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New Diagnostic and Therapeutic Tools against Multidrug-Resistant Tumours

First Working-Group Meeting

WG 1 – WG 4

Abstract Book

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WG 1

Weighted Gene Coexpression Analysis to determine hub genes and potential biomarkers

Thomas Mohr

ScienceConsult – DI Thomas Mohr KG, Guntramsdorf, Austria

The classical approach to discover new key driver genes, biomarkers, and potential therapeutic targets is based on gene-centric and pathway-centric analyses of tumor tissue samples compared to normal tissue samples. This approach suffers from a series of setbacks. Genes act in cooperation and not individually. Therefore a view on a restricted set of genes may result in non-detection of important components of a process. Pathway analysis ameliorates these problems to some extent. However, cross-pathway communication remains largely a terra incognita.

Investigating cooperating units of genes (a.k.a. "modules") circumvent overcome these shortcomings. Therefore network-based analysis methods are natural approaches to understanding such a "system". During the last decade, gene co-expression analysis has had a significant impact on the analysis of -omics data. Whereas most analysis methods focus on the expression level and significance of gene groups, Weighted gene co-expression analysis correlates gene expression by calculating a co-expression network and detecting co-expression patterns. This approach results in gene co-expression modules, i.e. groups of genes with similar co-expression patterns. Network derived measures such as eigengene expression, centrality, connectivity, or topological overlap allow the detection of genes playing potentially a key role in a biologic process. Differential co-expression analysis, i.e., the analysis of changes between co-expression networks of respective treatments reveals groups of genes that remain conserved and groups of genes whose co-expression patterns change due to the treatment. Taken together, these analysis methods may lead to the discovery of new, unexpected biomarkers and therapeutic targets.

In our example, we examine the differences between normal and tumor-associated endothelial cells (quiescent and activated) derived from hepatocellular carcinoma. WGCNA revealed several modules associated with activation and tumor location, as well as hub genes within these modules.

Multiple targets of cancer drugs: construction and analysis of complex protein–drug bipartite networks

De Las Rivas, J[1], Alonso-Lopez, D[1], Arroyo, MM[2], Sanchez-Luis E[1]

[1] Cancer Research Center (CiC-IBMCC) University of Salamanca and Consejo Superior de Investigaciones Cientificas (USAL/CSIC), Salamanca, Spain.[2] Pontifical Catholic University of Puerto Rico (PCUPR), Ponce, Puerto Rico.

Abstract: A critical step to obtain a better understanding of the molecular mechanisms that drive multi-drug resistance (MDR) in cancer is to have better maps of the specific bio-molecular targets (proteins) that each drug affects. In recent years, omic experimental studies and bioinformatic comprehensive efforts have provided compendiums of the human protein interactome, which include all known protein–protein interactions (PPIs) and allow the construction of protein interaction networks [1]. Other bioinformatic efforts have compiled several drug–target resources to provide a current perspective of the associations between human proteins and specific drugs or chemicals. The identification of the specific protein targets of each anticancer drug is a critical step to improve our understanding of therapies at molecular level and also to discover the mechanisms that cause drug resistance. Cancer is a complex disease in which multiple genetic changes rewire cellular networks during carcinogenesis. This indicates that cancer drug therapy needs the implementation of network-driven studies to reveal multiplex interactions between cancer genes/proteins and drugs. To make progress in this direction, we provide a bipartite network of cancer genes and drugs in a graph landscape that discloses the existence of specific drug–target modules [1].

[1] De Las Rivas J, Alonso-López D, Arroyo MM. (2018) **Human Interactomics: Comparative Analysis of Different Protein Interaction Resources and Construction of a Cancer Protein-Drug Bipartite Network.** *Adv Protein Chem Struct Biol.* 2018;111:263-282. PMID: 29459035.

New anti-Cancer Agents through an Interaction with Tubulin

La Regina G, Coluccia A, Naccarato V, Silvestri R[1]

[1]Department Drug Chemistry and Technologies, Sapienza University of Rome, Italy

Microtubules (MTs) are involved in key cellular functions, such as cell division, cell motility and intracellular transport. Interfering with the MT dynamic equilibrium, by either inhibiting tubulin polymerization or blocking MT disassembly, has resulted in a productive strategy for the development of efficient anticancer agents. However, they can elicit drug resistance, toxicity and undesired side effects. Thus, new MT inhibitors would provide a better alternative to current anticancer treatments.

New tubulin targeting heterocyclic compounds uniformly inhibit at nanomolar concentration the cancer cells including P-glycoprotein (Pgp) overexpressing lines NCI/ADR-RES and Messa/Dx5. Besides the inhibition of tubulin polymerization, the new agents stimulate the cytotoxic activity of natural killer cells at doses which do not severely affect cell viability, increasing NKG2D and DNAM-1 ligand up-regulation on HeLa cells. At higher concentrations, these compounds stably arrest mitotic progression, prevent mitotic slippage and the ensuing formation of aneuploid cells and induce cell death, with effectiveness comparable or superior to that obtained with VBL. Moreover such agents show strong inhibition of the Hedgehog signaling pathway and medulloblastoma D283 cells [1-3].

3-Aroyl-1,4-diarylpyrrole (ARDAP) derivatives exhibit potent inhibition of tubulin polymerization and inhibit the proliferation of BCR/ABL-expressing KU812 and LAMA84 cells from chronic myeloid leukemia (CML) patients in blast crisis and of hematopoietic cells ectopically expressing the imatinib mesylate (IM)-sensitive KBM5-WT or its IM-resistant KBM5-T315I mutation. These results provide a robust scaffold to develop tubulin inhibitors with potential as novel treatments for CML [4].

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Concomitant resistance to paclitaxel in an ovarian cancer cell variant selected with carboplatin

Juran Kralj, George E. Duran, Darija Stupin Polančec, Niko Bačić, Branimir I. Sikic, Anamaria Brozovic

Most epithelial ovarian cancer patients are diagnosed with advanced-stage disease due to the late appearance of symptoms and lack of early diagnostic methods/markers. The major problem for successful therapy is the development of tumour drug resistance during carcinogenesis (20-30%) and upon exposure to chemotherapy. The ovarian cancer cell line OVCAR-3/CBP was established by treatment of the ovarian adenocarcinoma cell line OVCAR-3 with long-term, stepwise selection in carboplatin (CBP) up to 25 μM. The variant is ~1.5-2-fold resistant to CBP, with cross-resistance to paclitaxel (TAX, ~4-fold), and presents with a mesenchymal-like phenotype. The increased expression of NHE-1, ATP7-B and decreased expression of ABCC2 and CTR-1 implied decreased total cell platination as a possible mode of CBP resistance, which was confirmed by flame atomic absorption spectrometry. Despite the increased level of ABCB1 transcripts, OVCAR-3/CBP did not efßux [3H]-docetaxel differently compared to parental cells, and the P-glycoprotein inhibitor PSC-833 did not alter these drug accumulation profiles. This indicates that the TAX resistance in OVCAR-3/CBP is non-MDR, but is associated with elevated TUBB3 (class III beta-tubulin) content along with total α- and β-tubulin relative to parental OVCAR-3 cells. In summary, drug selection with carboplatin in an ovarian cancer cell line resulted in non-MDR cross-resistance to paclitaxel. Experiments investigating the functional significance of altered tubulin content and microtubule dynamicity in response to drug exposure in OVCAR-3/CBP are on-going.

Application of machine learning algorithms to classify tissue samples using methylation patterns - a case study

Alexander Tolios [1,2], Claas Schmidt, Berthold Streubel, Gerwin Heller, Georg Dorffner, and Thomas Mohr

1 Medical University of Vienna, Department of Blood Group Serology and Transfusion Medicine 2 Heinrich Heine University Düsseldorf, Institute of Clinical Chemistry and Laboratory Diagnostics

Introduction: Epigenetic DNA modification is central to tissue-specific RNA expression. Patterns in DNA methylation, one of the most thoroughly investigated epigenetic modification, could potentially be used to distinguish the tissues of origin. Machine learning algorithms could be used to find relevant patterns within DNA methylation profiles.

Methods: Data from 'The Cancer Genome Atlas' was used for this analysis (650 samples in total). Data was split into a training set (approx. 2/3 of the dataset) and an independent test set (approx. 1/3 of the dataset). Sample similarities were calculated using an euclidean distance metric. Principal component analysis (PCA) was performed for dimensionality reduction, t-distributed stochastic neighbor embedding (tSNE) was used for clustering. Different supervised machine learning algorithms were used for tissue classification (neural networks, random forests, support vector machines, k-nearest neighbors). Linear models were used for performance comparison. All algorithms were trained in sample classification performance with the target of Cohen's kappa maximization. A cross validation approach was used to enhance model prediction performance. The final performance was quantified using the results on the unknown test dataset.

Results: The first 6 principal components could explain approx. 80% of the variance within the dataset. Using both PCA and tSNE a differentiation between the distinct populations was possible. Using the training dataset, non-linear supervised machine learning methods performed mostly well on the given data resulting in a Cohen's kappa of > 0.95 and a similar accuracy of > 95% (CI 92-98%). The linear models could not archive an equal level of accuracy. When using the best-performing algorithm (random forest) on the unknown test dataset, Cohen's Kappa and Accuracy on the same level (> 0.95).

Conclusion: Machine learning algorithms are able to utilize methylation patterns for the prediction of tissue sample origin.

Selected strategies used to design anticancer molecules.

Ostaszewski R.

