumor-targeting prodrug systems represent a promising approach to improve tumor-specific drug accumulation/release by exploiting the specific properties of the malignant tissue. One innovative strategy is to employ the enhanced accumulation, turn-over and even degradation of the serum protein albumin in the tumor tissue, which is caused by the so-called enhanced permeability and retention (EPR) effect as well as the increased energy requirement of cancer cells. Successful prodrug examples in clinical use are the albumin nano-formulation of paclitaxel Abraxane® and the albumin-binding derivative of doxorubicin Aldoxorubicin. For that reason, the here presented study aims to achieve knowledge about the mechanism and parameters influencing albumin homeostasis in cancer cells.

For this purpose, the murine melanoma B16 cell model has been chosen, as we discovered that the typical cell culture contains several subpopulation of cells with different albumin uptake. Using FITC-labeled albumin as a selection marker, these subpopulations were sorted with respect to their different albumin uptake (high, medium and low) via flowcytometry.

As a next step, the newly established cell clones were analyzed for different parameters including doubling time (by viability assays), expression of different proteins involved in albumin homeostasis (by Western blotting) as well as their metabolic characteristics (Seahorse). These preliminary data indicate that there exist distinct biological differences between the B16 populations with different albumin uptake. This renders our new isogenic B16 subline panel not only a convenient tool to further exploit in-depth changes in albumin homeostasis of cancer cells with the aim to identify new biomarkers but is also an ideal test system for the investigation of new albumin-targeted chemotherapeutics.

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Preparation and testing of liposome carriers targeting tumor cells

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Abstract:

One possible way of cancer treatment represents chemotherapy, which uses drugs to stop the growth and induce cellular death of cancer cells. However, during long-term treatment, cancer cells may become resistant to chemotherapeutics that reduce the chance of a successful outcome. Multiple drug resistance (MDR) can be caused by efflux pumps from the ABC family (ATP binding cassette) proteins. P-glycoprotein (P-gp) is one of the most frequently overexpressed transporters. Most P-gp modulators cannot be administered systemically because P-gp occurs naturally in other tissues and organs (*e.g.*, the blood-brain barrier) where it has a protective function. Therefore, it is necessary to use a system that would specifically target P-gp inhibitors to tumour cells, thereby reducing their side effects.

The aim of this project was to create and test an actively targeted system containing a P-glycoprotein inhibitor. For this purpose, liposomes carrying monoclonal antibodies against human epidermal receptor 2 (HER2) were prepared to target human cell lines overproducing this receptor. The activity of encapsulated inhibitor was compared with the free inhibitor using a set of P-gp positive/negative and HER2 positive/negative cells. The values obtained were compared in terms of selectivity for HER2 and modulation of P-gp. The presence of the HER2 was verified by immunochemical detection. Furthermore, the effect of prepared liposomes on cell viability and their immunomodulatory properties was studied. At the same time, the course of the sensitizing effect on resistant lines was measured in real time. The experiments have shown that the modulator itself is an inhibitor of P-glycoprotein, in a non-competitive mode. After encapsulation, the inhibitor retained the ability to modulate MDR. Selectivity for HER2 positive lines was not observed.

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Anticancer activity of novel bipyridine-silver(I) compounds against resistant colorectal and ovarian cancer cell lines

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Abstract:

Platinum-based anticancer drugs (e.g. Carboplatin) are frequently applied in chemotherapy regimens against several type of cancer including ovarian carcinoma (OC). However, intrinsic or acquired tumor resistance severely limit their clinical application. Consequently, we investigated the potential of several novel silver(I) 2,2'-bipyridine derivatives containing either triphenylphosphane (PPh_3) or 1,2-bis(diphenylphosphino)ethane (dppe) ligands for their potential to overcome platinum resistance. Their cytotoxic activity was tested in two human OC models (SKOV-3 and MESOV), their carboplatin-resistant counterparts, as well as non-malignant fibroblasts F331. These experiments revealed that all compounds displayed high anticancer activity and tumor selectivity. Moreover, they, were not affected by carboplatin resistance. Noteworthy, MES-OV showed exceptional sensitivity to several silver(I) 2,2'-bipyridine derivatives carrying a 1,2-bis(diphenylphosphino)ethane (dppe) ligand. This finding indicates that some of the new silver compounds might have a high selectivity for special cancer (sub)types, which makes them interesting drug candidates against drug-resistant OC.

