STRATAGEM
CA17104

ABSTRACT BOOK

COST Action 17104 (STRATAGEM) WG3 Meeting - International Online Symposium on "New Therapeutic Tools Against Preclinical Models of Multidrug Resistant Tumors"
4th November 2020

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IN SCIENCE AND TECHNOLOGY

COST is supported by the EU Framework Programme
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STRATAGEM Action Summary

This Action will build the first multidisciplinary network, including academic laboratories, research institutes, small and medium enterprises (SMEs), with a wide range of excellent and non-overlapping expertise, aiming at improving at the same time the diagnosis and therapy of multidrug resistant (MDR) solid tumors. Until now, there are fragmented knowledge on biomarkers and therapeutic tools used against MDR tumors; there are not algorithms predictive/diagnostic of MDR tumors ex ante; all the past therapies against MDR tumors failed. The key challenge of this Action is to fill these gaps, by producing a comprehensive, open and user-friendly platform of knowledge on MDR tumors, identifying new diagnostic/predictive biomarkers, producing new and safe compounds applicable to personalized treatments of MDR tumors. Up to 70% of solid tumors are resistant at the diagnosis: this means poor life quality and poor prognosis for patients, high management costs for the European healthcare systems. This Action is working to improve diagnosis and treatment of patients with MDR tumors and reduce the costs for their management. Second, by creating fruitful collaborations between basic and industrial research, we will give impulse to the creation of new Start-up and SMEs in Europe. Finally, the Action aims at raising the level of European research on MDR, reducing the disparity in the research quality between EU countries and ITC, providing the necessary training for European early stage researchers (ESRs) to grow as future independent research leaders, regardless of location, age or gender.

Action website: https://stratagem-cost.eu/
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Dear Friends,

Welcome to the third WG3 online Meeting. Given the current pandemic restrictions, we decided to extend the WG3 Meeting into a small Symposium, to allow us to better know each other and exchange scientific ideas. As you know, the WG3 aims to explore and validate the efficacy of new therapeutic tools against pre-clinical models of MDR tumors. However, this event is open to members from all WGs of STRATAGEM, with complementary scientific aims.

This one-day online Symposium includes two outstanding plenary lectures given by Prof. Elisa Giovannetti and Prof. Godefridus J. Peters, on topics highly relevant to our objectives. We have eight excellent selected oral presentations given by young scientists/students from different member's laboratories, from various parts of the world.

Twenty posters are available at our webpage (following login) and will be shortly presented during the Symposium, in the form of 5-minute "Speed-talks". At the end of the Meeting/Symposium, we will award prizes for the best presentations. These will be selected by the Prizes Selection Committee, consisting of members of the Scientific Committee who will attend sessions.

The WG3 members Meeting itself, will take place at the end of the morning session, during which an update on relevant topics will be made. We wish that this online Meeting/Symposium enhances the scientific knowledge of all participating scientists and students, providing a valuable experience and new opportunities for future collaborations.

Finally, and most importantly, we hope you enjoy this scientific day!

FINAL PROGRAMME

(IN CENTRAL EUROPEAN TIMES)

	Wednesday 4th November 2020
9:30	**Welcome** **Chiara Riganti, Chair of STRATAGEM and Helena Vasconcelos, WG3 Leader**
9:40-13:00	**MORNING SESSION**
9:40	**Keynote lecture I** **Moderator: Helena Vasconcelos, WG 3 Leader**
	Elisa Giovannetti: Pancreatic cancer: why is it so hard to treat? Molecular mechanisms underlying resistance and new therapeutic strategies.
10:30 – 11:50	**Selected Oral Presentations**
	Moderator: Nuray Erin, WG3 Vice-Leader
10:30	**Raquel Alves:** Drug response prediction in CML: the value of microRNAs expression profile.
10:50	**Martina Godel:** Small nucleolar RNAs mediate doxorubicin resistance in osteosarcoma.
11:10	**Cristina P.R. Xavier :** Chitinase 3-like-1 and Fibronectin present on extracellular vesicles shed by human macrophages induce gemcitabine resistance in pancreatic cancer cells.
11:30	**Preeta Ananthanarayanan:** Novel Acoustic Wave Programmed 3D Vascularised Cancer Model in Malignant Pleural Mesothelioma: a platform for drug screening.
11:50	**Coffee-Break**
12:00-13:00	**WG3 members meeting (for WG3 members)** **Moderators: Helena Vasconcelos, WG3 Leader and Nuray Erin, WG3 Vice-Leader**
13:00- 14:00	**Interval (Lunch Break)**
14:00– 18:00	**AFTERNOON SESSION**
14:00	**Keynote lecture II** **Moderator: Chiara Riganti, Chair of STRATAGEM**
	Godefridus J. Peters : The role of drug sequestration and physicochemical properties in drug uptake and resistance.
14:50– 16:10	**Selected Oral Presentations**
	Moderator: Helena Vasconcelos, WG3 Leader
14:50	**Nur Ogan:** Urokinase-type Plasminogen Activator and Peroxiredoxin 2 in cancer cell exosomes may lead resistance to CXCR2 antagonists by release of CXCR2 ligands.
15:10	**Marialessandra Contino:** MRP1-Collateral Sensitizers as a Novel Therapeutic Approach in Resistant Cancer Therapy: An In Vitro and In Vivo Study in Lung Resistant Tumor.
15:30	**H. Schueffl:** Development and anticancer activity of hypoxia-activatable prodrugs of the tyrosine kinase inhibitor crizotinib.
15:50	**Muhlis Akman:** Self-assembling nanoparticles encapsulating zoledronic acid overcome immune resistance in human osteosarcoma
16:10	**Coffee-break**
16:20 – 18:00	**Speed-Talks of Poster Presentations**
	Moderators: Milica Pesic, Short Term Scientific Missions Coordinator and Jitka Viktorova, Early Career Investigators (ECI) Coordinator
16:20	**Sofia La Vecchia:** Gender and estrogens as key factors in the response to

	immunotherapy in non- small cell lung cancer.
16:25	**Nuray Erin:** Expression of Fibulin-4, LTBP and BMP-1 levels in human breast carcinoma samples as well as liver metastasis.
16:30	**Diana Sousa:** Multidrug Resistant cells show different ability to release and capture EVs than their sensitive counterparts: effect of endocytic pathway regulation.
16:35	**Rita Rebelo:** Drug sequestration by Extracellular Vesicles: preliminary evidence for a role of P-glycoprotein in this process.
16:40	**Esra Nizam:** Inhibition of bone morphogenetic protein 1 activity decreases colony and spheroid formation in metastatic breast carcinoma cells.
16:45	**Sonja Hager:** Elucidating mechanisms of resistance against the anticancer thiosemicarbazone COTI-2 by structural modifications and metal complex formation
16:50	**Mónica Suárez Korsnes:** Cell speed as phenotypic signature in drug discovery.
16:55	**Raquel Alves:** Everolimus - a mTOR inhibitor as a new drug for CML treatment.
17:00	**Emre Ozgenc:** Development of New Trastuzumab-Chelating Agent Complexes for Breast Cancer Treatment.
17:05	**Cecilia Carpinella:** Active principles obtained from plants of Argentina as new therapeutic tools against multi-drug resistant tumors.
17:10	**Patrícia Rijo:** Novel class of P-glycoprotein inhibitors from Plectranthus spp.
17:15	**David S. P. Cardoso:** Overcoming ABC transporters-mediated MDR in cancer: alkylated indole alkaloid derivatives as ABCB1 inhibitors .
17:20	**Jitka Viktorova :** Cancer and Bacterial Multidrug Resistance Modulation By Natural and Semi- synthetic Flavonolignans.
17:25	**Nikoletta Szemerédi:** Resistance modulating activity of selenoesters in bacteria.
17:30	**Seren Haksever:** TRPV1 modulators synergistically enhances anti-proliferative effects of Amitriptyline on metastatic breast carcinoma cells.
17:35	**Andrea Rodríguez-Alonso:** A novel small-molecule compound targeting Hakai for anticancer therapy.
17:40	**Madalena M. M. Pinto**: Inhibitors of tumor cell growth and P-glycoprotein as potential anti MDR agents.
17:45	**Radka Vaclavikova:** Efficacy and molecular mechanism of action of MDR-reversal stony brook taxanes in resistant in vitro and in vivo models of ovarian cancer cells.
17:50	**Gabriella Spengler:** Efflux pump inhibition by symmetric selenoesters on colon adenocarcinoma cells.
17:55	**Ana Podolski-Renić :** Novel TrxR1 inhibitors induce oxidative stress and sensitize human multidrug resistant glioblastoma cells to chemotherapy.
18:00	**Prizes for best presentations (to be announced on the Stratagem website) and Closing remarks**
	Chiara Riganti, Chair of STRATAGEM and Helena Vasconcelos, WG3 Leader
18:10	**End of Day**

Abstracts; Oral presentations

The abstracts presented herein are organised as per the event programme.

Pancreatic cancer: why is it so hard to treat? Molecular mechanisms underlying resistance and new therapeutic strategies

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Despite its low incidence rate (3% of cancers worldwide), pancreatic ductal adenocarcinoma (PDAC) is the fourth cause of cancer death in Europe and is the only cancer with increasing mortality rates in both men and women. Because of this dismal trend, this malignancy is expected to become the second most common cause of cancer-related death by 2030.