Institute of Organic Chemistry Polish Academy of Sciences, 01-224 Warsaw, Poland

Recent progress in a biomedical research is focused, among others, on the synthesis of peptidomimetics; small peptide type molecules that reveal wide spectrum of biological activities. In our research we are focusing in particular on three key steps: the target validation and selection; chemical hit and lead generation; lead optimization to identify clinical drug candidates. In the first step of our studies, a therapeutic target proteasome was identified and several inhibitors with peptidomimetc structure were synthesized and validated as antitumor molecules. [1, 2] The next studies were devoted to peptidomimetic inhibitors of thioredoxin-thioredoxin reductase system [3,4]. One of our the most potent compound, SK053, triggers tumor cells apoptosis by oxidative stress-mediated endoplasmic reticulum stress. [5, 6] Subsequent experiments allowed us to identify peptidomimetic kinase inhibitors with high Cytostatic/Cytotoxic Activity. This effect was evaluated on tumor cell line (RAS-3T3) displaying overactivation of the MAP kinase RAS/MEK/ERK pathway in comparison to the parental, non-tumorigenic cells. [7] We intended to use gold and silver nanoparticles as anticancer molecules and revile the high instability of silver nanoparticles in buffers as well as low affinity of gold nanoparticles towards selected enzymes. [8]

Acknowledgements

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Rational design of multi-target compounds with potential anticancer activity

Dobričić, VD, Oluić, J, Nikolic, KM, Vučićević, J, Gagić, ŽP, Filipić, SV, Agbaba, DD, Čudina, OA

Department of Pharmaceutical Chemistry, University of Belgrade – Faculty of Pharmacy

Multi-target compounds are designed to act on at least two targets in synergistic way, in order to produce stronger biological effect. The link between cancer and inflammation has been intensively studied in last years and inhibition of both COX-2 and 5-LOX enzymes may be an effective therapeutic approach for colon cancer treatment [1]. Acridines are known as DNA-topoisomerase II inhibitors. These compounds intercalate into the DNA and inhibit topoisomerase II by their side chain. Recent studies showed additional activities of acridine derivatives, depending on the side chain structure, such as inhibition of Src, MEK and VEGFR2 kinases [2,3]. Altered activity of PI3K/mTOR signaling pathway is one of the most common aberrations found in various forms of neoplastic lesions. Dual inhibition of PI3K and mTOR represents a reasonably attractive concept in potential cancer treatment [4]. In this paper, design of three groups of multi-target compounds with potential anticancer activity is presented. Designed compounds are potential inhibitors of COX-2 and 5-LOX, DNA-topoisomerase II complex and kinases (Src, MEK and VEGFR2), as well as dual inhibitors of mTOR and PI3K. A set of 27 compounds with published inhibitory activity on COX-2 and 5-LOX was formed and two QSAR models, for both activities, were created in Pentacle program. On the basis of models' interpretation, nine new compounds were designed and activity on both targets predicted by developed models. Twenty-three acridine derivatives were designed and their interactions with DNA-topoisomerase II complex, Src, MEK and VEGFR2 were tested using AutoDock Vina program. Nineteen designed compounds bind to DNA similarly to inhibitor amsacrine and among them, ten derivatives show key binding interactions with tested kinases and therefore possess potential to inhibit them. A dataset of eighty-five compounds with dual PI3K/mTOR inhibitory activities was formed, divided into two groups based on their structural analogy and 3D-QSAR analysis of each group was performed in Pentacle program,

resulting in four QSAR models. On the basis of these results, new compounds were designed and further evaluated by use of molecular docking, virtual screening and ADMET predictions. Finally, seven compounds were chosen as the most promising new dual mTOR/PI3K inhibitors.

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New ruthenium metallodrugs against cancer multidrug resistance

Valente, Andreia[1]

[1] Centro de Química Estrutural, Faculdade de Ciências da Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal.

The discovery of new potent and selective anticancer agents, able to decrease the noxious side effects of the chemotherapeutics in clinical use, and capable to overcome resistance mechanisms, is the driving force for research in this field.

In this frame we have been developing new of $[Ru(\eta^5\text{-}C_5H_5)(2,2'\text{-bipiridine})(PPh_3)]^+$ based compounds endowed with specific targeting components to take advantage of the singular characteristics of tumor cells and tissues, such as their permeability to macromolecules and overexpression of several receptors.[1-4] Thus, by introducing a biodegradable and biocompatible polymer and a biomolecule recognized by cancer cells in the structure of our compounds, one can benefit from a passive and active targeting, respectively. This family of ruthenium metallodrugs possess very attractive features: high cytotoxicity against several cancer cell lines with different degrees of aggressiveness, strong inhibition of several proteins known for their role in mechanisms of cell resistance, interference with proteins that regulate the microtubule or actin dynamics leading to cell death and low in vivo toxicity. Thus, this talk discloses the potential of these new ruthenium(II) compounds for the targeted therapy of metastatic and resistant cancers.

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Ruthenium complexes as potential antitumor agents

Iztok Turel

Faculty of Chemistry and Chemical Technology, University of Ljubljana, Večna pot 113, SI-1000 Ljubljana, Slovenia

Approximately forty years ago (1978), cisplatin was approved for use in certain types of cancers (testicular and ovarian). Platinum complexes were the most intensely studied from all potential metal based anticancer drugs for many years but in last decades more and more research was done with other metals, also ruthenium. Important reasons that alternatives for platinum complexes were requested were amongst other resistance of cancer cells and severe side effects of platinum drugs. Few ruthenium(III) compounds have long ago entered clinical trials. Additionally, many other ruthenium(II) and (III) compounds are involved in preclinical level studies nowadays. It is encouraging to hear that photodynamic therapy based ruthenium compound TLD-1433 has also recently entered clinical trials.

Our group was dealing with interactions of ruthenium with variety of biologically active ligands that coordinate to metal ions through different donor atoms (e.g. O,O-donor quinolones), N-donor azoles, O,O-donor diketonates, N,O-donor hydroxyquinolines, O,S-donor pyrithione and also with N,N-donor ligands. Many of these complexes were tested for their anticancer properties and we have also tried to determine the targets that are important for their activity (DNA, proteins, etc). It was determined that ruthenium compounds are potential inhibitors of various enzymes, such as aldo-keto reductases, lipoxygenases, acetylcholinesterase (AChE), carbonic anhydrases, glutathione-S-transferases (GSTs) and cathepsins.

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Nitrogen donor ligands and their coordination compounds as a tool for treatment of multi drug resistant tumors

Filipović, NR[1], Todorović, TR[2], Bjelogrlić, S[3], Silvestri, R[4], La Regina, G[4], Pellizia, S[5], Padron, JM[6], Schembri-Wismaye,r P[7], Donard, M[8,9], Gulea, M[9], Muler, CD[10]

[1] Dept. of Chem. and Biochem., Faculty of Agriculture, University of Belgrade, Serbia [2] Faculty of Chemistry, University of Belgrade, Serbia [3] National Cancer Research Center of Serbia, Serbia [4] Dip. di Chimica e Tecnologie del Farmaco, Sapienza UniversitaA˜ di Roma, Italy [5] Dip. di Farmacia, Universita` di Napoli ''Federico II", Italy [6] BioLab, IUBO AG, CIBICAN, Universidad de La Laguna, Spain [7] Anatomy Dep., Faculty of Medicine and Surgery, University of Malta, Malta [8] Institut de recherche Donnet, Universite de Haute-Alsace, France [9] Faculté de Pharmacie, Université de Strasbourg, France [10] Institut Pluridisciplinaire Hubert Curien, Universite´ de Strasbourg, France

In our previous work we prepared several libraries of compounds: organic ligands with N-donor atoms (in some cases with additional chalcogen donor atoms) and corresponding metal complexes. These compounds have been tested against various cancer and cancer stem cells (CSCs) and the most potent compounds have been identified. For selected compounds a detailed mechanistic study regarding antitumor activity has been performed together with *in silico* and experimental molecular target(s) identification. The most promising results will be presented [1-13].

A novel line of our research represents building of drug delivery systems based on coordination polymers (CPs). CPs represent assembly of different building blocks - metals and organic linkers, with different porosities and dimensionalities (from 1D to 3D). CPs have demonstrated inimitable advantages in drug delivery systems. These advantages are nontoxicity, well-controlled drug release and stability, which can be the most pivotal characteristics of every efficient carrier. The mentioned properties make CPs one of the most applicable carriers in anticancer drug delivery systems [14]. A novel class of Ag-based 1D-3D CPs with organic thiocyanates and substituted thiomorpholine ligands has been developed in collaboration of several groups [15]. Studies of their drug delivery properties are in progress.

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Formulation of lipid nanocapsules and self-assemblies in order to reach MDR cancers

Catherine PASSIRANI[1], Elise LEPELTIER[1]

[1] MINT Laboratory, INSERM 1066 CNRS 6021, Angers University, FRANCE

Ever since they were developed by MINT laboratory, lipid nanocapsules (LNC) [1] have demonstrated their ability to successfully encapsulate various therapeutic agents and can be considered as a promising therapeutic platform [2]. Indeed, LNC are formulated through a solvent free process of phase inversion that is easily scalable for commercial purposes [3]. These nano-objects have for example proven to be a suitable vehicle for metallocomplexes such as ferrocifens showing remarkable anticancer activities: ferrocifens are able of multitargeting in cancer cells (e.g. leading to senescence and/or apoptosis depending on the concentration) [4]. Moreover, the architecture of LNC offers the option of surface modification, making it possible to adapt the pharmacological behavior of the carrier to suit the intended application.
Another approach developed by MINT is the synthesis of amphiphilic prodrugs, able to self-assemble in water without the need of any excipient, avoiding potential toxicity [5]. For example, the covalent conjugate of ferrocifens with various hydrophilic cell penetrating peptides has been performed, leading to self-assemblies in water by nanoprecipitation. The multiplicity of the targets is a mean to fight against multidrug resistance phenomenon. Thus, these nanosystems combining two active substances could be twice as efficient. Moreover, in order to protect the carriers from enzymatic degradation and in order to prevent macrophage clearance, a co-nanoprecipitation is possible with a conjugate formed by the covalent coupling of the drug with a poly(ethylene glycol) molecule (PEG).
Finally, any new bio-active compounds targeting multidrug resistance (MDR) tumors could be formulated using our nanocarriers in order to improve their solubilisation, delivery, selectivity and anticancer action, by passive or active targeting, via the optimization of their pharmacokinetic properties.

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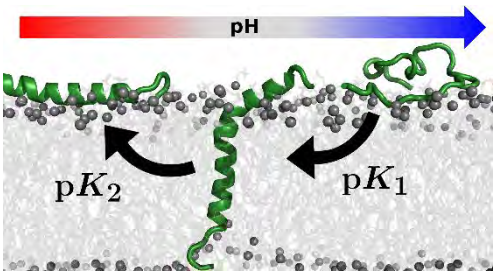
In silico studies of pHLIPs: the pH-sensitive peptides that target the acidity of tumor cell surfaces

Machuqueiro, M[1], Vila-Vicosa, D[1], Silva, TFD[1], Slaybaugh, G[2], Reshetnyak, YK[2], Andreev, OA[2]

[1] CQB & BioISI, Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal [2] Department of Physics, University of Rhode Island, 2 Lippitt Road, Kingston, Rhode Island 02881, United States



The pH (low) insertion peptides (pHLIPs) is a family of peptides that are able to insert into cell membranes with an acidic vicinity, such as tumor cells, thus working as an efficient tumor-specific biomarker or drug carrier [1]. The molecular mechanism of pHLIPs insertion, folding, and stability in the lipid bilayer at low pH is based on multiple protonation events, which are challenging to study experimentally at the molecular level.