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Therapeutic potential of the NRF2 inhibition in association with ibrutinib in chronic lymphocytic leukemia – studies *in vitro*

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Abstract:

Chronic lymphocytic leukemia (CLL) is the most common leukemia in Western Countries, being more prevalent in men. Various genetic alterations can be found in this pathology, including chromosomic alterations, miRNA alterations, epigenetic changes, and somatic mutations. Oxidative stress, which results from the disequilibrium between reactive oxygen species (ROS) production and antioxidant defenses, seems also to play a crucial role in the development of this disease. The NRF2-KEAP1 pathway is a redox regulator being the principal mechanism of antioxidant and cytoprotective response against ROS. However, this pathway presents a dual role in cancer – can prevent tumor development and, after tumor establishment, can protect the tumor cells from therapeutic agents leading to treatment resistance. The aim of this work was to evaluate the therapeutic potential of an NRF2 inhibitor, the Brusatol (BRU), in monotherapy and association with Ibrutinib (IBR; BTK inhibitor used in clinical practice for CLL treatment), in an *in vitro* CLL model, the HG-3 cell line. The expression levels of *NFE2L2* (which encodes NRF2) and *KEAP1* genes were initially evaluated in the HG-3 cells, using the qPCR. Then, the HG-3 cells were incubated, for 72 hours, in the absence and presence of increasing concentrations of Brusatol in monotherapy and association with Ibrutinib. The metabolic activity was determined by resazurin assay. The type of cell death was analyzed by optical microscopy (May-Grünwald-Giemsa staining) and by flow cytometry (FC), using the annexin-V (AV) and 7- AAD double staining. The cell cycle was evaluated by flow cytometry using the propidium iodide (PI)/RNAse solution. The mitochondrial membrane potential was quantified by FC using JC-1probe. Results were analyzed statistically considering a 95% significance level ($p<0.05$). The results showed that HG-3 cells express *NFE2L2* and *KEAP-1* genes. The exposure to increasing concentrations of BRU induced reduced metabolic activity depending on concentration and time. The IC_{50} of BRU at 48 hours was 110 nM. The association of BRU (50 nM) with Ibrutinib (0.5 μM) showed a synergic effect at 48 and 72 hours, leading to a higher reduction of metabolic activity than drugs alone. BRU in monotherapy and in association with IBR induce a cytostatic and cytotoxic effect, mediated by cell cycle arrest in phase G_0/G_1 and apoptosis, respectively. Finally, cells treated with both conditions showed a decrease in the mitochondrial membrane potential. Our results suggest that BRU could be a potential pharmacological agent for the therapeutic of CLL, in monotherapy and as an adjuvant to treatment with IBR. However, further studies are needed to clarify the therapeutic potential of these approaches.

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Overcoming imatinib resistance with a P-gP and BCRP inhibitor

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Abstract:

In chronic myeloid leukemia (CML), the most relevant mechanisms associated with the acquisition of resistance to tyrosine kinase inhibitors (TKIs) are those dependent on the therapeutic target, the BCR-ABL oncoprotein. Point mutations and overexpression of the *BCR-ABL1* are the mechanisms taken into consideration for therapeutic selection according to guidelines. However, drug transporters influence intracellular drug concentration, which proved to be very important in response and acquisition of resistance. The inhibition of efflux transporters, such as P-gP and BCRP, may contribute to greater efficacy of TKIs. The aim of this study was to evaluate the therapeutic potential of Elacridar (a P-gP and BCRP inhibitor) in monotherapy and in combination with Imatinib (IMA, a first-line therapy for CML patients), trying to overcome resistance in CML *in vitro* models.