The poor prognosis of PDAC is cause by several factors.[1] First, no methods for screening are available in order to improve early detection in high-risk groups, due to the absence of disease-specific biomarkers. Second, clinical presentation typically occurs late in the history of the disease: the symptoms are vague and nonspecific, making early detection extremely difficult. Third, despite the approval of novel polychemotherapeutic regimens, which led to a slight improvement of survival, surgical resection remains the only potentially curative option, but it is only possible in 20% of patients.

Notably, very few patients experience an objective response after chemotherapy. Thus, the resistance of these tumors is primary (innate), rather than the secondary (acquired) resistance that is classically observed in most cancers. This chemoresistance occurs due to cell-autonomous and non-cell-autonomous mechanisms. A striking feature of PDAC is the high penetrance of genetic alterations in four genes (K-Ras, TP53, cdkn2a and SMAD4/DPC4), whose combined effects make these tumors extremely aggressive. Other genetic aberrations have been evaluated in several -omics studies, but, with the exception of BRCA1/BRCA2 mutations for PARP1 inhibitors, this increased knowledge in the underlying genetics of PDAC has not yet been translated to the identification of “actionable” targets. Differential expression of transporters and enzymes associated with the metabolic activation/inactivation of drugs (such as hENT1 and CDA for gemcitabine) can also impact tumor sensitivity. However, the peculiar desmoplastic, immunosuppressive, stromal reaction surrounding PDAC has major effects on tumor metabolism as well as on drug delivery and efficacy. Unfortunately, most trials using anti-stroma approaches failed, implying that, although the stroma may be a physical barrier hampering drug delivery, it may also have protective effects in restraining tumor progression. It is thus imperative to carefully consider both the tumor and the different properties of the surrounding stroma when designing novel therapeutics for PDAC. Last but not least, metastatic relapse occurs in the majority of patients undergoing resection, and their 5-year survival is still below 30%. This implies that achieving cure in the absence of new therapeutic strategies against metastasis is likely to be impossible.

References:

[1] Giovannetti E et al (2017). Sem Cancer Biol. 44: 43-59

Drug response prediction in CML: the value of microRNAs expression profile

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Despite the higher therapeutic efficacy of Imatinib and other tyrosine kinase inhibitors (TKIs), chronic myeloid leukemia (CML) cells develop mechanisms that overcome the therapeutic effect becoming resistant to therapy. Currently, the challenge in CML management is to improve therapeutic selection in order to avoid TKI resistance. Several mechanisms are described as necessary for resistant phenotypes, like *BCR-ABL1* point mutation or influx/efflux drug transporters expression. Additionally, microRNAs (miRs) are essential epigenetic regulators of gene expression that could play a critical role in Imatinib response/resistance phenotype.

The aim of this work was correlate the expression levels of miR-21, miR-451 and miR-26 in CML patients at diagnosis with TKI response. The expression levels of miR-21, miR-26, miR-451 and miR-16 (endogenous control) were determined by TaqMan MicroRNA Assays, in 30 CML patient samples at diagnosis. The population in the study presented a median of 54 years old and 83.3% of the patients were diagnosed at chronic phase. The proper statistical analysis was performed with significance level of 95%. The miR-451 was the miR with higher expression levels (median: 7.3), while the miR-26 show a median of expression of 0.086, and the miR-21 presented the lowest levels (median: 0.0003). miR-21 and miR-451 presented potential as biomarkers of response to Imatinib at 12 months of treatment follow-up. Higher levels of miR-21 were observed in patients without optimal response while higher levels of miR-451 were correlated with TKI response. Using the combined profile of both miRs, we create a predictive model of optimal response after one year of treatment. This study highlights the role of miR-21 and miR-451 expression levels at diagnosis in predicting which CML patients achieve the optimal drug response.

Acknowledgements:

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Small nucleolar RNAs mediate doxorubicin resistance in osteosarcoma

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Osteosarcoma, one the most frequent tumors in childhood, is usually treated with conservative surgery and neo-adjuvant or adjuvant chemotherapy. Doxorubicin (Dox) is one of the front-line drugs, but in 60% of patients' resistance arises. The main mechanism of Dox resistance is the presence the ATP binding cassette transporter B1 (ABCB1)/P-glycoprotein (Pgp), which effluxes Dox outside the cells, impairing its accumulation and cytotoxicity. Although Pgp levels are predictive of osteosarcoma response to Dox, resistance is not fully explained by the Pgp-mediated mechanism. In order to explore other mechanisms of drug resistance, we performed a whole-genome expression profile analysis of Dox-sensitive human osteosarcoma U-2OS cells and their resistant variants (U-2OS/DX30, U-2OS/DX100, U-2OS/DX580), characterized by progressively increasing resistance to Dox. As expected, we found the upregulation of specific drug resistance-related genes, such as *ABCB1*, but also a general upregulation of small nucleolar RNAs (snoRNAs) in resistant variants. SnoRNAs have a role in organizing nucleolar RNA and in guiding methylation, pseudo-uridylation and acetylation of rRNA; moreover, they mediate mRNA processing, and some of them can produce regulatory RNAs [1]. Our analysis showed that snoRNAs upregulation was higher in the mildly resistant U-2OS/DX30 and U-2OS/DX100 variants than in the strongly resistant U-2OS/DX580 cells, suggesting that snoRNAs upregulation may be involved in the first phases of acquisition of Dox resistance. Three specific snoRNAs were progressively upregulated with increasing Dox resistance: *SNORD3A*, *SNORA13*, and *SNORA28*. Indeed, their overexpression in U-2OS sensitive cells demonstrated that each snoRNA is able to induce resistance to Dox in a Pgp-independent way: snoRNA-overexpressing U-2OS cells showed the same viability of their resistant variants, notwithstanding Pgp levels remained low in the former, high in the latter. Moreover, by analyzing gene set important in osteosarcoma progression, we found that resistant U-2OS/DX30, U-2OS/DX100 and snoRNA-overexpressing U-2OS cells had *GADD45A* and *MYC* up-regulated, *TOP2A* down-regulated. Silencing of *GADD45A* and *MYC* and overexpression of *TOP2A* restored Dox sensitivity in all five variants. These results suggest a new mechanism of acquired resistance to Dox in osteosarcoma: the continuous exposure to the drug may lead to the upregulation of *SNORD3A*, *SNORA13* and *SNORA28*, and consequently to the modulation of downstream effectors, such as *GADD45A*, *MYC* and *TOP2A*, that induce resistance to Dox in a Pgp-independent manner.

References:
[1]. Kufel J et al. (2019). Trends Genet. (35): 104-117.

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Chitinase 3-like-1 and Fibronectin present on extracellular vesicles shed by human macrophages induce gemcitabine resistance in pancreatic cancer cells.

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Tumor-associated macrophages (TAMs) are immune cells from the tumor microenvironment, which have been implicated in pancreatic ductal adenocarcinoma (PDAC) therapy resistance [1]. TAMs may communicate with cancer cells through secretion of extracellular vesicles (EVs), which are currently recognized as a source of biomarkers [2,3]. This work aims to understand the impact of the protein cargo of EVs released by human macrophages on PDAC cellular response to gemcitabine (GEM), the standard-based chemotherapy for this cancer, and identify novel targets for therapeutic intervention.

Results showed that large EVs shed by human macrophages decreased BxPC3 PDAC cellular sensitivity to GEM, as verified with a SRB assay. Proteomic analysis of those EVs identified Chitinase 3-like 1 (CHI3L1) and Fibronectin (FN1) as the most abundant proteins. Experiments using either recombinant human proteins or pharmacological inhibitors confirmed that CHI3L1 and FN1 induced PDAC cellular resistance to GEM, involving the ERK signaling pathway. Moreover, CHI3L1 and FN1 expressed in the stroma of human PDAC tumor patient samples were associated with high presence of macrophages, as demonstrated by immunohistochemistry analysis. Interestingly, analysis of The Cancer Genome Atlas (TCGA) corroborated our *in vitro* data, showing that high expression levels of both genes, *CHI3L1* and *FN1*, have been associated with low overall survival of PDAC patients and also with high levels of macrophage infiltration. Besides, patient's response to GEM was dependent on the expression levels of both genes, further suggesting the involvement of CHI3L1 and FN1 on GEM resistance in PDAC.

This work suggests that CHI3L1 and FN1 should be further studied as potential therapeutic targets for PDAC treatment.

References: [1]. Farajzadeh Valilou S, et al. (2018). Cytokine Growth Factor Rev 39:46-61; [2]. Vasconcelos MH et al. (2019). Drug Resist Updat 47:100647; [3]. Xavier CPR et al. (2020). Cells 9 (5): 1141.