Constant-pH molecular dynamics (CpHMD) methods have been used successfully to sample protonation behavior of titrable amino acids inserted into a lipid bilayer [2]. Here, we use this computational approach to help identify the role of key protonable residues in the membrane stability of pHLIP [3]. Also, by proposing new peptide sequences (see Figure), we are helping our experimentalist collaborators (Prof. Oleg Andreev in Rhode Island, USA) strengthening the technological application of pHLIPs as biomarkers and possible drug-delivery systems for tumor cells.

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Cell-based Strategies for the Delivery of Theranostic Nanoparticles

Popovtzer, R[1]

[1] Faculty of Engineering & The Institute of Nanotechnology and Advanced Materials.
Bar-Ilan University, Ramat Gan 52900, Israel

A critical problem when administrating anticancer drugs is the lack of drug specificity, leading to high systemic toxicity and adverse side effects, which limit the maximum tolerated dose. T-cells exhibit homing abilities to sites of lesion, injury, and inflammation, and exert anti-inflammatory effects, and in particular, can efficiently and specifically home to and infiltrate into tumors. We propose to harness the innate ability of immune cells to specifically home to the tumor area, in combination with nanotechnology based strategy for cell tracking and drug release. In this presentation, I will show our recent results regarding tumor targeting[1][2], the therapeutic impact of nanoparticles [3] and image-guided stratification[4].

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Natural product inspired chemical approaches against MDR cancer

Fási L[1,#], Vágvölgyi M[1,#], Latif A[1,2,#], Issaadi M[1], Zoofishan Z[1], Zupkó I[2], Spengler G[3], Martins A[3,+], Hunyadi A[1]

[1] Institute of Pharmacognosy, University of Szeged, Hungary [2] Department of Pharmacodynamics and Biopharmacy, University of Szeged, Hungary [3] Department of Medical Microbiology and Immunobiology, University of Szeged, Hungary [#] Equal contribution; [+] Current affiliation: Synthetic Systems Biology Unit, Institute of Biochemistry, Biological Research Centre, Szeged, Hungary

Natural products have been and keep being as the richest source of new anticancer drugs.

In our group, several natural product inspired chemical approaches are currently pursued to find new antitumor agents. Ecdysteroids, analogs of the insect molting hormone ecdysone, can be engineered by semi-synthetic modifications to obtain potent, MDR-selective chemo-sensitizers towards a variety of chemotherapeutic agents [1]. To this end, our related studies on this class of compounds resulted in a chemical library of >100 compounds and rich related SAR data. Protoflavones, rare natural flavonoids with a non-aromatic, *p*-quinol type B-ring, exert potent antitumor activity *in vitro* and *in vivo*. Over the last few years, we found that these compounds effectively inhibit the activation of Chk1 and can hence be successfully applied in combination with DNA damaging chemotherapeutics, and that many of them exert selective cytotoxicity against adapted MDR cancer cell lines [2]. Finally, we recently initiated an antioxidant-inspired research program studying the antitumor potential of free radical scavenging-related, chemically stable antioxidant metabolites. To this end, we identified one lead, the cinnamic acid derivative graviquinone. Graviquinone could bypass ABCB1-mediated MDR, exert DNA damaging effect in NCI-H460 and MDR NCI-H460/R cells while exerting DNA protective effect in normal HaCaT cells, and modulate DNA damage response through Chk1/Chk2 [3].

Our recent results in these three research directions, and possible related research collaboration opportunities are presented.

Acknowledgments

This work was funded by the NKFIH, Hungary (K119770). AH was supported by the UNKP-18-4 and VM by the UNKP-18-3 of the New National Excellence Program of the Ministry of Human Capacities, and by the János Bolyai Fellowship of the Hungarian Academy of Sciences.

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MDR-modulating natural flavonoids and flavonolignans

Valentová K[1], Viktorová J[2], Chambers CS[1], Biedermann D[1], Křen V[1]

[1] Institute of Microbiology of the Czech Academy of Sciences, Czechia [2] University of Chemistry and Technology, Prague, Czechia

Multidrug resistance (MDR) is a major challenge for the 21th century in both cancer chemotherapy and antibiotic treatment of bacterial infections. Efflux pumps and transport proteins play an important role in MDR and compounds inhibiting them are prospective for adjuvant treatment of such conditions. A rich source of efficient non-toxic biologically active molecules is the plant kingdom producing various natural compounds, such as the (poly)phenols, flavonoids and flavonolignans. First studies on the ability of some flavonoids to cause partial reversal of MDR resistance in cancer cells appeared at the end of 20th century and specific flavonoids are since then researched for their MDR interactions. Flavonoids so far found to exhibit MDR inhibiting activities originate from a wide variety of plants, many of them are commonly consumed dietary flavonoids, such as quercetin, apigenin, chrysin or kaempferol. A distinct class belong among flavonolignans, formed by the oxidative coupling of a flavonoid e.g. taxifolin, quercetin and luteolin with a phenylpropanoid (lignan) such as coniferyl alcohol or sinapyl alcohol [1]. The main source of flavonolignans is silymarin - complex extract of *Silybum marianum* (L.) Gaertn. (Asteraceae) fruits [2]. Other types of flavonolignans, "non-taxifolin derived", are isolated from white milk thistle or the tropical tree *Hydnocarpus wightiana* (chaulmoogra oil containing hydnocarpins in combination with cyclopentenoic fatty acids traditionally used for leprosy treatment [1]). Flavonoids and flavonolignans are prospective natural low-cost nontoxic compounds able to reverse both antineoplastic and bacterial multidrug resistance by inhibiting ABC, bacterial drug efflux and glucose transporters. No direct antioxidant activity, but rather receptor modulation seem to play a role in MDR inhibition by flavonoids.

Supported by Czech Science Foundation (18-00150S).

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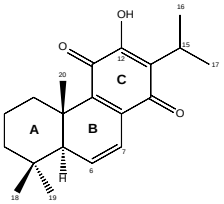
Diterpenoids from *Plectranthus* spp. as P-glycoprotein inhibitors in multidrug resistant NCI-H460/R cells

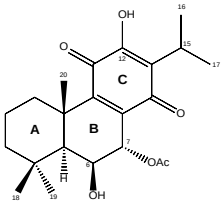
Rijo, P[1,2], Isca, V[1,2], Ntungwe E[1], Ferreira RJ[3], Stankovic T[4], Afonso CAM[2], dos Santos DJ[5], Pesic M[4]

[1] Center for Research in Biosciences & Health Technologies (CBIOS), Universidade Lusófona de Humanidades e Tecnologias, Portugal, [2] Instituto de Investigação do Medicamento (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Portugal [3] Laboratory of Molecular Biophysics, Uppsala University, Sweden [4] Institute for Biological Research "Siniša Stanković", University of Belgrade, Serbia [5] BioISI: Biosystems and Integrative Sciences Institute, Faculty of Sciences, University of Lisbon, Portugal

Royleanones are bioactive compounds frequently found in *Plectranthus* genus [1]. The essencial oil of *P. madagascariensis* is rich in 6,7-dehydroroyleanone (DHR, Figure 1), a cytotoxic royleanone [2]. The abietane diterpene 7α-acetoxy-6β-hydroxyroyleanone (AHR, Figure 1) can be isolated from the extract of *P. grandidentatus* and has high value due to its bioactivity [3]. Both diterpenes have hydroxyl groups suitable for derivatization, which have drawn attention to the possibility of exploring its reactivity, with the aim of improving the cytotoxicity of the lead compounds.



DHR



AHR

In this work, several hemi-synthetic reactions were performed with the intention of achieving a small library of compounds with enhanced cytotoxic potential, resourcing to reactions of carbamoylation, benzoylation or hydrogenation. The general toxicity of the synthesized products was evaluated through an *Artemia salina* model. The benzoylated products of AHR

had increased the toxicity of the original scaffold, which led us to believe that the benzoylation is a key step for increasing the toxicity of this diterpene. A dibenzoylated derivative of AHR, previously synthetized, also demonstrated to enhance the cytotoxic properties, with selective activation of the PKC-δ [4]. The synthetized compounds were also assayed by docking in a murine PgP structure [5] and promising results were obtained for the benzoylated and dibenzoilated products, indicating that benzoyl residues are important groups for the bioactivity.

Figure 1– Natural bioactive royleanones: 6,7-dehydroroyleanone (DHR) and 7α-acetoxy-6β-hydroxyroyleanone (AHR)

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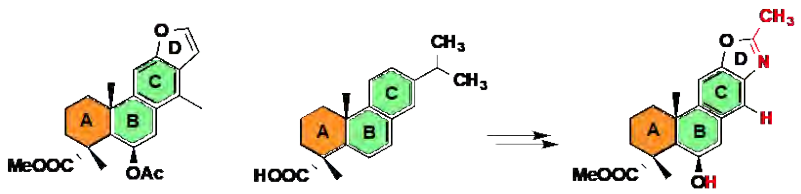
Abietic acid: a natural template for the development of downstream Hedgehog pathway inhibitors

Athanassopoulos, C.M.[1], Antoniou, A.[1], and Pitsinos, E.N.[2]

[1] *Laboratory of Synthetic Organic Chemistry, Department of Chemistry, University of Patras, GR-265 04, Patras, Greece* [2] *Natural Products Synthesis & Bioorganic Chemistry Laboratory, I.N.N, NCSR "Demokritos", P.O. Box 60228, GR-153 10 Ag. Paraskevi, Athens, Greece.*

Among the pathways responsible for drug resistance in CSCs, the Hedgehog (Hh) pathway has emerged as a favorable pharmaceutical target, since its aberrant activation has been linked to several human cancers [1]. Since the early introduced Hh small molecule modulators, such as cyclopamine, several new Hh inhibitors of synthetic or natural origin have been identified, including Vismodegig and Sonidegib, which have been approved by FDA for the treatment of basal cell carcinoma (BCC). The majority of these molecules target the membrane receptor smoothened (Smo) that controls canonical pathway activation [2]. However, recent evidence suggests that targeting glioma-associated oncogene 1 (Gli1), which is the terminal effector of the pathway, might be more advantageous target. It is anticipated that Gli-1 inhibitors not only evade the potential emergence of drug resistance associated with Smo inhibitors but also display fewer side effects [1]. In this context, we were inspired by the Gli-mediated transcription inhibitor taepeenin D **(1)**, a cassane-type diterpenoid originally isolated from *Caesalpinia crista*, with significant Hh/Gli-mediated transcription inhibitory activity [3]. Thus, synthesis and evaluation of a series of taepeenin D analogues, using Abietic acid (**2**, a readily available resin acid) as chiral template, has led to structure–activity relationships for this natural product and has identified an oxazole analogue **(3)** as an improved lead compound [4].