To achieve this goal, we used three CML cell lines: K562 cells (sensitive to IMA), K562-RC (8x resistant to IMA), and K562 RD (18x resistant to IMA). P-gP and BCRP activity was evaluated by flow cytometry (FC). The therapeutic potential of Elacridar was assessed in cells incubated in the absence and presence of Elacridar, in monotherapy and in combination with increasing doses of IMA, by the resazurin method. Cell death was evaluated by optical microscopy (May-Grünwald-Giemsa staining) and by FC (Annexin V/7-AAD). The Apoptosis, DNA Damage, and Cell Proliferation Kit was used to analyze the mechanism of cell death and proliferation. The cell cycle was evaluated by FC (PI/RNAse). SynergyFinder was used to calculate the synergy score between both inhibitors. The data were analyzed statistically, and the differences were considered significant when $p<0.05$.

Resistant cell lines show higher expression and activity of P-gP and BCRP compared to the sensitive one. Elacridar in monotherapy, in tested concentrations, did not reach the IC_{50} in any cell line. However, the association of 250 nM of Elacridar with IMA modulated the resistance and re-sensitized resistant cells to IMA. In mechanistic terms, Elacridar in monotherapy induced cell death by apoptosis, showing no effect on cell cycle progression. In combination with IMA was observed cell death by apoptosis, accompanied by increased caspase-3 activation, cleaved PARP, and DNA damage (phosphorylated H2AX). This effect was accompanied by a cell cycle arrest in S phase.

In conclusion, our results suggest that Elacridar in therapeutic combination with IMA re-sensitize resistant cell lines to this TKI, namely in cell lines in which the main mechanism of drug resistance was associated with efflux transporters.

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Use of 2D and 3D cell models to test the potential of cannabinoids to modulate multidrug resistance

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Abstract:

Cancer is one of the major challanges of modern medicine. Chemotherapeutic treatment is often accompanied by the development of multidrug resistance (MDR), which makes therapy considerably more difficult. Within the described mechanisms of MDR phenotype, the most common is the increased expression of *ABC* transporter genes, specifically the P-glycoprotein (P-gp) efflux pump. P-gp is responsible for the transport of several physiological substrates, and many xenobiotics as well. Its excessive amount presented in tumour cells reduces the effectiveness of administrated drugs. Thus, the suppression of MDR is possible by administration of suitable P-gp inhibitors. That kind of inhibitors should provide low cytotoxicity and at the same time high efficacy at low concentrations. Based on previous research, phytocannabinoids could serve as promising P-gp inhibitors.

The aim of this project was to determine the toxicity of phytocannabinoids on 2D models of ovarian cancer cells, breast cancer cells and human dermal fibroblasts. Furthermore, the effect of phytocannabinoids on MDR modulation have been demonstrated, although their P-gp inhibition has not been observed using rhodamine 123 accumulation assay. This fact suggests a possible interference with P-gp gene expression. The preparation of 3D cell models (spheroids and microtumors) mimicking *in vivo* conditions was optimized for further measurements. Decreased cytotoxicity and lower MDR modulatory effect were observed in microtumors of resistant ovarian and breast cancer sublines cultured together with human dermal fibroblasts mimicking tumour conditions *in vivo*. CBD, CBDV and CBN had the greatest effect among the tested phytocannabinoids and should be further studied for their ability to modulate the resistant phenotype of tumour cells.

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Anti-tumoral activity of wasp venom Chartergellus-CP1 peptide to different breast cancer cell lines

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Abstract:

Breast cancer remains the most common cancer in women.[1] Conventional treatments are characterized for their poor response rates, increasing resistance and associated complications. Triple-negative breast cancer, that has the lowest survival rate, does not respond also to hormonal-therapy, highlighting the need for the development of new anti-tumoral therapeutic agents.[2,3] Several studies have been reporting strong anti-tumoral properties of venoms of arthropods such as scorpions, bees, and wasps.[4] In this study, we evaluated the anti-tumor potential of the Chartergellus-CP1 peptide isolated from the wasp venom of *Chartergellus communis* in human breast cancer cell lines MCF-7 (HR+) and MDA-MB-231 (triple-negative). Cells viability, cell cycle dynamics and apoptotic cell death were assessed for both cell lines after exposure to Chartergellus-CP1 during 24 and 48h. The results showed that Chartergellus-CP1 peptide was cytotoxic for both cell lines, presenting highest antiproliferative potential to HR+ tumor (MCF-7), specially at low doses. This work demonstrates, for the first time, the cytotoxic effects of Chatergellus-CP1 on human breast cancer cell lines showing the potential of Chatergellus-CP1 as an antitumor peptide.

Acknowledgement: The authors acknowledge the financial support to CESAM by FCT/MCTES (UIDP/50017/2020 &UIDB/50017/2020 & LA/P/0094/2020), through national funds, and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. The Portuguese Foundation for Science and Technology (FCT) is also acknowledged for the research contract of V. Bastos (CDL-CTTRI-161-ARH/2018) funded by the FCT project (POCI-01-0145-FEDER-031794). The authors would like to express their sincere gratitude to the following Brazilian funding agencies: FAP-DF (project number 00193-00001343/2019-53 e 00193-00000724/2021-30) and CNPq (project number 306547/2020-1 and 403543/2021-5) for their financial support.

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Chartergellus-CP1: a wasp venom peptide in melanoma therapy

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Abstract:

Melanoma is the most severe type of skin cancer. Their invasive properties, poor response rates to conventional treatments and the high risk of death, demands the need for research on new therapeutic approaches.[1] Venoms, of a wide variety of species, are mostly composed by natural and biologically active compounds, some of which with anti-tumoral properties.[2] In this study, the anti-tumor potential of the Chartergellus-CP1 peptide, isolated from the venom of the wasp *Chartergellus communis*, was evaluated using human malignant melanoma cell lines A375 (amelanotic) and MNT-1 (melanotic). Cells viability, reactive oxygen species production and apoptotic cell death were assessed for both cell lines after exposure to Chartergellus-CP1 during 24 and 48h. Chartergellus-CP1 cytotoxic activity was demonstrated including anti-tumoral profiles on both melanoma cell lines. This work demonstrates, for the first time, the cytotoxic effects of Chatergellus-CP1 on human melanoma cell lines showing the potential of Chatergellus-CP1 as an antitumor peptide.

Acknowledgement: The authors acknowledge the financial support to CESAM by FCT/MCTES (UIDP/50017/2020 &UIDB/50017/2020 & LA/P/0094/2020), through national funds, and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. The Portuguese Foundation for Science and Technology (FCT) is also acknowledged for the research contract of V. Bastos (CDL-CTTRI-161-ARH/2018) funded by the FCT project (POCI-01-0145-FEDER-031794). The authors would like to express their sincere gratitude to the following Brazilian funding agencies: FAP-DF (project number 00193-00001343/2019-53 e 00193-00000724/2021-30) and CNPq (project number 306547/2020-1 and 403543/2021-5) for their financial support.

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Evaluation of Anticancer Agents against BCR-ABL tyrosine kinase by computer aided drug design (CADD) approaches

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Abstract:

Cancer is one of the most important and prominent type amongst health problems. The number of cancer patients is increasing every year, and the number of new cancer cases is expected to reach 23.6 million annually by 2030. Chronic myeloid leukemia (CML) is a type of cancer caused by abnormal proliferation of pluripotent stem cells and account for 15% of adult leukaemia.

Inhibition of BCR-ABL tyrosine kinase enzyme with tyrosine kinase inhibitors (TKIs) is the main treatment for CML. Four new CML drugs (Imatinib, Dasatinib, Nilotinib and Bosutinib) have been developed since 2001 as first and second-line treatments. However, the side effects of these drugs and especially the drug resistance are the handicaps of the CML treatments. Therefore, there is a need for more effective and selective new drug substances for the treatment of CML disease. In this context, it is aimed to develop new tyrosine kinase inhibitors with computer aided drug design methods (CADD) for the BCR-ABL tyrosine kinase enzyme, which has an important role in the treatment of CML.