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Novel Acoustic Wave Programmed 3D Vascularised Cancer Model in Malignant Pleural Mesothelioma: a platform for drug screening

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Malignant Pleural Mesothelioma (MPM) is a highly aggressive and resistant cancer, with a median survival of 9–12 months in both untreated and treated patients with surgery, radiotherapy or chemotherapy [1, 2]. There is a growing need for advanced 3D models to overcome shortcomings of traditional 2D methods and animal testing. Creating such models simulating the whole tumour microenvironment (TME) is challenging and have mostly been developed using microfluidics with limited scalability, making drug discovery time-consuming and expensive [3]. We exploited the Sound Induced Morphogenesis (SIM) technique to develop an advanced 3D *in-vitro* model that resembles *in-vivo* MPM [4, 5]. This tunable multicomponent model consisted of spatially organized cancer cells, cancer associated fibroblasts (CAFs) and microvasculature within a matrix, to reproduce the highly heterogenous TME and investigate response to conventional and novel chemotherapeutics [6].
Using SIM, HUVEC–GFP endothelial cells and CAFs were patterned in a fibrin hydrogel within 2 min to obtain a circular ring (diameter: 2mm; thickness: 100 ± 50µm) forming a microcapillary network in 48hrs. Patient derived heterotypic tumour spheroids (MPM:CAF) of around 582.3µm were co-cultured in fibrin matrix over the assembled endothelial cells and followed for 4 days. Using confocal microscopy, microcapillary area evolution, spheroid sprouting and apoptosis was evaluated. Preliminary analysis of the vascular network over the time showed that the patterned HUVECs/spheroids system showed greater stability and allowed to monitor MPM migration. The vascularized model was treated with Cisplatin, the gold standard therapy, and Bevacizumab, a recently approved anti-angiogenic drug in MPM. They produced a 75% and 45% decrease in spheroid sprouting, respectively. Cisplatin alone produced a 4-fold increase in apoptosis. Although the combinatorial treatment produced only a 3.2-fold increase in apoptosis, spheroid sprouting decreased by 87%.
This platform offers an advanced vascularized *in-vitro* model to investigate tumour growth, invasion and resistance, suitable for high-throughput drug screening.

References:
[1]. Thellung S et al. (2016). Curr Drug Targets. 17:824–849.
[2]. Spugnini EP et al. (2006). Int J Biochem Cell Biol. 38:2000–2004.
[3]. Singh A et al. (2017). Transl lung cancer Res. 6:248–258.
[4]. Lata JP et al. (2016). Adv Mater. 28:8632–8.
[5]. Guo F et al. (2016). Proc Natl Acad Sci. 113:1522–1527.
[6]. Chu GJ et al. (2019). Front Oncol. 9 (1366): 1-12.

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The role of drug sequestration and physicochemical properties in drug uptake and resistance.

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Tyrosine kinase inhibitors (TKI) were introduced into the clinic as specific inhibitors of critical signaling pathways in tumors. Most TKIs are highly protein bound and substrates for Phase I and II metabolism. However, these molecules are all slightly polar and hydrophobic in nature, making them excellent substrates for multiple influx and efflux transporters, which ultimately determine their pharmacokinetic profile, efficacy and resistance. Various organic cation transporters (OCT1,2 & 3) played a role in uptake and P-glycoprotein and ABCG2 (BCRP) in the efflux of dasatinib and sorafenib, respectively. A high concentration increased uptake by diffusion, but at a lower concentration active transport mediated elimination back into the gut was the predominant process. TKIs such as erlotinib and gefitinib had permeability efflux ratios of between 1-2 whereas dasatinib had an efflux ratio of greater than 10, explaining a poor gut transfer and cellular uptake.
Absolute total cellular accumulation was very different with erlotinib and dasatinib reaching 20-150 ng/mg protein, while gefitinib was 20 fold higher. Sunitinib, sorafenib and crizotinib accumulation were even higher reaching 2 to 12 µg/mg protein. This pattern matched accumulation of some TKIs observed in patients in the clinical setting. For sunitinib and to a lesser extent crizotinib, a high lysosomal accumulation was found, which masked true cellular uptake. This sequestered portion of the drug is isolated from its intended target leading to resistance. With a biomimetic membrane model we investigated their physico-chemical properties and protein binding in order to predict their theoretical volume of distribution and % of lysosomal accumulation, which was determined via cellular accumulation studies, with and without a lysosomal inhibitor.
Development of TKIs often neglected these common pharmacological parameters and many potential drugs failed to achieve efficacy in clinical trials. Uptake of the various TKIs in tumor cells is highly variable, as well as transfer rates in the gut epithelial model, with a very high negative flux for dasatinib. Hence proper evaluation of these properties in suitable model systems will help to predict how drugs will behave in the patient.

Urokinase-type Plasminogen Activator and Peroxiredoxin 2 in cancer cell exosomes may lead resistance to CXCR2 antagonists by release of CXCR2 ligands

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Tumor-derived factors are involved in resistance to cancer treatments. We previously found that factors released by metastatic breast carcinoma cells increased MIP2 (Macrophage Inflammatory Protein2), a chemokine ligand for CXC receptor 2 (CXCR2) whose antagonists are in clinical trials for cancer treatment. Hence tumor-derived factors by increasing endogenous ligand of CXCR2 may lead resistance to CXCR2 antagonists. We here examined the effects of autocrine factors on MIP2 and KC (Keratinocyte Chemoattractant) secretion. We previously found that eighty-five autocrine factors significantly altered, mostly increased, in murine breast metastatic carcinoma cells compared with non-metastatic cells. In this study we selected five of these autocrine factors to investigate their role on MIP2 and KC release. These factors were Urokinase-type Plasminogen Activator (uPA), Beta-galactoside-alpha-2,-sialyltransferase-1(ST6GAL1), Peroxiredoxin-2 (PRDX2), Complement component-3a (C3a), and Thymic Stromal Lymphopoietin (TSLP). Furthermore, combination studies with CXCR2 inhibitor were performed with the two of the factors that were most effective in inhibiting proliferation and chemokine secretion.
4TBM and 4THM cells were treated with specific antagonists of uPA, ST6GAL1, PRDX2, C3a, and TSLP-Receptor (IPR-803, 3-Fax Neu-5 AC, Conoidin-A, SB290157, and TSLPR-ab, respectively), then changes in cell proliferation and chemokine release were determined. The effects of antagonists were evaluated in the presence of: 1.High (%5) FBS, which allows fast cell proliferation, 2.Very low (%0.2) FBS, which allows growth under the influence of autocrine factors.
Treatment with IPR-803, inhibitor of uPA and Conoidin-A, inhibitor of PRDX2 were the most effective in suppressing the cell proliferation and chemokine secretion. Both inhibitors suppressed cell proliferation in 4THM and 4TBM cell lines concentration dependently. Anti-proliferative effects of both inhibitors were observed in the presence of low and high FBS. IPR-803 when combined with CXCR2 inhibitor (SB 225002) did not markedly alter the anti-proliferative effects of SB 225002 but reversed the rebound increases in chemokine secretion due to CXCR2 blockage. The anti-proliferative effects of Conaidin-A when combined with CXCR-2 antagonist was higher than when it was used alone. These results demonstrated that uPA, and PRDX2 may mediate chemoresistance in metastatic breast carcinoma in an autocrine manner.

1. Erin N, Ogan N, Yerlikaya A. Secretomes reveal several novel proteins as well as TGF-β1 as the top upstream regulator of metastatic process in breast cancer. Breast Cancer Res Treat. 2018 Jul;170(2):235-250.

2. Erin N, Kale S, Tanrıöver G, Köksoy S, Duymuş O, Korcum AF. Differential characteristics of heart, liver, and brain metastatic subsets of murine breast carcinoma. Breast Cancer Res Treat. 2013 Jun;139(3):677-89.

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MRP1-Collateral Sensitizers as a Novel Therapeutic Approach in Resistant Cancer Therapy: An In Vitro and In Vivo Study in Lung Resistant Tumor

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MultiDrugResistance (MDR) is the main cause of the failure of the anticancer therapy and it is mainly due to the overexpression of some transporters as MDR1, BCRP and MRP1 also claimed MDR proteins [1,2]. MDR proteins inhibition has been used to overcome MDR but the translation of this strategy into clinic unfortunately failed because of pharmacokinetic aspects [1,2]. Recently, Collateral Sensitivity (CS) emerged as a new approach to hamper MDR [3-5]. CS can be described as the ability of some compounds to selectively kill MDR cells but not the drug-sensitive parental cells from which they derived. Therefore, an agent able to selectively kill MDR cells without any cytotoxic effect on the wild type cells, is claimed as CS-promoting agents or Collateral Sensitizers [3-5]. With the aim to identify CS-promoting agents, we screened our library of MDR ligands for their cytotoxicity in the "pure" model of MRP1-expressing cells (MDCK-MRP1) and in MRP1-expressing/drug resistant non-small cell lung cancer cells (A549/DX) and in the corresponding counterparts (MDCK and A549 cells). By this preliminary screening, we identified three compounds **F397**, **F400**, **F421** acting as Collateral sensitizers in the both couples of cells and thus we evaluated in vitro their cytotoxic activity in co-administration with the gold antineoplastic agent used for lung cancer therapy, Cis-Pt. Cis-Pt, as MRP1 substrate, is effluxed from the tumour cells by MRP1, not inducing cytotoxicity while, in the presence of the three compounds able to block MRP1, Cis-Pt is able to enter the tumour cells, exerting its antineoplastic activity. Since the three compounds were also able to induce per se cytotoxicity in the resistant cells, we study their mechanism of action in vitro by evaluating their ability to induce ROS production. We also deepened the CS mechanism in vivo in lung tumor xenografts. The in vitro and in vivo results showed that compounds **F397**, **F400**, **F421**: (a) were highly cytotoxic; (b) exerted an action due to MRP1; (c) improved the cytotoxic effect of Cis-Pt; (d) were able to induce a ROS mediated effect. All these findings candidate the three ligands as CS-promoting agents in resistant lung cancer.