Further optimization of the lead compound **3**, involving the synthesis of third generation analogues, as well as, their biological evaluation is currently in progress.



Taepeenin D, **(1)** Abietic acid, **(2)** **(3)** Gli-luc reporter IC_{50} = 0.64 ± 0.22 μM

Fig. Structures of taepeenin D, abietic acid and lead oxazole 3

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Discovery of novel ATP-competitive human DNA topoisomerase II inhibitors through biological screening of marine alkaloid oroidin analogues library

Skok, Ž, Durcik, M, Zidar, N, Ilaš, J

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, SI-1000 Ljubljana, Slovenia

Human DNA-topoisomerase II is an ATP-dependent enzyme that plays vital roles in DNA transcription, replication and chromosome segregation and therefore represents an attractive target in anticancer drug discovery.[1] Because of the presence of GHKL ATPase domain, DNA topoisomerase II belongs to the same protein superfamily as bacterial DNA Gyrase, Hsp90, histidine kinase and MutL proteins.[2] Based on this fact we used the biochemical screening of existing ATP-competitive bacterial DNA Gyrase inhibitors as a starting point in discovery of new human DNA-topoisomerase inhibitors.

Faculty of Pharmacy in Ljubljana possesses a library of about 1000 ATP-competitive DNA-gyrase inhibitors, that is a product of extensive work of our research group on preparation of novel antibacterial agents.[3-4] The library is based on the structure of marine alkaloid oroidin, isolated from *Agelas* sponges, which shows antibacterial activity. Initial screening of approximately 100 bacterial DNA-gyrase inhibitors resulted in identification of 12 hit compounds, 9 of which contained a common *N*-phenylpyrrolamide scaffold that was later used in design and synthesis of new series of human DNA-topoisomerase II inhibitors. Structure-based optimization of newly discovered hit compounds led to synthesis of new series of human DNA topoisomerase II inhibitors. New inhibitors posses significantly lower molecular weights than original hits which gives them an improved potential for hit-to-lead optimization. Cytotoxic activity of novel inhibitors was tested on MCF-7 and HepG2 cancer cell lines. Best compounds of the series show activity comparable to one of etoposide, a clinically successful DNA-topoisomerase II inhibitor.

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Thursday 31st January
WORKING GROUP MEETING – *Day 2*
WG 3

ABC transporters: biomarkers of a multi-stress resistant phenotype and targets of new synthetic chemotherapeutic drugs

Chiara Riganti
Department of Oncology, University of Torino
Italy

Our research group investigates:
1) if ABC transporters - besides inducing resistance to chemotherapy – are markers/mediators of resistance to other stressing conditions;
2) which are the molecular circuitries involved;
3) which are possible therapeutic tools able to overcome the simultaneous resistance to chemotherapy and other stresses.
We found that cells overexpressing ABC transporters:
- do not induce nitric oxide (NO), a mediator of doxorubicin's toxicity, in response to the drug [PMID:15695394]. Synthetic doxorubicins conjugated with NO-releasing groups and their liposomal formulations reverse doxorubicin-resistance, by nitrating ABC transporters on tyrosines critical for their activity [doi:10.1021/mp300311b; doi:10.1016/j.jconrel.2017.11.042]. Specific components of the liposomal shell also alter the lipid environment where Pgp works [doi:10.1021/mp2001389] and inhibit allosterically Pgp [doi:10.1016/j.nano.2013.06.013];
- have a different pH in the membrane regions containing Pgp: the acidification of these membrane regions, by inhibiting NHE1 [doi:10.1002/ijc.20959] or CAXII [doi:10.1158/1535-7163.MCT-18-0533; doi:10.1002/ijc.31607], reduces Pgp efficiency;
- are resistant to endoplasmic reticulum (ER) stress: indeed, ABC transporters-expressing cells constitutively ubiquitinate C/EBP-β LIP, that induces apoptosis and down-regulates Pgp [doi:10.1093/jnci/djv046]. By inhibiting LIP degradation with lysosome and proteasome inhibitors [doi:10.1186/s13046-018-0967-0] or using synthetic doxorubicins engineered to induce ER stress [doi:10.1007/s00018-018-2967-9], chemosensitivity is restored. On the other hand, cells adapted to survive under ER stressing conditions acquire a MDR phenotype by constitutively activating PERK/Nrf2/MRP1 axis [doi:10.1186/s12943-017-0657-0];
- have an oxidative phosphorylation-based metabolism: impairing oxidative phosphorylation by mitochondria-targeted doxorubicins [doi:10.1158/1535-7163.MCT-16-0048] or doxorubicin-based chrono-chemotherapy [doi:10.1016/j.canlet.2015.02.008] reverse chemoresistance;
- have a higher synthesis of cholesterol and isoprenoids that increase Pgp activity and expression by inducing Ras/ERKs/HIF-1α axis: cholesterol-lowering strategies, i.e. statins associated with a LDL-masked doxorubicin [doi:10.1016/j.jconrel.2010.10.003], ω3-fatty acids [doi:10.1186/1476-4598-12-137] or zoledronic acid [doi:10.18632/oncotarget.5058], induce chemosensitization;
- have a higher stem cell-like phenotype: the constitutive activation of Wnt/GSK3β/β-catenin/c-myc axis up-regulates Pgp [doi:10.1093/neuonc/not104] and ABCB5;
- are resistant to the immune-system: the presence of Pgp on cell surface impairs the phagocytosis mediated by dendritic cells [doi:10.1111/j.1582-4934.2010.01137.x], while Pgp down-regulation increases ABCA1, that activates the tumor killing by Vγ9Vδ2 T-cells via Ras/Akt/mTOR/LXRα axis [doi:10.1038/ncomms15663].
Understanding the molecular circuitries that determine such multi-stress resistant phenotype may help to design and validate new multitarget chemosensitizing agents.

Reversal of resistance: multidrug efflux pumps and the role of efflux pump inhibitors

Spengler, G[1], Kincses, A[1], Nové M[1], Molnár J[1], Burián K[1]

[1]Department of Medical Microbiology and Immunobiology, Faculty of Medicine, University of Szeged, Hungary

Multidrug resistance (MDR) of cancer cells is a major cause of therapeutic failure. One of the mechanisms of MDR is the overexpression of efflux pumps such as ABCB1 (P-glycoprotein). Control over the efflux pump that bestows multidrug resistance has been a goal of research during the past decades. Efflux pump inhibitors (EPIs) as adjuvants represent a promising approach in the experimental chemotherapy because these compounds can interfere with the function of the MDR transporter proteins or disturb different cellular functions contributing to efflux mechanisms.

As a consequence of this search for inhibitors of efflux pumps, it has been noted that many agents which affect the efflux pump system of bacteria also have similar activity against efflux pumps of drug resistant cancer cells. Based on our studies, several groups of compounds have been investigated for reversal of MDR in different cancer model systems *in vitro*, for example natural compounds [1], phenothiazines [2], selenocompounds [3], and metal complexes [4].

The combination therapy using a conventional chemotherapeutic drug with an agent that can improve the action of the anticancer drug (e.g. efflux pump inhibitor) could be a good approach to treat MDR cancer.

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Natural compounds to treat or prevent the multi-drug resistance in cancer cells

Mihai, CT[1], Vochița, G[1], Alexa-Stratulat, T[2], Luca, A[2]

[1]Institute of Biological Research Iași, branch of NIRDBS, Romania [2]"Grigore T. Popa" Medicine and Pharmacy University, Romania

Chemotherapy is the standard treatment for different types of cancer, but the development of resistance to nearly all kinds of chemotherapeutic drugs and targeted drugs has become prevalent [3]. Occurrence of the multidrug resistance (MDR) is linked to multiple factors as individual's genetic differences, changes in ATP-binding cassette transporters, target proteins, detoxification, DNA repair and gene amplification, drug metabolic enzymes, apoptosis suppression, epigenetic targets [1, 2]. These modifications of the cancer cells drive the tumor to become unresponsive to chemotherapeutic drugs with different chemical structures or mechanisms and are the main cause for chemotherapy failure and, also, hampers the new drug development.

Addressing to the development of new resources to counteract the MDR and adverse reactions of chemotherapy, natural products have attracted extensive research. Natural products could play an important role in treatment or prevention of multidrug tumors by some specific traits: often have multiple targets, have beneficial impact on tumor inhibition, limit the chemotherapy side effects, enhance immunity, improve symptoms and prolong survival [4]. Also, the great biodiversity of natural products offers the possibility to develop more effective therapies for MDR cancers or to use this natural resources as chemosensitizers of MDR cells [5].

We briefly review the status of natural products that could reverse or prevent the MDR in cancer cells and present the future perspectives in this field.

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ROS and antiproliferative agents

Óscar López,[1] Paloma Begines,[1] Jesús Roldán-Peña,[1] Inés Maya,[1] Laura L. Romero-Hernández,[2] Ana I. Ahuja,[2] Alma Fuentes-Aguilar,[2] Ailed Arenas-González,[2] Socorro Meza-Reyes,[2] José L. Vega-Báez,[2] Penélope Merino-Montiel,[2] Sara Montiel-Smith,[2] Gabriela B. Plata,[3] Irene Lagunes,[3] José M. Padrón,[3] José G. Fernández-Bolaños[1]

[1] Dpto. Química Orgánica, Facultad de Química, Univ. de Sevilla Apartado 1203, E-41071, Seville, Spain [2] Fac. Ciencias Químicas, Ciudad Universitaria, BUAP, 72570 Puebla, PUE, Mexico [3]BioLab, IUBO-AG, CIBICAN, Univ. La Laguna, C/Astrofísico Fco. Sánchez 2, Apto. 456, 38200 La Laguna, Spain

osc-lopez@us.es

Reactive Oxygen (and Nitrogen) Species (ROS and RNS, respectively) are comprised by an ample number of derivatives, like hydrogen peroxide, alkyl peroxides, single oxygen, or peroxynitrite. Such species, endowed with a strong pro-oxidant character, are produced endogenously in normal biological processes (e.g. mitochondria), and even undergo relevant tasks, like cell-to-cell signaling. Nevertheless, in numerous degenerative diseases, like diabetes, Alzheimer's and Parkinson diseases, cardiac damage, or cancer, there is an abnormal accumulation of ROS in tissues, leading to a cellular state known as oxidative stress [1].