Accordingly, in this study, the in silico biological activity and molecular mechanism of several anticancer agents against the BCR-ABL tyrosine kinase enzyme was investigated by molecular docking method. As a result of this analysis, some of these compounds showed better binding activity than the drug molecules used in the treatment of CML, and interacted with Tyr315, Lys271, Phe382 and Tyr253 amino acids, which have an important role in enzyme activity. In addition, pharmacokinetics for these agents were evaluated by *in silico* ADME analyse. This information will guide further clinical studies to develop more effective, reliable, and selective drugs for the treatment of CML.

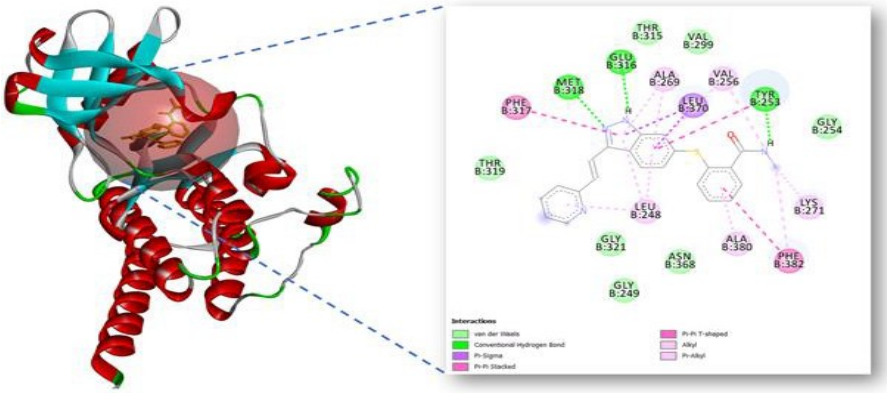

Figure 1. The 2D analysis of Molecular Docking simulation of the 3D structure of human ABL1 with imatinib.

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Sdox, a H_2S releasing anthracycline, with a safer profile than doxorubicin toward vasculature

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Abstract:

Sdox is a synthetic H_2S-releasing doxorubicin (Dox) less cardiotoxic and more effective than Dox in pre-clinical, Dox-resistant tumour models.
The well-known anthracycline vascular toxicity, however, might limit Sdox clinical use. This study aimed at evaluating Sdox vascular toxicity in vitro, using Dox as reference compound. Both vascular smooth muscle A7r5 and endothelial EA.hy926 cells were more sensitive to Dox than Sdox, although both drugs equally increased intracellular free radical levels.
Sdox released H_2S in both cell lines. The H_2S scavenger hydroxocobalamin partially reverted Sdox-induced cytotoxicity in A7r5, but not in EA.hy926 cells, suggesting a role for H_2S in smooth muscle cell death.
Markers of Sdox-induced apoptosis were significantly lower than, in A7r5 cells, and comparable to those of Dox in EA.hy926 cells. In A7r5 cells, Dox increased the activity of caspase 3, 8, and 9, Sdox affecting only that of caspase 3.
Moreover, both drugs induced comparable DNA damage in A7r5 cells, while Sdox was less toxic than Dox in Ea.hy926 cells.
In fresh aorta rings, only Dox weakly increased phenylephrine-induced contraction when endothelium was present. In rings cultured with both drugs for 7 days, Sdox blunted phenylephrine- and high K+-induced contractions though at a concentration 10-fold higher than that of Dox.
In conclusion, Sdox may represent the prototype of an innovative anthracycline, effective against Dox-resistant tumours, displaying a more favourable vascular toxicity profile compared to the parent compound.

Doxorubicin and quercetin double loading in mesoporous silica nanoparticles (MCM-41) enhances antiproliferative effects *in vitro*

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Abstract:

The purpose of this study was to develop double loaded doxorubicin (Dox) and quercetin (QR) drug delivery system, using mesoporous silica nanoparticles (MCM-41) as carrier. Further aim was to compare their cytotoxic potential on L5178Y and multidrug resistant L5178 MDR1 lymphoma cells.