References:

[1] Colabufo NA et al. (2010). J. Med. Chem. 53: 1883-1897.
[2] Riganti C et al. (2015) Frontiers in Oncology, 5: 105.
[3] Hall MD et al. (2009) Trends Pharmacol. Sci. 30: 546-556.
[4] Szakàcs G. et al. (2006) Nat. Rev. Cancer 5: 219-234.
[5] Szakàcs G et al. (2014) Chem. Rev. 114: 5753-5774.

Development and anticancer activity of hypoxia-activatable prodrugs of the tyrosine kinase inhibitor crizotinib

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Over the last decades, tyrosine kinase inhibitors have fundamentally changed cancer therapy. However, the clinically approved representatives lack in tumor specificity, causing similar severe side effects like chemotherapeutics, dose-dependent toxicity and drug resistance. One possible strategy to ensure that the drug is selectively active in the tumor is to exploit the hypoxic conditions which are usually only present in solid tumors. Thus, we recently converted crizotinib, an ALK and MET inhibitor, into two different 2-nitroimidazole-based hypoxia-activatable prodrugs. In prodrug A the nitroimidazole moiety is connected to the aniline NH_2 of crizotinib via a carbamate, in prodrug B the NH_2 is directly alkylated. The aim of this study was to proof the prodrug stability and binding to the ALK and MET kinase and to investigate the anticancer activity of prodrugs. Cell-free kinase inhibition and docking studies revealed that the inhibitory potential against ALK and MET of both crizotinib prodrugs was diminished. Moreover, the prodrugs were stable in phosphate-buffered saline. However, reductive activation by E. coli nitroreductase under cell-free conditions could be only observed for prodrug A, while prodrug B could not be activated. Next, the anticancer activity and signaling inhibition of the compounds against three human cancer cell lines were tested under normoxic and hypoxic conditions via MTT assay and Western blot analysis, respectively. These experiments showed that prodrug A has strong anticancer activity against MET-dependent cells in a hypoxia-dependent manner. In contrast, prodrug B had anticancer activity comparable to free crizotinib, regardless of the oxygen conditions. In line, hypoxia dependency of MET signaling inhibition was observed with the prodrug A, while oxygen levels had no impact on the activity of prodrug B. Furthermore, phosphorylation of MET was also inhibited by prodrug A in vivo using H1993 xenografts. In conclusion, prodrug A is a promising new hypoxia-activatable form of crizotinib and should further investigated.

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Self-assembling nanoparticles encapsulating zoledronic acid overcome immune-resistance in human osteosarcoma

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Background: Zoledronic acid is a clinically used aminobisphosphonate indicated for treatment of patients with bone metastases from solid tumors. Moreover, by up-regulating the efflux of the phosphoantigen isopentenyl pyrophosphate [IPP] via ABCA1, it is a strong activator of the antitumor Vγ9δ2 T-lymphocytes [1]. **Aims:** The aim of this work is to validate the use of self-assembling lipid nanoparticles [NanoZol, NZ] as an immune-therapeutic treatment against osteosarcoma refractory to standard chemotherapy, in order to propose new chemo-immune-therapy adjuvant protocols. **Methods:** In ex-vivo experiments, we compared doxorubicin-sensitive human U-2OS and Saos-2 osteosarcoma cells, their doxorubicin resistant [DX] sublines and the 3D cultures derived from parental doxorubicin-sensitive cells. Vγ9δ2 T-lymphocytes were obtained from healthy donors. We examined the effects of NZ on the molecular circuitries up-regulating ABCA1 and on the Vγ9δ2 T-lymphocyte-mediated killing. The immune-activating and the anti-tumor effects of NZ were validated in NSG mice engrafted with human hematopoietic $CD34^+$ cells (Hu-$CD34^+$ mice) bearing doxorubicin-resistant osteosarcoma. **Results:** Differently from 2D sensitive cells, 2D DX sublines and 3D cultures have low levels of ABCA1, a feature that makes them resistant to the Vγ9δ2 T-lymphocyte-mediated killing. By targeting the farnesyl pyrophosphate synthase step in the isoprenoid synthetic pathway, NZ inhibits Ras/Akt/mTOR axis and re-activates LXRα. In consequence of the LXRα-driven up-regulation of ABCA1, NZ restores the immune-killing by Vγ9δ2 T-lymphocytes. At the same time, NZ inhbits Ras/ERK1-2/HIF-1α, down-regulates ABCB1 (i.e. the main transporter effluxing of doxorubicin) and restores chemosensitivity. This phenotype is recapitulated in Hu-$CD34^+$ bearing chemo-immuno-resitant osteosarcomas derived from 3D cultures, where NZ reduces tumor growth, increases the ABCA1/ABCB1 ratio, the intratumor Vγ9δ2 T-lymphocytes and the sensitivity to doxorubicin. **Conclusion:** We propose nanoformulations of aminobisphosphonates as multi-target chemo-immune-sensitizing tools against high-grade osteosarcomas, thanks to their high intratumor delivery, enhanced Vγ9δ2 T-lymphocyte tumor cell killing and simultaneous rescue of chemosensitivity.

References: [1]. Castella B et al (2017) *Nat. Commun*. doi: 10.1038/ncomms15663
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Abstracts; Speed-talks of poster presentations

The abstracts presented herein are organised as per the event programme.

Gender and estrogens as key factors in the response to immunotherapy in non- small cell lung cancer

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The recent introduction of immune-checkpoint inhibitors (ICPI) – mainly the anti-programmed death 1 (PD1)/PD-ligand 1 (PD-L1) Pembrolizumab, Nivolumab and Atezolizumab – has improved the prognosis of non-small cell lung cancer (NSCLC) patients in 20-35% cases [1]. Recent network meta-analyses reported a differential response to immunotherapy (IOT) between the two genders but the results are controversial [2,3]. NSCLC are rich of estrogen receptor (ER)α and β and of aromatase that reduce the efficacy of chemotherapy [4]. It is not known if they are responsible for the gender-differential benefit of IOT.

To investigate if there was a molecular link between ERα and Pembrolizumab efficacy we analyzed a panel of 30 human NSCLC cell lines of female and male origin. The amount of ERα and 17-β-estradiol, produced by endogenous aromatase, was directly related to the expression of PD-L1. ERα transcriptionally up-regulated *CD274/PD-L1* gene, with higher effects in females. In cells with high EGFR activity, EGFR-downstream effectors Akt and ERK1/2 increased the amount of transcriptionally active phospho(Ser118)ERα, which in turns up-regulates *PD-L1*. The efficacy of Pembrolizumab in humanized mice bearing NSCLC xenografts was significantly enhanced by the aromatase inhibitor letrozole that reduced PD-L1 and increased the percentage of anti-tumor immune-infiltrating cells. The benefit was maximal in *17-β-estradiol/ERα high* female xenografts, minimal in *17-β-estradiol/ERα low* male xenografts.

Our data demonstrate that 17-β-estradiol/ERα status predicts the response to Pembrolizumab in NSCLC, potentially explaining the gender-related differential benefit of the IOT. Aromatase inhibitors should be explored in pivotal studies as adjuvant agents in gender-tailored IOT studies for NSCLC.

References:

[1]. Dafni U et al. (2019). Lung Cancer. 134:127-140.
[2].Conforti F et al. (2018). Lancet Oncol. 19(6):737-746.
[3]. Wallis CJD et al. (2019). JAMA Oncol. 1;5(4):529-536.
[4]. Zhao XZ et al. (2015). Onco Targets Ther. 9;8:2849-63.

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Expression of Fibulin-4, LTBP and BMP-1 levels in human breast carcinoma samples as well as liver metastasis

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We previously observed that molecules, which were involved in formation of extracellular matrix (ECM) as well as deposition of growth factors within ECM, were markedly increased in the exosomes of breast cancer cells derived from visceral metastasis compared to exosomes of non metastatic cells (1, 2). Based on the literature, we grouped the molecules that are functionally related, and selected the ones that are most likely to be involved in metastasis but not well-studied in breast carcinoma. We found that Fibulin-4 might be one of these molecules. Furthermore, LTBP-1, which is involved in secretion and deposition of TGF- β1 in ECM, and BMP-1, an activator of matrix-bound TGF-ß, were also markedly increased in metastatic cells. Recent studies demonstrated that Fibulin-4 is involved in deposition of TGF-ß in ECM, which is important for maintaining TGF-ß1 activity. It is known that TGF-ß1 induces epithelial-mesenchymal transition (EMT) leading to metastasis in aggressive carcinomas. Hence, we evaluated expressions of Fibulin-4, LTBP-1 and BMP-1 in 21 liver metastases of human breast carcinoma, which are originated from invasive ductal adenocarcinoma. In addition, levels of expressions of these three proteins were evaluated in 56 primary tumors (invasive ductal adenocarcinoma n=29; and lobular ductal carcinoma n=27) and in carcinoma in-situ samples (n=20). Lastly, we also examined expression of Fibulin-4, LTBP-1 and BMP-1 levels in normal mammary tissue. To our best knowledge, this is the first study examining the levels of these proteins in both metastasis and primary tumors.

Diffuse staining of BMP-1 was observed in majority of the cancer cells of both invasive ductal and invasive lobular types. Majority of breast cancer cells metastasized to liver demonstrated intense BMP-1 staining which was significantly higher than primary tumors of invasive ductal. Interestingly nuclear expression of BMP-1 in normal breast epithelia was observed. Epithelium and myoepitelium expressed LTBP-1 in control mammary tissue. Expression was mostly nuclear which is similar to staining with BMP-1. In majority of the tumor samples, intensity of staining was low and the staining was not diffuse. Significant increases in expression of LTBP-1 was observed in liver metastasis. Fibulin 4 staining was confined to extracellular space in both control tissue and breast cancer samples. Increased expression and altered intracellular localization of BMP-1 and LTBP-1 in liver metastasis of breast cancer demonstrate that the TGF-b-BMP-1 and LTBP-1 pathway may involve in breast cancer metastasis.