Oxidative stress has a pronounced deleterious effect, as it provokes the oxidative degradation of virtually all biomolecules. Regarding cancer, mitochondrial dysfunction, leading to increased levels of ROS, is involved in the initial stages of many cancer types. In spite of that, handling oxidative stress in cancer is still a matter of discussion.

Taking this into consideration, we have incorporated several pharmacophores featured with antioxidant properties (organoselenium motifs, polyphenolic scaffolds) and have evaluated their antiproliferative properties against a panel of human solid tumor cell lines, including drug resistant lines [2-8]. Moreover, we have also analyzed their anti-ROS properties, using a wide range of model assays, like antiradical, H_2O_2-scavenging, inhibition of lipid peroxidation, or the capacity of mimicking the natural antioxidant enzyme glutathione peroxidase (GPx). Cellular ROS levels of cancer cell lines in the presence of such derivatives has also been measured.

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TPGS-based approaches in battling resistant breast cancer

Neophytou, CM[1], Gregoriou, Y[1], Matsentidou, A[1], Constantinou, AI[1]

[1] University of Cyprus, Cyprus

Breast cancer is the most frequently diagnosed and second in mortality rate malignancy among women. Despite the many advances in breast cancer treatment in recent years, there is still need for the discovery of novel approaches to improve drug effectiveness and reduce negative side effects. D-alpha-tocopheryl polyethylene glycol succinate (TPGS) is a vitamin E synthetic derivative, known to inhibit the P-gp efflux pump [1]. TPGS is frequently used in the development of drug delivery systems to improve the pharmacokinetics of anti-cancer drugs and reduce multi-drug resistance [2]. We have previously shown that TPGS not only acts as a carrier molecule but also exerts anti-cancer effects by inducing apoptosis and cell cycle arrest in breast cancer cells that overexpress the anti-apoptotic protein Survivin [3]. We have recently investigated the effect of TPGS with a small molecule inhibitor of Survivin (YM155), in various breast cancer cell lines representing different subtypes of the disease. Our aim was to evaluate the synergistic effect of the TPGS-YM155 combination and reveal its mechanism of action. Our results show that the TPGS-YM155 combination acts synergistically to reduce the viability specifically in Her2Neu-overexpressing SKBR3 cells. The combination of agents inhibited the p-AKT pathway, decreased Survivin mRNA levels, increased the subG1 phase of the cell cycle, and induced PARP cleavage. In addition, the TPGS-YM155 treatment led to the cleavage of Caspases -8, -9 and 7 and reduced the levels of the anti-apoptotic protein Bcl-2

indicating that the intrinsic pathway of apoptosis is induced. Importantly, the TPGS-YM155 combination did not significantly affect the viability of MCF-10A normal immortalized cells. Since SKBR3 cells overexpress the Her2neu protein, we could potentially synthesize a TPGS-based micelle, loaded with the YM155 and conjugated with the antibody Herceptin for targeted delivery to the cancer site. Currently, we are developing TPGS-based nanoparticles, loaded with natural agents such as Resveratrol and Gallic acid. These nanocarriers will be fully characterized and their effectiveness will be evaluated in pre-clinical models. Further studies will elucidate the effectiveness of TPGS-loaded nanoparticles against multi-drug resistant and aggressive breast cancer.

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Improvement of drug penetration into 2D and 3D cell culture models

Paškevičiūtė M[1], Januškevičienė I[1], Sakalauskiene K[2], Raišutis R[2], Santos HA[3], Petrikaitė V[1,4]

[1] Lithuanian University of Health Sciences, Lithuania [2] Prof. Kazimieras Baršauskas Ultrasound Research Institute, Kaunas University of Technology, Lithuania [3] University of Helsinki, Finland [4] Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania

Cancer is the second most common cause of death worldwide [1]. The most important factors which lead to high mortality rate are the delayed diagnosis and cancer resistance to chemotherapeutic drugs.

Recent studies show that drug delivery to the tumour tissue may be enhanced by using ultrasound exposure [2]. Acoustic cavitation occurs when ultrasonic wave propagates through a liquid medium. It is thought that acoustic cavitation may create pores in the cell membrane by causing expansion, contraction and explosion of microbubbles [3].
Also, targeted delivery systems are very important in cancer chemotherapy. In such systems nanoparticles are usually conjugated with a ligand/carrier interacting with the specific target in cancer cells. This strategy could improve drug localization in tumor the drug at specific site of the body and reduce toxic effects.

Most solid tumors are characterized by hypoxia, and it is a prognostic indicator of a poor clinical outcome for patients [4]. Carbonic anhydrase IX (CA IX) is a membrane protein which is highly overexpressed in numerous cancers, but is largely absent in normal tissues.
In our presentation we will discuss the possibilities to apply different strategies to enhance drug penetration into 2D and 3D cancer cell cultures: the application of ultrasound and CA IX inhibitors as a specific cancer targeting agents in targeting nanosystems.

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From nanoformulation of small molecules to personalized drug screening: A tumoroid Point of View

Caligiuri I.[1], Palazzolo S.[1], Rotondo R.[1], Mouawad N. [1], El Boustani M. [1], Mauceri M.[2], Adeel M. [2], Canzonieri V. [1,2] and Rizzolio, F.[1,3]

[1] Department of Pathology, IRCCS CRO Aviano-National Cancer Institute, Aviano, PN, Italy [2] Department of medical, Surgical and Health Sciences, University of Trieste, Trieste, TS, Italy [3] Department of Molecular Sciences and Nanosystems, Ca' Foscari University of Venice, Venice-Mestre, VE, Italy

Tumoroids are lab-built mini-organs that can serve as models of cancer. In collaboration with Prof. Vincenzo Canzonieri (Pathologist) at CRO of Aviano, we are now building an innovative biobank of 3D tumoroids and organoids from healthy tissues. In oncology, introducing specific mutations into organoids made from healthy tissues, permits to study how cancer arises and develops. In this order, it appears evident the great application of tumoroids and organoids into predicting how an individual will respond to a drug, making organoids a highly valuable tool in the implementation of precision medicine. Nowadays, organoids offer the possibility of identifying specific associations between the genetics of a

cancer and its response to anticancer therapy, predicting the sensitivity or development of resistance to experimental or FDA-approved drugs and providing preliminary guidance in therapeutic choices. For this reason, we are now using patient-derived tumoroids as high-grade serous ovarian cancer (HGSOC) and colorectal cancer models to predict which treatments will be most effective for patients. In fact, during the last two years, our efforts into recreating the best conditions to favour the ovarian and colorectal tumoroids growth and maintenance, led us to develop a very reliable method to obtain ex vivo ovarian and colorectal tumoroids, that morphologically and molecularly mimic the parent tumor from which they are derived. In support of our assertions, to the best of our knowledge, we are among the first research groups to developed human and mouse oviduct and colon "Organoids Kits", now commercialized by BioFuture Medicine. Considering our knowledge and experience in nanodelivery systems [1–5] and advantages of nanoformulated drugs, we are now evaluating the anticancer activity of our nano-drugs for specific targets on patient-derived tumoroids. On this side, our preliminary results suggest the prominent involvement of peptidyl-prolyl *cis-trans* isomerase NIMA- interacting 1, PIN1, in HGSOC patients-derived tumoroids.

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Single-cell tracking and beyond

Korsnes, Mónica Suárez [1,2] Korsnes, Reinert[1]
[1] Korsnes Biocomputing (KoBio), Norway [2] Norwegian University of Life Sciences (NMBU), Norway

We work on methods for tracking single cells grown as mono-layers. An intention for the work is to facilitate detection of rare sub-populations and changes among cells subject to treatments. This can be of prognostic value in oncology. Cell tracking directly provides pedigree tree profiles and which may bear signatures of subpopulations.Single-cell tracking can help to find biological meaningful classifications and parameterizations of cellular morphologies and movements. If parameters derived from video recordings of closely related cells, are correlated, it increases the likelihood of biological significance of the data reduction. Further statistical treatment can therefore be based on such parameterization. Single-cell tracking can contribute to understand MDR mechanisms in tumors. We need test data for systematic benchmarking and further development before making our software open source available via for example github. However, we have already made our software available as a prototype service via "cloud computing". Pilot users can access the software this way just by a common browsers such as Firefox or Chromium. They can then do tracking, make statistics, and visualization in addition to export data for further treatment.

The web page: https://korsnesbiocomputing.no/

includes illustrations from our software. A preliminary version of a user guide is available.

We have published three scientific papers based on single-cell tracking and possible applications [1,2,3].

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Monitoring anti-cancer drugs action by atomic force microscopy

S. Kasas[1], A.-C. Kohler[1], V. Pfannenstill[2], M. Pesic[3], J. Bankovic[3], and G. Dietler[1]

[1]Laboratoire de physique de la Matière Vivante, EPFL, CH-1015 Lausanne, Switzerland [2]Technische Universität Wien, Institute of Applied Physics, Wiedner Hauptstrasse 8-10, 1040 Wien, Austria [3]Institute for Biological Research «Sinisa Stankovic» (IBISS), University of Belgrade, Despota Stefana 142, 11060 Belgrade, Serbia

Our team recently demonstrated that the presence of living organisms onto an Atomic Force Microscope (AFM) cantilever induce nanometric scale oscillations of the lever. The oscillations are detected relatively easily by using traditional AFMs or dedicated homemade devices. Our laboratory apply such an instrument to rapidly detect pathogens antibiotic susceptibility and to assess the presence of living organisms in hostile environment in a chemistry independent way. The device can also be employed to rapidly detect cancer cells sensitivity to anti-cancer drugs. The working principle of the technique as well preliminary results involving application on cancer cells will be presented.