The mesoporous silica nanoparticles were loaded with Dox and QR (alone and in combination) as model drugs. Alamar blue assay was performed to evaluate the cytotoxicity after exposure of the cells to double loaded nanoparticles (Dox/QR), free drugs (Dox and QR), and combined solution of both active compounds for 72 h.

The double loaded nanoparticles were characterized with a small size (563 nm) and negative zeta-potential (- 18 mV). The cytotoxicity of free Dox (0.001-10 μM) was less pronounced in L5178Y MDR1 cells (IC50 4.0 μM), compared to L5178Y cells (IC50 2.2 μM). The solution containing both non-encapsulated Dox (0.001-10 μM) and QR (0.07-5.8 μM) caused enhanced cytotoxic effects compared to the effect of Dox administered alone, suggesting that quercetin can increase the chemosensitivity of lymphoma cells to doxorubicin. Interestingly, double loaded Dox/QR nanoparticles showed significant concentration-dependent cytotoxicity on both lymphoma cells. The highest cytotoxicity of double loaded Dox/QR nanoparticles was observed on resistant L5178Y MDR1 cells, compared to L5178Y cells.

This in vitro study suggests that the co-delivery of DOX and QR by mesoporous silica nanoparticles, is a promising approach for improving the antiproliferative effects of doxorubicin and its efficacy in lymphoma MDR1 resistant cell lines.

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Preliminary work to evaluate the effect of synthetic compounds selected by *in silico* studies on SARS-CoV-2 host cell targets

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Abstract:

Angiotensin-converting enzyme (ACE2) has been described as the key cellular receptor for entry of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of the COVID-19 global pandemic[1]. Other host factors may serve as critical entry cofactors for productive infection, like the glucose-regulated protein 78 (GRP78)[2]. Several virtual[3-4] and *in vitro* studies[1,2] hypothesized that inhibiting the interaction between SARS-CoV-2 spike protein and cell surface (cs) ACE2 and/or csGRP78 could possibly decrease the rate of viral infection. Thus, these two host proteins are suggested as putative molecular targets to counteract SARS-CoV-2 infection.

Herein, a docking study was performed using an in-house library of approximately 300 small molecules synthesized by "Grupo de Produtos Naturais e Química Medicinal (CIIMAR/FFUP)" on the host targets ACE2 (PDB 6m17) and GRP78 (PDB 5E84), using AutoDock Vina. The virtual screening revealed interaction of 29 compounds with the ACE2 and 31 compounds with the substrate binding domain (SBD) GRP78 binding pockets, with better or equal docking scores than the positive control used (ponatinib). These promising compounds were found to be mainly xanthones and steroids with bulky, aminated or sugar hydroxylated moieties. Preliminary studies with several of the *in silico* hit compounds were performed in two human cell lines (A549 and MDA-MB-231), to assess their effect on the expression levels of both cellular targets, ACE2 and GRP78, by Western blot. Four of these compounds reduced and two of them increased ACE2 expression by approximately 20% (although not statistically significant). Ongoing studies will determine the impact of these compounds on SARS-CoV-2 cell infection assays (BSL-3), by immunofluorescence detection of SARS-CoV-2 spike protein. Further work will verify if these compounds act as competitors of SARS-CoV-2 for binding to these host receptors.

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Off-label use of hydroxychloroquine in COVID-19: analysis of reports of suspected adverse reactions from the Italian National Network of Pharmacovigilance

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Abstract:

This study aimed to characterize adverse drug reactions (ADRs) to hydroxychloroquine in the setting of COVID-19, occurring in Italy in the period March to May 2020. The analysis of the combination therapy with azithromycin or/and lopinavir/ritonavir as well as a comparison with ADRs reported throughout 2019 was performed.

ADRs collected by the Italian National Network of Pharmacovigilance were analyzed for their incidence, seriousness, outcome, coadministered drugs, and Medical Dictionary for Regulatory Activities classification.