1. Erin N, Ogan N, Yerlikaya A. Secretomes reveal several novel proteins as well as TGF-β1 as the top upstream regulator of metastatic process in breast cancer. Breast Cancer Res Treat. 2018 Jul;170(2):235-250.

2. Erin N, Kale S, Tanrıöver G, Köksoy S, Duymuş O, Korcum AF. Differential characteristics of heart, liver, and brain metastatic subsets of murine breast carcinoma. Breast Cancer Res Treat. 2013 Jun;139(3):677-89.

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Multidrug Resistant cells show different ability to release and capture EVs than their sensitive counterparts: effect of endocytic pathway regulation.

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Multidrug resistance (MDR) is one of the main limitations of cancer treatment. MDR is defined as a phenomenon by which tumor cells develop cross-resistance to several drugs and is frequently caused by an overexpression of drug-efflux pumps, particularly P-glycoprotein (P-gp). Interestingly, Extracellular vesicles (EVs) carrying P-gp in their cargo may be mediators of horizontal transfer or MDR phenotype between MDR (donor) cells and drug-sensitive (recipient) cells [1].

The aim of the present work was to analyze and understand the mechanisms involved in the release of EVs by P-gp overexpressing MDR cells and their uptake by drug-sensitive counterparts. To accomplish this, two pairs of cell lines from two different tumor models [chronic myeloid leukemia (CML) and non-small cell lung cancer (NSCLC)] were used, consisting of a drug-sensitive cell line and its MDR (P-gp overexpressing) counterpart. EVs released by those pairs of cell lines were isolated by ultracentrifugation and properly characterized by our group [2,3].

We verified that MDR cells released more EVs than their drug-sensitive counterparts and that drug-sensitive cells had higher uptake of EVs than their MDR counterparts. In addition, we found that a distinct regulation of the endocytic pathway between drug-sensitive and MDR cells may influence their different ability to release and capture EVs. Importantly, manipulation of the recycling pathway reduced the response of drug-sensitive cells to doxorubicin treatment.

Our results indicate that the EVs-mediated transfer of a MDR phenotype follows characteristic and regulated mechanisms, regarding both the release of EVs by MDR donor cells and the uptake of EVs by recipient drug-sensitive cells.

References:

[1] Sousa D., et al. Trends Mol Med. 2015; 21(10):595-608. [2] Lopes-Rodrigues V., et al. Sci Rep. 2017; 7 44541. [3] Lopes-Rodrigues, et al. Biochim Biophys Acta. 2016; 1860 (3):618-27.

Drug sequestration by Extracellular Vesicles: preliminary evidence for a role of P-glycoprotein in this process.

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Multidrug resistance (MDR) is one of the main challenges of cancer treatment [1]. Extracellular Vesicles (EVs) are known key players in the transfer of MDR traits from donor multidrug-resistant tumor cells to recipient drug-sensitive (DS) counterparts. In fact, several mediators of MDR were already found in the cargo of EVs released by drug-resistant cells, including the drug-efflux P-glycoprotein (P-gp) [2]. Moreover, EVs can sequester drugs into their cargo, decreasing drug concentration in the intracellular environment, and thus contributing to drug resistance [2]. Nonetheless, this process is not fully understood.
This work aims to investigate the role of P-gp in drug sequestration by EVs shed by MDR cancer cells. To achieve this goal, EVs from a non-small cell lung cancer MDR cell line (NCI-RH460) and from its sensitive counterpart (NCI-H460), were isolated by differential ultracentrifugation, and further characterized by dynamic light scattering, transmission electron microscopy (TEM) and Western blot (WB) using specific EVs-markers [3]. Also, overexpression of P-gp was detected in the MDR cells and on the EVs isolated from those cells, compared with their DS counterpart cells and released EVs, using WB. Then, flow cytometry analysis of EVs incubated with rhodamine (a substrate of P-gp) and captured by aldehyde/sulfate latex beads showed that EVs released by the P-gp overexpressing MDR cells exhibit higher levels of rhodamine in their cargo than EVs released by their DS counterpart cells, suggesting that drug sequestration by EVs might depend on the MDR phenotype of their donor cells. Using a similar approach, EVs released by MDR cells were found to have higher presence of the internal epitope of P-gp in their membrane than EVs released by their counterparts. Preliminary data obtained by immuno-TEM corroborated these findings, suggesting that P-gp might be inverted, i.e. with an inside-out orientation, in some (but not all) of the EVs released by MDR cells, which is in agreement with other authors and could partly explain drug sequestration by EVs shed by drug-resistant cells [4].
Therefore, our preliminary results suggest a role for P-pg in drug sequestration by EVs released by MDR cells; further studies are ongoing to validate these findings.

References: [1] Assaraf, Y.G. et al. (2019).Drug Resistance Updates, 46, 100645; [2] Sousa, D. et al. (2015). Trends in Molecular Medicine, 21(10), 595-608; [3] Thery, C. et al. (2006). Current protocols in cell biology, 30(1), 3-22; [4] Gong, J. et al. (2013). European Journal of Pharmacology, 721(1-3), 116-125.

Inhibition of bone morphogenetic protein 1 activity decreases colony and spheroid formation in metastatic breast carcinoma cells

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Triple negative breast cancer (TNBC) is the most malignant subtype of breast cancer in which drug resistance is observed commonly. Transforming growth factor-β (TGF-β) by inducing cancer stem cell population leads to multidrug resistance. Bone morphogenetic protein 1 (BMP-1) is a metaloprotease involved in release of TGF-ß from extracellular macromolecular complexes. We previously observed that level of BMP-1 in exosomes of metastatic murine breast cancer cells is higher compared to non-metastatic cells [1]. We initially found that inhibition of BMP-1 activity decreases proliferation of 4TLM and 4TBM cells 72 hours after treatment. The aim of the present study was to evaluate the effects of BMP-1 inhibitor on metastatic potential of breast carcinoma cells using *in-vitro* models. We have used UK 383367, which is a selective inhibitor of BMP-1. The 4TBM and 4TLM cells were treated with various concentrations of the inhibitor and changes in colony as well as spheroid formation and TGF-ß secretion was determined. The 4TBM and 4TLM cells were derived from brain and liver metastatic lesions originated from 4T1 primary tumor, which is a murine model for TNBC [2].

UK 383367 dose dependently decreased both the number of colony formation and TGF-ß secretion from colonies in 4TBM cells. We observed somewhat similar effects of UK 383367 on spheroid formation such that number of spheroids markedly decreased following treatment with UK 383367. TGF-ß secretion from spheroids however was not markedly altered in 4TBM cells. Liver metastatic cells of 4T1 breast carcinoma act more aggressively including EMT phenotype. UK 383367 effectively inhibited the colony formation but did not markedly altered TGF-ß secretion. Similar changes were observed in spheroid formation such that UK 383367 markedly decreased spheroid formation without altering TGF-ß secretion. These results demonstrate for the first time that inhibition of BMP-1 activity may decrease metastatic growth of breast carcinoma.

1. Erin N, Ogan N, Yerlikaya A. Secretomes reveal several novel proteins as well as TGF-β1 as the top upstream regulator of metastatic process in breast cancer. Breast Cancer Res Treat. 2018 Jul;170(2):235-250.

2. Erin N, Kale S, Tanrıöver G, Köksoy S, Duymuş O, Korcum AF. Differential characteristics of heart, liver, and brain metastatic subsets of murine breast carcinoma. Breast Cancer Res Treat. 2013 Jun;139(3):677-89.

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Elucidating mechanisms of resistance against the anticancer thiosemicarbazone COTI-2 by structural modifications and metal complex formation

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COTI-2 is a thiosemicarbazone (TSC) that is currently tested as an anticancer therapeutic in a clinical phase I trial. The best-studied anticancer TSC is Triapine, against which cancer cells form resistance (a major obstacle in anticancer therapy) that strongly depends on the modification of Triapine's amino groups. The anticancer activity of TSCs is influenced by their interaction with endogenous metal ions, such as copper and iron. Therefore, in this study the effect of terminal N^4-modifications and metal complexation of COTI-2 on anticancer activity and resistance formation were investigated. As a first step, a new COTI-resistant cell model, named SW480/Coti, was established and its sensitivity/resistance tested against COTI-2 and its derivatives in comparison to the Triapine-resistant SW480/Tria cell model. The viability measurements revealed distinct structure-activity relationships in the resistance profile of the compounds. Interestingly, SW480/Coti cells were also resistant against the copper(II) (but not the iron(III)) complex of COTI-2, which is assumed to be the active metabolite in case of highly active TSCs. Further elucidation of the resistance mechanism revealed the ATP-binding cassette (ABC) transporter ABCC1, which exports glutathione-drug conjugates, as an important driver of COTI-2 resistance. While we found no glutathione adduct formation with the metal-free TSCs, COTI-2, in contrast to Triapine, formed stable ternary complexes with glutathione and copper(II), which can be effluxed by ABCC1. Consequently, the formation of copper(II) complexes is not only important for the anticancer activity of TSCs such as COTI-2, but also for acquired resistance due to their export by ABC transporters.