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Horizontal transfer of MDR traits by extracellular vesicles:

implications for diagnosis and treatment of MDR cancers

Sousa D[1,2,3], Lopes-Rodrigues V[1,2,4], Xavier CPR[1,2], Caires HR[1,2], Vasconcelos MH[1,2,3]

[1] i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal [2] Cancer Drug Resistance Group, IPATIMUP - Institute of Molecular Pathology and Immunology, University of Porto [3] FFUP - Faculty of Pharmacy of the University of Porto, Portugal [4] ICBAS-UP – Institute of Biomedical Sciences Abel Salazar of the University of Porto, Portugal

Cells may release different types of extracellular vesicles (EVs) into the extracellular environment [1] and some tumour cells seem to do so in greater amounts [2]. EVs are surrounded by a lipid bilayer, ranging in size from 30 to 1000 nm [3]. Their cargo (proteins, various types of RNA, lipids and fragments of DNA) is selectively packaged from the donor cells. EVs have been isolated from biological fluids, where they are more abundant than circulating tumour cells and protect their molecular cargo against degradation, thus being a potential source of biomarkers. We found that P-glycoprotein (P-gp) overexpressing MDR cells shed larger EVs than their sensitive counterparts, carrying specific EV's markers and P-gp [4]. Consequently, our laboratory is currently working towards the potential detection of MDR in EVs from liquid biopsies of acute myeloid leukaemia patients (unpublished work). Once released by donor cells EVs may transfer their cargo including MDR traits to recipient cells, thus being responsible for the horizontal transfer of those traits [5]. Our work indicates that MDR cells shed more EVs than their sensitive counterparts and that sensitive cells capture more EVs than their resistant counterparts, which may contribute to "dissemination" of MDR (submitted for publication). Furthermore, co-culture of EVs released by MDR cells with sensitive cells conferred increased resistance (NSCLC and Leukaemia models, unpublished work) and alterations in the metabolic profile of recipient cells [6]. Proteomics and microRNA NGS analysis of EV´s cargo is currently providing molecular target candidates (unpublished work). In addition, we verified that EVs released by human macrophages decreased the sensitivity of pancreatic tumour cells to gemcitabine, providing evidence for EVs-mediated intercellular communication between the immune microenvironment and tumour cells. Proteomic analysis of their cargo is currently indicating molecular candidates for possible therapeutic intervention (unpublished results).

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Metastatic Subset of Breast Carcinoma: Model for Treatment-Resistant Grade IV Breast Cancer

Nuray Erin

Akdeniz University, School of Medicine, Dept. Medical Pharmacology

Breast carcinoma is comprised of heterogeneous groups of cells with different metastatic potential. To develop effective therapeutic strategies targeting metastatic disease, it is crucial to understand the characteristics of breast cancer cells that enable metastasis to distant organs. 4THM breast carcinoma cells are the cells of 4T1 primary tumors that metastasized to the heart. Cells of 4THM tumors which metastasized to liver (4TLM) and brain (4TBM were isolated to obtain a cell lines. Phenotypically we observed both similarities and differences among different metastatic subsets. Specifically 4TLM cells produced significantly more lung and liver metastasis compared to 4TBM and 4THM cells. In vitro, proliferation as well as migration rate of 4TLM cells was also significantly higher than the other cell lines. Remarkably primary tumors formed by 4TLM cells expressed significant amounts of CD34, a marker for mesenchymal malignancies. Markers of epithelial-mesenchymal transition were expressed in all metastatic cells, but the degree of expression differed. Majority of 4TLM, 4THM, and 4TBM cells expressed cancer-stem cell phenotype (1). Recently we characterized exosomes of metastatic cells and compared them with non-metastatic breast carcinoma. Eighty-five of over 500 secreted proteins were significantly altered in metastatic breast cancer cells. Many of these identified proteins were also present in human metastatic breast carcinomas. Ingenuity Pathway Analysis showed that proteins differentially secreted from metastatic cells are involved primarily in carcinogenesis and TGF-β1 is the top upstream regulator in all metastatic cells (2). Hence this model provides an opportunity to find new target and to validate new therapeutic approaches. Up to now, we have explored several new treatment approaches using heart, brain and liver metastatic cells and some of them were published or under revision (3-6).

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Translational research in Vienna: How can we improve tumor targeting of anticancer drugs?

Heffeter, P[1], Berger, WB[1], Keppler, BK[2], Kowol, CK[2]

[1] Institute of Cancer Research, Medical University of Vienna, Austria [2] Institute of Inorganic Chemistry, University of Vienna, Austria

Chemotherapy and therapy with small targeted molecules are two major strategies for therapy of human cancer at the disseminated stage. During the last decades, thousands of compounds have been developed and consequently have improved therapy effectiveness. However, even when using new, targeted therapeutics, treatment is often limited by strong side effects, resistance development and insufficient tumor specificity. In order to overcome these limitations, in 2009 an interuniversity research platform between the Institute of Cancer Research (Medical University of Vienna) and the Institute of Inorganic Chemistry (University of Vienna) was established. The aims of this research platform are to investigate the mechanisms underlying sensitivity and resistance of cancer cells to therapy and, subsequently, to use this new knowledge to develop novel drugs with improved efficacy and tolerability. During the last years, multiple new compounds have been designed resulting in the discovery of several promising new drugs, which are currently in (pre)clinical development as novel anticancer agents. This talk will give a short overview on our research hotspots as well as the tools which are available at our facilities in Vienna.

From molecules to targeted therapies in hematological neoplasias - implications in prognosis and therapeutic failure

Sarmento-Ribeiro AB[1]

[1]Faculty of Medicine, University of Coimbra (FMUC); Center of Investigation on Environment, Genetics and Oncobiology (CIMAGO)/Coimbra Institute for Clinical and Biomedical Research (iCBR) and Hematology Department of Centro Hospitalar Universitário de Coimbra (CHUC), Portugal

Our research group is a multidisciplinary group included in the Oncobiology research strand of the Center of Investigation on Environment, Genetics and Oncobiology (CIMAGO) of Coimbra Institute for Clinical and Biomedical Research (iCBR) of Faculty of Medicine, University of Coimbra (FMUC), in close association with the Hematology Department of Centro Hospitalar Universitário de Coimbra (CHUC), Portugal.

Our main goals are the characterization of cellular and molecular mechanisms involved in cancer risk/develoment, and in drug sensitivity and resistance to conventional chemotherapy and to targeted therapies, in order to identify new prognostic and drug response markers and targets for new therapies.

We found that oxidative stress (OS) and apoptosis may be involved in chemoresistance in acute leukemia [1] and that influx/efflux transporters (decreased OCT1 and OCNT2 and increase of GL-P and BCRP, respectively) were involved on Chronic Myeloid Leukemia (CML) resistance to imatinib (TKI) [2], and that simultaneous administration of TKI and everolimus re-sensitize resistant cells [3]. Furthermore, in CML patients, miR-21 and miR-451 appear to be good biomarkers of response, with great power to discriminate optimal TKI patients responders [4].

Additionally, we found that OS and DNA methylation may be involved in the development of acute myeloid leukemia and Myelodysplasic Syndromes, as well as may influence the prognosis, therapy response, and patients' survival [5]. Also, oxidative stress levels were associated with P15 and P16 methylation [6] and OS levels and GSH content could be related with *NFE2L2* gene and NRF2 protein expression levels.

Moreover, the increased expression of activated caspase 3 was identified as a biomarker of Multiple Myeloma treatment failure. Furthermore, we observe that higher levels of NF-kB expression in neoplastic plasma cells are associated with a greater likelihood of response to bortezomib and, in these patients a significant increase in overall survival, highlighting the relevance of NRF2/NF-kB pathways in bortezomib response.

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A Modular Approach to Trim Cellular Targets in Anticancer Drug Discovery

Padrón JM[1], Fernandes, MX[1]

[1] Universidad de La Laguna, Spain

The challenge in a Phenotypic Drug Discovery strategy is to fully understand and elucidate the mechanism of action identifying with high resolution the molecular target(s) affected by any given small molecule (drug or candidate) and responsible for its pharmacological activity [1]. At present, existing models have the limitation of the low statistical significance of the results, which can be attributed to the low relevance of the input data. Our model arises to address the limitations of the existing strategies and uses the combination of three data sources, ordered from higher to lower importance. 1) The phenotypic responses obtained from the interaction between the drug and the biological targets; 2) The biological targets involved in the affected biological routes; and 3) The chemical structure of the small molecule, which is the responsible –among other considerations– of the molecular interaction with the target.

The objective of our group (BioLab) is developing a working model that combines experimental and computational data to correlate phenotypic response patterns anticipating the mechanism of action of new compounds of potential therapeutic application in the areas of cancer and rare diseases [2]. We anticipate that our results will allow the actors involved to design more efficient, faster, less laborious, and less expensive strategies for the search of new therapeutic options with small molecules.

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Development and application of *in silico* approaches to predict pharmacologial/toxic effects of bioactive compounds

Tsakovska I, Alov P, Pencheva T, Al Sharif M, Lesigiarska I, Jereva D, Diukendjieva A, Pajeva, I

Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Bulgaria

The *in silico* (computer aided) approaches in the fields of drug design and human health and environmental risk assessment of chemicals combine various ligand- and structure-based methods, including classical and three dimensional (3D) quantitative structure-activity relationships (QSAR), pharmacophore and homology modeling, docking and virtual screening. The aim of the developed in silico models is quantitative characterization of the relationship between the chemical structure of the compounds and their effect - therapeutic, toxic, etc. They effectively help in understanding and elucidation of mechanisms by which the bioactive compounds interact with target biomacromolecules, thereby explaining fundamental processes in the living organisms. This presentation provides an overview of the research that is being performed at the Institute of Biophysics and Biomedical Engineering – Bulgarian Academy of Sciences to develop and implement *in silico* models, algorithms and software tools in the field of computer aided design and computational toxicology. Several studies will be discussed: (i) development of QSAR models to predict MDR reversal activity of P-glycoprotein ligands [1, 2]; (ii) development of *in silico* screening protocol to identify potentially hepatotoxic PPARgamma agonists that can function as steatosis inducers [3]; (iii) prediction of ADME/Tox properties and biochemical interactions of naturally-derived compounds [4]; (iv) development of QSAR model for membrane permeability prediction to evaluate gastrointestinal absorption of bioactive compounds [5].

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Strategies for the generation of vascularized 3D-in vitro cancer models

Serra, T, Eglin, D, Alini, M

AO Research Institute Davos, Switzerland

Cell patterns are important for the development of novel approaches in biomedicine. Finding new ways to pattern cells means opening to the generation of highly complex 3D cell technologies. This will revolutionize drug discovery and precision medicine. Surface acoustic wave (SAW) technologies, based on Faraday wave principle, enable the generation of spatially orchestrated particulate systems (cells, spheroids, inorganic aggregates). Patterns shape can be tuned on demand by varying a set of parameters, such as sound frequency, amplitude, chamber shape. Here we report a proprietary SAW-based technology, named 3D Sound Induced Morphogenesis (3D-SIM), which allows producing hierarchically complex 3D cellularized constructs[1]. We propose the use of 3D-SIM to create precise and reproducible microvascular networks formed by interconnected and perfusable vessels. Hierarchically shaped vessels with a multiscale organization (meso-micro scale) can be integrated into fluidic chip where perfusion can be performed in a reproducible manner with a controlled flow rate. Tuning on demand spheroids composition (i.e. including tumor-environment related cells and extracellular matrices) will allow the generation or more reliable and translationally relevant *in vitro* models.