A total of 306 reports were gathered for the quarter of 2020: 54% non-serious and 46% serious, and half of the latter required either the hospitalization or its prolongation. However, most of them were either completely recovered (26%) or in the process of recovery (45%), except for 9 fatal cases. Throughout 2019, 38 reports were collected, 53% non-serious and 47% serious, but no deaths had been reported. Diarrhea, prolonged QT interval, and hypertransaminasemia were the most frequently ADRs reported in 2020, significantly higher than 2019 and specific for COVID-19 subjects treated with hydroxychloroquine.

The logistic regression analyses demonstrated that the likelihood of serious ADRs, QT prolongation, and diarrhea significantly increased with hydroxychloroquine dosage. Coadministration of lopinavir/ritonavir and hydroxychloroquine showed a positive correlation with diarrhea and hypertransaminasemia and a negative relationship with the ADR seriousness. The combination therapy with azithromycin was another independent predictor of a serious ADR.

Off-label use of hydroxychloroquine for COVID-19, alone or in combination regimens, was associated with increased incidence and/or seriousness of specific ADRs in patients with additional risk factors caused by the infection.

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Is HIF-1α a master regulator of DNA repair capacity and chemotherapy response in testicular germ cell tumors?

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Abstract:

Testicular germ cell tumors (TGCT) are malignity highly curable with cisplatin (CDDP), gaining excellent response rates even in advanced metastatic stages. However, approximately 20-30% of patients, predominantly young men, do not respond to this treatment, relapse and develop CDDP resistance. CDDP generates DNA adducts expected to lead the cell to apoptosis. Homologous recombination (HR) and nucleotide excision repair (NER) can repair these adducts but their aberrant repair capacity can critically contribute to resistance. Therefore, targeting DNA damage response (DDR) and repair pathways remains a plausible and efficient strategy to overcome drug resistance. TGCTs have one of the lowest overall somatic mutation rates of all solid tumors, but possess unique epigenetic signature that may play a prominent role in their biology. Increasing evidence demonstrates that gene promoter methylation patterns as well as microRNAs (miRNAs) posttranscriptional regulation play an important role in therapy response. The most of DDR genes are under epigenetic control. To identify molecular mechanisms modulating the efficiency of DNA repair we decided to evaluate the regulatory role of hypoxia and find out whether HIF-1α can be the master regulator of CDDP response in TGCTs and to what extent epigenetic regulation contributes. To best of our knowledge, significant increase of miR-218 has not been reported in chemoresistant TGCTs until now. We propose that regulatory axis HIF/miR-218/PPP2R2A-PPP2R5A can be critical for chemoresistance in TGCT cells. In parallel, we propose significant regulatory effect of HIF/miR-218 on mitochondria that due to changes in their morphology, dynamics and ROS generation represent another contributor to chemoresistance and provide novel potential therapy targets.

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COVID-19 recommendations

Currently in Portugal, the use of masks is only **mandatory** in very specific situations, such as in health establishments and services (including pharmacies), and also, in the collective passenger transport, including air transport, as well as in the transport of passengers by taxi, Uber or similar.

Despite this rule, continues to be recommended social distancing, frequent hand washing, and respiratory etiquette. To tackle some of these aspects, in the annual meeting the coffee breaks and lunches will be in open-air spaces, and we will guarantee the air circulation in the meeting spaces. In addition, although it is not a legal obligation in Portugal, we **recommended the use of masks** in closed spaces.

UC testing site

The University of Coimbra created special conditions for the security of the UC community to be maximized and provides a COVID-19 screening service at Clinical Analysis Laboratory (2nd floor of the Faculty of Medicine, Campus I, Rua Larga, 3004-504 Coimbra). It is not necessary to schedule the sample collection.

Sample collection schedule

Working days: 08h30 - 13h00 e 14h00 - 16h00 / **Saturday:** 08h30 - 12h00

Price

Laboratorial Rapid Antigen Test: 15€

PCR (results in 24h): 60€ / **Fast PCR (result 1-2h):** 95€

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