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Cell speed as phenotypic signature in drug discovery"

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Single-cell tracking throughout several cell cycles allows to trace kinships of cells in lineage trees. It therefore allows to utilize the fact that related cells bear information on the underlying mechanisms behind single cell phenotypes. Cell speed is a phenotypic feature that correlates between sister cells presumably due to similarities between the driving mechanisms behind their movement. It therefore deserves to be called a phenotype. This understanding conforms with the idea of stereotypical behaviour [1]. Speed data reflects single and collective cell motility modes which have been conserved through evolution and they depend on active reorganization of the cytoskeleton [2]. Such behaviour plays a crucial role in many vital processes as well as in cancer invasion and metastasis. A metastatasic cascade can enable some cells to migrate and create their own migration tracks [3]. Quantitative analysis of migration tracks can help to discover biological
functions or processes involved in diseases. This can guide evaluation of drug treatment, detection of rare sub-populations and discovery of drug-tolerant persister states [4].

References:

[1]. Tweedy, Luke, et al. "Screening by changes in stereotypical behavior during cell motility." *Scientific reports* 9.1 (2019): 1-12.
[2]. Mencattini, A., et al. "Discovering the hidden messages within cell trajectories using a deep learning approach for in vitro evaluation of cancer drug treatments." *Scientific reports* 10.1 (2020): 1-11.
[3].Paul, Colin D., Panagiotis Mistriotis, and Konstantinos Konstantopoulos. "Cancer cell motility: lessons from migration in confined spaces." *Nature Reviews Cancer* 17.2 (2017): 131-140.
[4].Vallette, François M., et al. "Dormant, quiescent, tolerant and persister cells: four synonyms for the same target in cancer." *Biochemical pharmacology* 162 (2019): 169-176.

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Everolimus - a mTOR inhibitor as a new drug for CML treatment

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The hallmark of chronic myeloid leukemia (CML) is the presence of *BCR-ABL1* fusion gene, which encodes an oncoprotein with a deregulated tyrosine kinase activity. This BCR-ABL oncoprotein activates multiples signaling pathways responsible for tumor cells characteristic, namely high cellular proliferation rate and resistance to apoptosis. PI3K/AKT/mTOR is one of downstream targets of BCR-ABL oncoprotein and is responsible for increase CML cell survival, being correlated with tyrosine kinase inhibitors (TKIs) therapeutic failure. The aim of this study was to evaluate the therapeutic potential of Everolimus (EVE), a mTOR inhibitor, in sensitive and resistant CML models. In this study, we use three CML cell lines: K562 cells sensitive to imatinib and two imatinib-resistant models, K562-RC and K562-RD cells. Cell lines were treated with different concentrations of EVE and the effect in cell viability was analyzed by the resazurin assay. Cell death was determined by flow cytometry (FC) using annexin V/propidium Iodide (PI) staining. The cell cycle analysis was assessed using PI incorporation by FC. Peripheral blood samples of 56 CML patients under TKI treatment were culture with increasing concentrations of EVE during 48h. The cytotoxic effect on these primary cultures was evaluated by FC using annexin V staining. Our results show that EVE induced a reduction in cell lines viability, with an IC_{50} of 20µM for sensitive cells and 25µM for Imatinib resistant cell lines. The cell death was induced by apoptosis and this drug has also an antiproliferative effect through an arrest in cell cycle progression in G_0/G_1 phase. In *ex-vivo* studies, EVE reduced cell viability by increasing apoptosis of hematopoietic stem cells ($CD34^+$ cells) with low cytotoxicity to lymphocytes. We observed a better response to EVE in patients under 2nd generation TKI associated with lower toxicity to lymphocytes. Our results suggest that EVE could be an alternative therapeutic approach in CML resistance.

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Development of New Trastuzumab-Chelating Agent Complexes for Breast Cancer Treatment

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Background: Human epidermal growth factor receptor 2 (HER2) is overexpressed in most cases of breast cancer. Patients with breast cancer who overexpress HER2 have an aggressive form of the disease. Trastuzumab (TRZ) is a monoclonal antibody targeting HER 2; While providing an effective treatment in practice, cytotoxicity causes negativities such as cardiotoxicity and development of resistance, which cause death [1].
Aims: This study aims to develop new TRZ complexes that can be treated and targeted to breast cancer cell. For this purpose, TRZ complexes were prepared by using different chelating agents and characterized with particle size, polydispersity index (PDI), FTIR and UV spectrophotometer. In vitro antitumoral activity of TRZ-chelating agent complexes were evaluated with cytotoxicity studies.
Methods: In TRZ complexes studies, 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), diethylenetriaminepentaacetic acid (DTPA), Ethylenediaminetetraacetic acid (EDTA) and 1,2-Dimethyl-3-hydroxypyridine were used as chelating agents. The particle size and PDI values were measured by Malvern Zeta Sizer (Nano-ZS). The proof of TRZ complexes form was evaluated by FTIR and UV spectrophotometer. MCF-7 was used for cytotoxicity studies and IC50 values were calculated by GraphPad Prism program.
Results and Conclusions: 13 of TRZ-different chelating agents were newly developed. The particle size and PDI of complexes were found approximately 1000 nm and 0,7, respectively. IC50 values were found to be between 8,5 and 24,54 µM. We plan to performed radiolabeling studies with Lutetium-177. The further studies will be continued with these 13 formulations.

References:

[1]. Mohan, N., Jiang, J., Dokmanovic, M. Wu, W. J. 2018. "Trastuzumab-mediated cardiotoxicity: Current understanding, challenges, and frontiers", Antibody therapeutics. NIH Public Access, 1(1), 13-17.

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Active principles obtained from plants of Argentina as new therapeutic tools against multi-drug resistant tumors

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Chemotherapy is one of the most powerful tools for successful treatment of cancer; however, this approach is often challenged by the development of multidrug resistance (MDR). The resistant phenotype represents an adaptive response of tumor cells, mediated *via* a range of cellular modifications, such as altered metabolism of drugs and changes in targets that result in less effective anticancer agents. Another major mechanism that leads to resistance is the presence of membrane transporters that extrude a broad range of drugs from cancer cells, thereby preventing to fulfill their cytotoxic activity. P-glycoprotein (P-gp) is probably the best known among these. Despite it constitutes the most important cause of failure in therapies, there are no P-gp inhibitors suitable for chemotherapies so far. Under this scenario, the search for agents able to hit resistant cancer cells and overcome P-gp-mediated MDR is a high priority. Since antiquity, plant products have been an invaluable source of leading molecules for drug development with a significant contribution to the treatment of cancer. As part of our continuous search for agents with anticancer properties, a panel of more than 160 extracts obtained from plants of Argentina and of bioactive metabolites isolated from these, were analyzed in order to determine their ability to inhibit the development of cancer cells resistant to conventional cytotoxic agents and their potential to reverse MDR in cells with over-expression of P-gp. New and known compounds, in particular sesquiterpene lactones, showed to be highly effective against MDR cancer cells. The antiproliferative effect was mediated by cell cycle arrest followed by apoptosis. Other metabolites, among them a triterpene and a lignan, and synthetic derivatives thereof designed using computer-assisted techniques, showed to inhibit the efflux of chemotherapeutic drugs from leukemia cells overexpressing P-gp, resulting in an increased sensitization to these. Molecular modeling studies showed that subject compounds mainly bind to the P-gp at transmembrane helices (TMH) 4, 5, and 6.

Novel class of P-glycoprotein inhibitors from Plectranthus spp.

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Multidrug resistance (MDR) is one of the main challenges in cancer treatment, in which overexpression of P-glycoprotein (P-gp) plays an important role. Therefore, there is an urgent need to identify new compounds that can exert anticancer effects and at the same time revert MDR. In this context, *Plectranthus* genus (Lamiaceae) is a great source of cytotoxic compounds that could be used as lead molecules for drug development, such as 6,7-dehydroroyleanone (**1**) (*P. madagascariensis* (Pers.) Benth. essential oil) and 7α-acetoxy-6β-hydroxyroyleanone (**2**) (*P. grandidentatus* Gürke) [1].
The aim of this work was to prepare a small library of new 12-*O*-substituted derivatives with potential P-gp inhibitory effect by exploring the reactivity of the natural royleanones **1** and **2**. In this study, we identified a new derivative that exhibited a P-gp inhibitory activity higher than the natural diterpenes **1** and **2**, and comparable to Dexverapamil. Furthermore, this compound showed the ability to sensitize the resistant cell line NCI-H460/R to doxorubicin. This activity was evaluated in the human non-small cell lung carcinoma (NCI-H460) cell line and its MDR counterpart NCI-H460/R with the P-gp overexpression by using the MTT and Rhodamine 123 accumulation assays. Further derivatizations and quantitative structure–activity relationship analysis are ongoing to discover new derivatives with improved activity.

References: [1] Isca VMS et al. (2020). ACS Med Chem Lett. 11 (5): 839-845.

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Overcoming ABC transporters-mediated MDR in cancer: alkylated indole alkaloid derivatives as ABCB1 inhibitors

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Multidrug resistance (MDR) has become the main limitation for cancer treatment. The overexpression of ABC transporters, by pumping out of the cells anticancer drugs, is considered one of the principal mechanisms behind MDR. P-glycoprotein (P-gp/ABCB1) is one of the main ABC transporters associated with MDR.