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Characterization of metabolic pathways of small molecule drugs

Raunio H, Juvonen RO

University of Eastern Finland, Faculty of Health Sciences, School of Pharmacy, Finland

Absorption, distribution, metabolism, and excretion (ADME) are the steps of pharmacokinetics that determine the concentration of drugs at the site action. A critical step is metabolism, as active parent compounds can turn to inactive metabolites, active metabolites or even toxic metabolites. Sometimes a drug candidate is unstable, being metabolized during first pass in liver. The key metabolizing enzymes in human liver are the cytochrome P450s (CYPs) and some conjugating enzymes, especially those catalyzing glucuronidation and sulfonation [1].

Together with our collaborators, we have developed and applied several in vivo, in vitro and in silico methods to evaluate metabolic stability and pathways of small molecule compounds. The tools used are animal and human tissue fractions (especially liver), recombinant metabolizing enzymes, and several analytical methods including high precision mass spectrometry [2]. Computational modeling approaches include ligand-based and target-based methods such as 3-dimensional quantitative structure-activity relationship and molecular docking [3].

Our approaches facilitate efficient elucidation of metabolic pathways and the enzymes mediating them in humans. Similar studies in different animals identify inter-species differences and allow for selection of most suitable species for nonclinical studies. In addition, potential drug-drug interactions can be screened, and prodrug approaches can be applied to improve ADME characteristics of drug candidates [4].

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Assessment of cardiovascular liabilities of new candidate drugs

Simona Saponara[1], Miriam Durante[1], Maria Frosini[1], Beatrice Gorelli[1], Fabio Fusi[2]

[1]Department of Life Sciences, University of Siena, Italy [2]Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Italy

Functional and structural cardiovascular liabilities are a leading cause of drug attrition during preclinical and clinical development, and post-approval stage. They pertain to both cardiovascular and non-cardiovascular drugs and can be due to interaction of the drug with either intended or unintended targets (on-target and off-target effect, respectively).
These liabilities can be unveiled during the lead optimization phase by addressing cardiovascular safety endpoints [1].
We have developed a systematic approach based on *in vitro* functional assays as well as *in silico* methods to identify these potential hazards and characterize their molecular mechanism [2-4].
Freshly isolated or cultured vascular and cardiac cells-, whole-tissue- and isolated organ-based models are routinely used to assess:
1) cell viability, patterns of cell death, mitochondrial function, redox state, Ca^{2+} handling, endothelial mesenchymal transition
2) vascular responsiveness to various agents (in aorta rings, single myocytes),
3) cardiac conduction and contractility and related biomarkers (in Langendorff perfused heart)
4) patch-clamp analysis of ionic currents [Na^+, Ca^{2+}, K^+ channels, i.e. Kv11.1(hERG) - the target of virtually all QT interval-prolonging torsadogenic drugs] in single cell
5) computational methods for molecular docking and dynamics simulations to predict drug interaction with ion channel protein.
These approaches represent a risk mitigation strategy to guide and inform pharmaceutical chemists as well as to increase quality and efficiency within the drug discovery and development process.

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Nanotoxicology tools for safety evaluation of novel drug-delivery systems

Virginia Tzankova and Denitsa Aluani

Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University – Sofia, Bulgaria

Different ABC transporters inhibitors (e.g. P-glycoprotein (P-gp) inhibitors) have been developed in the past, as a strategy to overcome P-gp mediated multidrug resistance (MDR). Unfortunately, most of the proposed inhibitors do not show good selectivity, thus blocking the normal cell function of P-gp in the intestines or at the blood–brain barrier (BBB). Therefore, several pharmacological inhibitors of ABC transporters have failed in clinical stages due to their low specificity and high toxicity**.** A refinement of this concept might be the encapsulation of the new molecules with promising efficacy in appropriate drug – carriers for target delivery (e.g., nanoparticles) or encapsulation of both the therapeutic drug and the P-gp inhibiting agent into the same drug carrier for simultaneous delivery into the cell.

Recently, different types of nano-sized drug carriers (polymers, liposomes, dendrimers, silicon or carbon materials, magnetic nanoparticles, etc.) have been developed to improve the drug delivery and to overcome some drug - dependent limitations. Cell specific targeting, enhanced therapeutic efficacy and improved safety are the main goals of the new drug – delivery systems development. The great challenge is to develop and introduce in the practice therapeutically efficient, non-toxic, biocompatible, biodegradable, and safe nano-sized drug delivery systems. The use of nanoparticles as drug carriers involves intentional contact to the biological system, so that the understanding of the effect of the nanoparticles before their clinical use is very important. Our special attention is focused on the complex *in vitro* and *in vivo* toxicity evaluation of different nano-sized drug-delivery systems (inorganic, biopolymers, polymers) as safe and target specific drug carriers.

Poster Presentations

WG 1

Identification of new diagnostic/predictive biomarkers and compounds applicable to personalized treatments of multidrug resistant tumors

Vaclavikova, R[1,2], Soucek, P[1,2], Hlavac, V[1,2], Ehrlichova, M[1,2], Brynychova, V[1,2], Kloudova, A[1,2], Holy, P[1,2], Seborova K[1,2]

[1] National Institute of Public Health, Prague, Czech Republic, [2]Biomedical Center, Faculty of Medicine in Pilsen, Charles University, Czech Republic

Our research team has been involved in research of multidrug resistance (MDR) in solid tumors and tumor cell lines since 2000. Firstly, on the basis of our comprehensive studies of gene expression profile in cancer patients (breast, ovarian, pancreatic and colorectal cancer cohorts), we have identified a broad spectrum of candidate genes associated with prognosis, progression and therapy outcome e.g. ABC and SLC membrane transporter genes or CYPs [1-7]. Very recently, we have performed next generation sequencing (NGS) studies of cancer patient cohorts and we have developed *in silico* tools for bioinformatics, prioritization, and validation of sequencing data. Associations of genetic variants located in *ATP7A, KCNAB1,* and *DFFB* genes with therapy response and rs1801160 in *DPYD* with disease-free survival of breast carcinoma patients were found [8]. In the frame of this COST action (WG1) we would like to characterize new identified molecular biomarkers of therapy outcome and MDR, validate them and study their function.

Secondly, we have investigated and compared the efficacy of classical taxanes and novel taxane analogs (Stony Brook Taxanes) *in vitro* in sensitive and resistant cancer cells and tumors (e.g. breast cancer MCF7 sensitive and paclitaxel resistant, ovarian cancer OVCAR-3 sensitive and NCI/ADR-RES taxane resistant cancer cell lines, rat lymphoma models). High efficacy of novel taxane analogs in resistant tumor cells was found [9,10]. We would like to study efficiency and toxicity of those promising potential MDR-reversal agents in different experimental models in the frame of our upcoming cooperation with COST (WG3) members for successful identification of tools overcoming MDR in cancer patients.

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Expression and gene variants of *VHL* tumour suppressor in papillary thyroid carcinoma

Todorović L[1], Stanojević B[1], Mandušić V[1], Živaljević V[2], Paunović I[2], Diklić A[2], Dzodic R[3], Saenko V[4], Yamashita S[4].

[1] Laboratory for Radiobiology and Molecular Genetics, "Vinča" Institute of Nuclear Sciences, University of Belgrade, Belgrade, Serbia. [2] Center for Endocrine Surgery, Clinical Center of Serbia, Belgrade, Serbia. [3] Institute for Oncology and Radiology of Serbia, Belgrade, Serbia. [4] Atomic Bomb Disease Institute, Nagasaki University, Nagasaki, Japan.

Papillary thyroid carcinomas (PTC) represent the most prevalent type of endocrine malignancy. Over the last decades their incidence has been increasing, underlining the significance of the identification of molecular markers which would improve the risk stratification and help optimize the therapeutic approach to PTC patients [1]. While the *VHL* gene is implicated in tumorigenesis of different types of carcinoma and has been reported to be associated with more aggressive biological behavior [2-6], its importance in the patogenesis and progression of PTC has not been explored so far. The subject of this study was to investigate the expression level of VHL in this type of carcinoma. The expression profiles of VHL mRNA and protein were determined using real time PCR and immunohistochemistry. In addition, it was investigated whether mutations in the coding sequence and post-transcriptional expression regulation mediated with miR-92a – which has been shown to regulate VHL expression in other cancers [7,8], were responsible for VHL expression alterations detected in this study. No mutations were detected by sequencing of all three exons of the *VHL* gene. The expression level of miR-92a, although altered, did not show significant correlation with VHL mRNA and protein levels. These results suggest that probably some other regulatory mechanisms are responsible for VHL expression alterations. The analysis of *VHL* mRNA expression levels in PTC tissues compared to matched non-tumour tissues revealed that there were two groups of patients – patients with a decreased *VHL* expression level and patients with an increased expression level in tumour tissue. The detected VHL expression profile alterations were in correlation with PTC clinicopathological parameters. Moreover, low VHL expression was associated with more aggressive tumour features and with a shorter disease free interval. The results of this study suggest that evaluation of VHL tumor suppressor expression level might have significance in the prognosis of more aggressive course of the disease in PTC patients.

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WG 2

Is Squalenization a Possible Way to Tackle Multidrug Resistance?