Aiming at optimizing monoterpene indole alkaloids for their MDR reversing activity in cancer [1], two major alkaloids, isolated from *Tabernaemontana elegans,* were derivatized by alkylation of the indole nitrogen. Twenty-six new derivatives were prepared. Their MDR reversal ability was assessed, using as models resistant human colon adenocarcinoma and human *ABCB1*-gene transfected L5178Y mouse lymphoma cells, overexpressing ABCB1. A noteworthy increase of activity was found for most of the derivatives, being the strongest ABCB1 inhibitors those having *N*-phenethyl moieties, exhibiting strong inhibitory activity concomitant with weak cytotoxicity. Furthermore, in combination assays, most of the compounds have shown strong synergistic interactions with doxorubicin, substantiating their potential as MDR reversers. In addition, 3D-QSAR models were established to identify structural elements contributing to the inhibitory effect of compounds.

References:
[1]. Paterna A et al. (2017). European Journal of Medicinal Chemistry 128: 247–257.

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Cancer and Bacterial Multidrug Resistance Modulation By Natural and Semi- synthetic Flavonolignans

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Flavonolignans are important natural compounds, which are now investigated in terms of the structural issues, intracellular signalization, and their inhibitory activities towards multi-drug resistance (MDR) proteins either in somatic cells (cancer cells) or in microorganisms [1]. We prepared by isolation, chemical and/or chemo-enzymatic derivatization new flavonolignans that were evaluated in detail for their MDR modulation activity.
The aim of the antimicrobial assay was to find compounds reducing the effective concentration of antibiotics used against different pathogenic multidrug-resistant strains of bacteria. For quantitative determination of synergistic effect of the new compounds and commercial antibiotics on the antimicrobial activity, the attention was focused on the concentration inhibiting growth of half of population (IC_{50}) and Fractional Inhibitory Concentration (FIC) index value. The special issue in MDR topic is the resistance of biofilms, whose decrease was measured by resazurin based viability assay. Possible inhibition of quorum sensing was determined by using of several strains of *Vibrio campbellii* serving as biosensors for detection of communication molecules.
Further, the antioxidant activities of flavonolignans were investigated by using oxygen radical absorption capacity (ORAC) and cellular antioxidant activity (CAA) assay. All substances efficiently reduced nitric oxide production and cytokines (TNF-α, IL-6) release in dose-dependent manner. Multidrug resistance (MDR) modulating potential was evaluated as inhibition of P-glycoprotein (P-gp) ATPase activity and regulation of ATP-binding cassette (ABC) protein expression. All the tested compounds showed strong dose-dependent inhibition of P-gp pump. Moreover, 2,3-dehydrosilybin A, silychristin A and 2,3-dehydrosilychristin A displayed the strongest sensitization of doxorubicin-resistant ovarian carcinoma. Despite these significant effects, silybin B, anhydrosilychristin and isosilychristin were the only compounds downregulating the expression of P-gp, MRP1 and BCRP.
Selected flavonolignans, especially derivatives of silybin AB and silychristin A, turned out to be rare MDR modulators and deserve attention in further research.

References:
[1]. Chambers CS et al. (2020). J. Agric. Food Chem. 68 (7):1763-1779.

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Resistance modulating activity of selenoesters in bacteria

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Infections caused by multidrug resistant bacteria can lead to serious problems regarding the antibacterial therapy. Multidrug resistance (MDR) mechanisms allow resistant bacteria to have limited uptake of drugs, modification of their target molecules, drug inactivation, or release of the drug into the extracellular space by efflux pumps (EPs). Previously, selenoesters have proved to be effective derivatives with anticancer and antimicrobial activities. Based on these results, new selenoesters have been synthesized to achieve a more potent antibacterial activity. Thus, the quorum sensing inhibiting and anti-biofilm effects *in vitro* of fifteen selenoesters (eight ketone-selenoesters and seven cyano-selenoesters) were investigated in this study.

The minimum inhibitory concentrations (MICs) of the selenoesters were determined on sensitive and resistant *Staphylococcus aureus* strains. Biofilms produced by *Pseudomonas aeruginosa* (CCM, 3955) and *S. aureus* (ATCC, 25923) were used to evaluate the eruption of mature biofilm and the anti-biofilm activity of the compounds. Additionally, two reference strains of *Vibrio campbellii* were used (BAA1118 and BAA1119) to establish the anti-quorum sensing activity of these novel selenoester derivatives. In this anti-quorum sensing assay, the viability of the cells and the quorum sensing inhibiting effects were determined in the presence of the selenoesters to differentiate between the toxic concentration and the quorum sensing inhibiting concentration.

According to the screening of the antibacterial activity, the ketone-selenoesters resulted to be more potent antibacterial compounds than the cyano-selenoesters. The biofilm inhibitory capacity and the ability to disrupt mature biofilms of the derivatives were noteworthy in all the experimental systems considered. Regarding the QS inhibition, four ketone-selenoesters and three cyano-selenoesters among the tested compounds exerted a noteworthy effect on both *V. campbellii* BAA-1118 and BAA-1119 strains.

The results obtained in this work point that these ketone- and cyano-selenoesters could be promising and valuable compounds in the search of novel antibacterials to combat the infections by resistant bacteria, as they have shown a promising anti-biofilm and anti-QS activity. Thus, selenium-containing compounds belonging to the family of the selenoester compounds could provide alternative and effective structural motifs to overcome bacterial MDR. Nevertheless, the mode of action of the compounds needs further investigation to be fully understood.

TRPV1 modulators synergistically enhances anti-proliferative effects of Amitriptyline on metastatic breast carcinoma cells

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TRPV1 (Transient Receptor Potential Vanilloid 1), a cation channel with a high affinity for Ca+2, is expressed in neurons, immune cells and breast cancer cells. [1] TrkA (tyrozin receptor kinase A), which belongs to tyrozin kinase receptor family, regulates the growth, survival and proliferation of neurons. TrkA is also expressed in breast carcinoma cells. [2] Recent studies showed that TrkA increases sensitivity of TRPV1. Because TRPV1 activation was shown to inhibit proliferation of cancer cells, we here investigated the effects of TrkA agonist Amitriptyline in combination with TRPV1 agonist Olvanil and TRPV1 antagonist AMG-9810 on brain (4TBM) and heart (4THM) metastatic subset of breast carcinoma cells.
The 4TBM and 4THM cells were seeded with DMEM-F12 medium without FBS in 96-well plates (4000 cells per well). Different concentrations of Amitriptyline, Olvanil and AMG-9810 were used alone or in combination with various concentrations of Amitriptiline. Changes in cell proliferation was determined using WST-1 after 48 hours. Each experiment was repeated 3-5 times independently. For determining the dose of combination with Amitriptyline, the isobologram method is used.
Treatment with Amitriptyline, Olvanil or AMG-9810 alone inhibited proliferation of 4TBM and 4THM metastatic breast cancer cells. When the effects of Olvanil and AMG-9810 are compared to each other, AMG-9810 was found to be more potent than Olvanil. Combination of Amitriptyline with Olvanil and AMG-9810 resulted in synergistic interactions depending on the metastatic sunset used. Specifically, 5 μM Olvanil and 5 μM Amitriptyline combination resulted in inhibition of cell growth larger than additive effects of each treatment which is determined by isobologram graph with CI value in 4TBM cells. Similarly, AMG-9810(1 μM) and Amitriptyline (1 μM) combination demonstrated synergistic effect in inhibition of 4THM cell growth.
In conclusion, treatment with TrkA agonist Amitriptyline in combination with TRPV1 agonist Olvanil and TRPV1 antagonist AMG-9810 synergistically inhibits the proliferation of metastatic breast carcinoma cell lines.

References:
[1]. Vercelli C, Barbero R, Cuniberti B, Racca S, Abbadessa G, Piccione F, Re G. Transient receptor potential vanilloid 1 expression and functionality in mcf-7 cells: a preliminary investigation. J Breast Cancer. 2014;17(4):332-8.

[2]. Mauri G, Valtorta E, Cerea G, Amatu A, Schirru M, Marrapese G, Fiorillo V, Recchimuzzo P, Cavenago IS, Bonazzina EF, Motta V, Lauricella C, Veronese S, Tosi F, Maiolani M, Rospo G, Truini M, Bonoldi E, Christiansen J, Potts SJ, Siena S, Sartore-Bianchi A. TRKA expression and NTRK1 gene copy number across solid tumours. J Clin Pathol. 2018;71(10):926-931.

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A novel small-molecule compound targeting Hakai for anticancer therapy

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The epithelial-mesenchymal transition (EMT) is a process with enormous relevance in tumor progression and metastasis, as well as in the acquisition of drug resistance [1]. EMT is characterized by the loss of the epithelial phenotype and cell-substrate adhesions, and the acquisition of a motile and invasive mesenchymal phenotype [2]. The most established marker of EMT is the loss of expression of E-cadherin, a tumor suppressor that mediates cell-cell contacts in the epithelium. Hakai is an E3 ubiquitin-ligase involved in E-cadherin ubiquitination, endocytosis, and degradation, altering cell-cell adhesions [3,4]. Furthermore, Hakai is gradually increased TNM stages of colorectal cancer compared to adjacent healthy tissues [5], highlighting the importance of Hakai during colorectal cancer progression [6,7]. Given that the E3 ubiquitin-ligase have emerged as promising therapeutic targets against cancer we performed a virtual screening to identify specific inhibitors of Hakai activity. By using *in vitro* models of metastatic colon cell lines, we show the effect of a novel Hakai inhibitor, named Hakin-1 that inhibits Hakai-mediated ubiquitination, and degradation of E-cadherin degradation at cell-cell contacts. Moreover, Hakin-1 reduces cell proliferation, colony formation, migration and invasion of metastatic colon cell lines. In conclusion, small-molecules targeting the E3 ubiquitin-ligase Hakai may have an impact on EMT process and are proposed as effective therapeutic drug to prevent tumor development and metastasis in colon cancer [8].

References:
[1]. Shibue T and Weinberg RA (2017). Nat Rev Clin Oncol. 14 (10): 611-629.
[2]. Marcucci F et al. (2016) Nat Rev Drug Discov 15(5):311-25.
[3]. Fujita Y et al. (2002). Nat Cell Biol (4): 222-231
[4]. Mukerjee M et al. (2012). EMBO J (31): 1308-1319
[5]. Castosa R et al (2018) Sci Rep. (22): 3466
[6]. Aparicio LA et al. (2012). Can Met Rev (31): 375-386
[7]. Figueroa A et al. (2009) Mol Biol Cell (20): 3533-3542
[8]. Martinez-Iglesias O et al (2020) Cancers (23): 1340

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Inhibitors of tumor cell growth and P-glycoprotein as potential anti MDR agents

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Our group has been focusing in developing small molecules that can simultaneously be active as antitumor agents and P-glycoprotein (Pgp) inhibitors. The rational follows structure based design using docking studies with P-gp homology models and derivatives of inspiring antitumor agents, both from natural [1], [2] and synthetic sources [3], [4].
Here we present a case study of the discovery of dual inhibitors of tumor cell growth and P-glycoprotein, based on existing drugs from the class of thioxanthones. In order to evaluate their role as inhibitors of multidrug resistance (MDR), as well as their interaction with other ABC transporters, a small library of derivatives was obtained by synthesis and studies were developed in order to understand their mechanism of action [3], [5], [6].
The strategy to design the molecules to be synthesized was based on molecular hybridization, using the thioxanthone scaffold, present in known antitumor agents and an amine group considered as important chemical moiety for P-gp inhibition [4]. More recently, chiral derivatives of aminothioxanthones were studied as P-gp modulators showing the influence of chirality on this activity [7]. In conclusion, from these studies it can be inferred that thioxanthone derivatives deserve to be explored in the search for inhibitors of multidrug resistance (MDR).

References:
[1] Sousa E et al (2013). Medicinal Chemistry Research. 22(5): 2115-2123.
[2] Lopes-Rodrigues V (2017). Bioorganic & Medicinal Chemistry. 25: 581–596
[3] Palmeira A *et al* (2012). J. Pharm Pharmaceut Sci. 15(1): 31-45
[4]. Palmeira A *et al*. (2012). Biochemical Pharmacology. 83:7-68
[5] Barbosa J *et al* (2016). Molecules. 21: 81
[6] Lima RT *et al*. (2016). Molecules. 21:1343
[7] Lopes A *et al* (2018). Molecules. 23:626

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Efficacy and molecular mechanism of action of MDR-reversal stony brook taxanes in resistant in vitro and in vivo models of ovarian cancer cells

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Taxanes are successfully used in therapy of different carcinomas, especially ovarian carcinomas. One of the main problems with lower efficiency of conventional taxanes (paclitaxel, docetaxel) is the development of multidrug resistance. New synthesized experimental taxanes (Stony Brook taxanes; SB-T-taxanes) seem to be potential drugs against solid tumors with resistant phenotype. The aim of our study was to compare the efficiency, cell cycle modulation ability and transport of conventional (paclitaxel) and new experimental taxanes (SB-T-1214, SB-T-121402, SB-T-121405, SB-T-121406, SB-T-1216, SB-T-121602, SB-T-121605 and SB-T-121606) in highly resistant ovarian carcinoma cells (NCI/ADR-RES). The most efficient taxane derivatives were tested *in vivo* in mice xenografts of ovarian resistant tumors. In addition, changes in mRNA and miRNA profiles were measured using Affymetrix arrays after the treatment of paclitaxel and taxane SB-T-1216.
The efficiency of new experimental taxanes (namely SB-T-121605, SB-T-121606) were up to 1000 times higher compared to paclitaxel in the *in vitro* model of resistant ovarian carcinoma NCI/ADR-RES cells. Significant changes in cell cycle after exposing the model to different concentration of new taxanes were also observed. Cell cycle arrest in G2/M phase was induced by 10-times lower concentrations of new taxanes compared to PCT. In addition, uptake of new taxanes was also higher (6.8-15.5-times) when compared to paclitaxel. The most efficient new taxane SB-T-121605 was selected for experiments in *in vivo* mice xenografts, where conventional taxane paclitaxel alone was not effective. SB-T-121605 application led to significant suppression of tumor growth in mice. Neverthless, this effect was accompanied by gastrointestinal toxicity in higher concentrations. In the frame of molecular mechanisms of taxanes, we have found significant differences in mRNA and miRNA profiles caused by the action of novel taxanes and identified key pathways and genes for molecular mechanism of taxane action (e.g., NOTCH and AhR signaling and Hedgehog pathways). These candidate pathways are investigated, at present, as potential therapeutic targets of human cancer.

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Efflux pump inhibition by symmetric selenoesters on colon adenocarcinoma cells

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The development of resistance to chemotherapy in tumour cells is often due to altered membrane transport, such as the overexpression of ABCB1 (P-glycoprotein). Previously, selenium and its derivatives have been reported as antiproliferative, cytotoxic compounds that can also reduce drug resistance in tumour cells, trigger apoptotic events and enhance synergistically the anticancer activity of chemotherapy drugs such as doxorubicin.
This work aims to elucidate how the symmetrical selenoesters exert their anticancer effect on sensitive and resistant human colon adenocarcinoma cells expressing ABCB1 protein.
MTT assay was applied to assess both the antiproliferative and cytotoxic effects of the compounds on sensitive and resistant colon adenocarcinoma and normal embryonic lung fibroblast cells. A flow cytometry based assay which determines the accumulation of rhodamine 123 was used to assess the efflux pump inhibitory activity of the tested selenoesters. Finally, annexin V-FITC staining was used to determine the apoptosis-inducing effect of the most promising derivatives. Furthermore, the interaction of the compounds with doxorubicin was assessed by checkerboard combination assay and the type of interaction was calculated by Calcusyn software.
Methyl ketone-containing compounds (EDAG-1, -5, -8) showed the most potent antiproliferative and cytotoxic effects. Out of them, EDAG-5 was the one that had the most synergistic interaction with doxorubicin. On the other hand, the methyloxycarbonylmethyl- and the methylcyano selenoesters had a very low cytotoxic activity on the normal MRC-5 fibroblast cell line. Methyl ketone- and methylcyano-selenoesters (EDAG-7, -10, 11) were more potent ABCB1 inhibitors than the reference compound verapamil. Finally, a methyl ketone selenoester (EDAG-1) was an effective inducer of apoptosis in the resistant Colo 320 cell line.
The biological activities observed are comparable or higher than the ones determined in previous works. Thus, it seems that the inclusion of symmetric centres in these selenocompounds may favour (or at least retain) the biological activity. Based on these results, it can be concluded that these compounds (or derivatives containing these symmetrical elements) could be interesting scaffolds for future research in medicinal chemistry.

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Novel TrxR1 inhibitors induce oxidative stress and sensitize human multidrug resistant glioblastoma cells to chemotherapy

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Glioblastoma (GBM) is the most common malignant brain tumor in adults, with limited treatment options due to aggressive invasiveness and resistance to therapy. Developing novel strategies for GBM treatment is of pivotal importance. Elevated expression of antioxidant defense system components thioredoxin (Trx) and thioredoxin reductase (TrxR) is a common feature of cancer cells and correlates with cancer progression and poor prognosis. We evaluated anticancer properties of novel TrxR1 inhibitors (DVD-444 and DVD-445) in human sensitive glioblastoma cell line and corresponding multidrug resistant (MDR) cell line (U87and U87-TxR, respectively).

Compounds DVD-444 and DVD-445 showed a similar growth inhibitory effect in U87 and U87-TxR cells. Both compounds were less effective in peripheral blood mononuclear cells showing selectivity towards cancer cells. Significant elevation of RONS level after treatment with TrxR1 inhibitors was observed only in MDR glioblastoma cells. However, DVD-444 and DVD-445 induced changes in expression of antioxidant enzymes in both cell lines, implying the existence of oxidative stress inside the cells. Besides antioxidative effect, DVD-444 and DVD-445 decreased cell proliferation and suppressed invasion and migration of glioblastoma cells. Both TrxR1 inhibitors increased accumulation of P-glycoprotein substrate Rho123 in U87-TxR cells. Furthermore, the compounds showed potential to modulate MDR by sensitizing U87-TxR cells to paclitaxel.

In conclusion, we found that novel TrxR1 inhibitors induced oxidative stress, leading to changes in expression of antioxidant enzymes. Consequently, these inhibitors affected cell proliferation and suppressed invasion and migration of glioblastoma cells. Moreover, TrxR1 inhibitors were capable to overcome MDR. Considering the fact that drug resistance and invasion are the main causes of ineffective GBM treatment, novel TrxR1 inhibitors, particularly DVD-444, could be valuable candidates for new GBM treatment strategy.

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