Desmaële Didier

Université Paris Sud, Institut Galien, France

Multidrug resistance (MDR) is a major cause of failure in cancer chemotherapy. A prevalent mechanism of cancer MDR is the expression of efflux pumps such as Pg-P, MDR-1 and MRP1 that cause lowered drug accumulation inside cells. Complexation or attachment of drugs to a nanosize delivery agent offers the possibility to compete transporter mediated efflux by enhancing drug uptake through either EPR effect or targeted nanocarriers. Furthermore, co-delivery in the same particle of an anticancer drug with a Pg-P-inhibitor is an ongoing promising strategy. [1] We have taken advantage of the remarkable dynamically folded conformation of squalene, a triterpene widely distributed in nature to chemically conjugate this lipid with various therapeutic molecules in order to construct nanoassemblies of 100–300 nm. "squalenoylation" found wide application to anticancer (ie. Gemcitabine, paclitaxel, cisplatine or oligonucleotide siRNA.), antiviral (ddI, ddC) or antibiotic compounds. In a lego-type approach, it is also possible to construct multifunctional nanoparticles endowed with additional imaging functionalities (ie. "Nanotheragnostics"). This nanotechnology platform is expected to have important applications in pharmacology including potential way to circumvent multidrug resistance [2]

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SEARCHING FOR NEW "CHEMICAL STRATAGEM" AGAINST MDR TUMORS. PART A : "LIGHT SOLUTION"

Konstantin Chegaev, Roberta Fruttero, Stefano Guglielmo, Loretta Lazzarato, Barbara Rolando DSTF, Department of Drug Science and Technology, University of Turin

Nitric oxide (NO) is an ubiquitous and pleiotropic messenger; it plays a variety of roles in human physiology and pathophysiology; among them, it may display its cytotoxic effects directly or indirectly, affording reactive nitrogen species (RNS) by reaction with oxygen and ROS. In human solid tumor there are hypoxic regions that have lower oxygen concentration than normal tissues; this imparts resistance to radiotherapy, chemotherapy and photodynamic therapy. So there is a great attention to NO-donors as anticancer agents. The photogeneration of NO achieved using NO photodonors (NOPDs), namely compounds able to release NO under the action of the light, has received a great attention as potential new anticancer therapy. Cellular viability is strictly dependent on mitochondrial functionality. Mitochondria have recently been considered as an important target for new antitumor drug development. On these bases, the mitochondrial accumulation of NO has been proposed as an effective strategy for the design of new anticancer drugs. This approach has been realized by linking NOPDs to vectors that

display high tropism for these organelles, like. Rhodamine B and alkytriphenylphosphonium.1,2 An interesting class of NOPDs is represented by photo-caged spontaneous NO-donors, like O-alkyl Cupferron (CP) derivatives. Our strategy consisted in a BODIPY substructure linked to CP by O-alkylation. This derivative irradiated with green light leading to the formation of the BODIPY, and to the release of NO as a consequence of the CP decaging.3 In recent years, a deal of attention has been devoted to the combination of PSs and NOPD as a very appealing strategy in view of multimodal therapeutic approaches entirely controlled by light. On these bases, we developed an intriguing molecular hybrid based on a BODIPY light-harvesting antenna that acts simultaneously as PS and NOPD upon single photon excitation with the highly biocompatible green light.4

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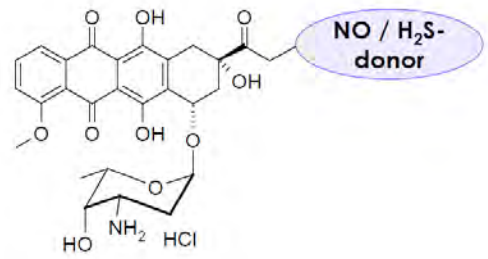
SEARCHING FOR NEW "CHEMICAL STRATAGEM" AGAINST MDR TUMORS. PART B: "GASEOUS SOLUTION".

Konstantin Chegaev, Roberta Fruttero, Stefano Guglielmo, Loretta Lazzarato, Barbara Rolando DSTF, Department of Drug Science and Technology, University of Turin

Doxorubicin (DOXO) is an antibiotic belonging to the class of antracyclines, used for the treatment of a wide range of cancers. The one of serious limitation to DOXO efficacy in cancer therapy is the easy development of the resistance through different mechanisms, the main of which is the overexpression of ATP-Binding Cassette (ABC) transporters that actively extrude the drug from tumour cells. Resistance to DOXO is often part of a cross-resistance towards several anti-cancer drugs known as Multidrug Resistance (MDR). 1 As part of a program aimed at developing new DOXO derivatives endowed active against DOXO-resistant tumor cells we designed a series of DOXOs able to release small gaseous messengers (NO or H2S), combining DOXO with appropriate gas-donor substructures.

These compounds were studied on a series of DOXO-sensible and DOXO-resistant human tumour cell populations in vitro as well as in vivo. 2-6



These experimental models have highlighted the ability of both NOand H2S- donating DOXO to trigger anticancer action and to overcome the drug resistance of the cells. Two different molecular mechanism of ABC protein deactivation were found, demonstrating that the "gaseous approach" could be a useful strategy to improve efficacy against drug-resistant tumours.

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SEARCHING FOR NEW "CHEMICAL STRATAGEM" AGAINST MDR TUMORS. PART C Efficiently targeting drug efflux

Konstantin Chegaev, Roberta Fruttero, Stefano Guglielmo, Loretta Lazzarato, Barbara Rolando DSTF, Department of Drug Science and Technology, University of Turin

One of the most studied mechanisms of chemoresistance in cancer cells is the overexpression of ATPBinding Cassette (ABC) transporters, which are actively involved in the efflux of chemotherapeutic agents. Among these, ABCB1/P-glycoprotein (P-gp) represents a main concern due to its ample spectrum of substrates and to its overexpression in Cancer Stem Cells (CSC), 1 a small population with great tumorigenicity, self-renewal ability and resistance to cancer therapy. In this setting our efforts aimed at the development of a library of P-gp ligands starting from an already studied inhibitor, MC70 ([4'-(6,7- dimethoxy-3,4-dihydro-1H-isoquinolin-2-ylmethyl)biphenyl-4-ol], EC50 = 690 nM). 2 The structural modification focused on several aspects: i) we developed a first series of compounds through the functionalization of the phenolic group of MC70 with alkyl and oxyalkyl chains; by tuning the length of the substituent we reached an EC50 of 5.4 nM;3 ii) through the introduction of more complex moieties containing variously substituted furazan (1,2,5-oxadiazole) ring, we obtained several compounds with EC50 between 0.5 and 1 nM;4 three of these proved able to induce doxorubicin accumulation in glioblastoma multiforme cells even in presence of blood brain barrier; 5 iii) the "decoration" of the biphenyl core of MC70 lead to compounds with comparable activity, but in this case we found that one of them was particularly efficient in restoring doxorubicin activity in co-administration assay; 6 iv) we fulfilled a more "drastic" modification of the phenolic group introducing a variety of structural features of key impact on molecular recognition: we obtained a ligand with an EC50 of 15 nM which reversed the chemoresistance mediated by P-gp in CSCs, the hardest tumour component to be eradicated, with a non-tumour specific effect. Its activity on P-gp was rationalized through an in-depth molecular dynamics study.7

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Potential of novel Heat Shock Protein 90 (HSP90) inhibitors for P-glycoprotein inhibition and cancer multidrug resistance reversal

Musso, L[1], Dinić, J[2], Podolski-Renić, A[2], Jovanović, M[2], Dallavalle S[1], Pešić, M[2].

[1]Department of Food, Environmental and Nutritional Sciences, Università degli Studi di Milano, Italy [2]Department of Neurobiology, Institute for Biological Research "Siniša Stanković", University of Belgrade, Serbia

Cancer chemotherapy is often compromised by development of multidrug resistance (MDR). Numerous strategies have been developed over recent decades to overcome cancer resistance but this issue remains unsolved in clinical practice. Dual-targeting by a single drug emerged as an unconventional approach to overcome incomplete efficacy of individual targeting agents. Heat Shock Protein 90 (HSP90) chaperone interacts with a broad range of client proteins involved in cancerogenesis and cancer progression. Its overexpression was found in several cancer types and thus it is considered a valuable target for anticancer treatment. However, HSP90 inhibitors were unsuccessful in clinical studies due to high toxicity, lack of selectivity against cancer cells and extrusion by membrane transporters such as P-glycoprotein (P-gp). P-gp is responsible for low efficacy of anticancer drugs in more than 50% of cancers. Recognizing the potential of new compounds to inhibit P-gp function and/or expression is essential in the search for effective anticancer agents. We have synthesized 11 novel HSP90 inhibitors containing an isoxazolonaphtoquinone core and identified candidates that inhibit P-gp and modulate MDR. HSP90 inhibitors were evaluated in MDR models comprised of sensitive and corresponding resistant cancer cells with P-gp overexpression (non-small cell lung carcinoma NCI-H460 and NCI-H460/R; colorectal adenocarcinoma DLD1 and DLD1-TxR) as well as human normal embryonic fibroblasts MRC-5. We have investigated the effect of HSP90 inhibitors on cell growth inhibition, P-gp function, and P-gp mRNA and protein expression. Additionally, optimization of HSP90 inhibitors' MDR modulation was performed by kinetics and dose response studies. Compounds **1** and **2** directly interacted with P-gp and inhibited its activity. Similar cytotoxicity of **1** and **2** in sensitive and MDR cancer cells indicated these compounds are not P-gp substrates. On contrary, the effect of compound **3** was significantly reduced in MDR cancer cells, indicating that this compound acts as P-gp substrate, exerting competitive inhibitory effect on P-gp. Inhibition of P-gp activity after **1**, **2** and **3** treatment lasted 24 h. These compounds also showed good relative selectivity towards cancer cells. Compound **4** had no direct effect on P-gp activity but significantly suppressed P-gp expression after 72 h treatment.

Discovery of isoform selective voltage-gated sodium and potassium channel modulators for the treatment of cancer

Zidar, N[1], Žula, A[1], Ilaš, J[1], Tomašič, T[1], Peterlin Mašič, L[1], Durcik, M[1], Kikelj[1], D, Peigneur, S[2], Tytgat, J[2], Rogers, M[3], Kirby, R[3]

[1] University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia [2] Katholieke Universiteit Leuven, Toxicology & Pharmacology, Leuven, Belgium [3] Metrion Biosciences Ltd, Cambridge, UK

Voltage-gated potassium (Kv) and sodium (Nav) channels are molecular complexes that have important roles in transduction of signals in neurons, myocytes, endocrine cells and in immune cells. The highest expression of Kv1.3 channels can be found in T- and B-lymphocytes, and in macrophages. Altered expression of Kv1.3 channels has been implicated in several types of cancers, including GI carcinomas and lymphomas [1]. Mitochondrial Kv1.3 protects cells from undergoing apoptosis, while the channel in the plasma membrane of immune cells is implicated in migration, proliferation and activation. Therefore, Kv1.3 inhibitors have the potential to eliminate the resistance to apoptosis in tumour cells and at the same time minimise the pro-tumoural actions of immune cells in the microenvironment. There are also many studies describing important roles of Nav channels in cancer [2].

We have designed and synthesized a series of potential Nav and Kv channel modulators. Compounds were evaluated for their modulatory activities on selected Nav1 (Nav1.1-Nav1.8) and Kv1 (Kv1.1, Kv1.2, Kv1.4, Kv1.5 and Kv1.6) channels using automated patch clamp or voltage clamp electrophysiology. Some compound displayed nanomolar IC_{50} values on Nav1.3 channels, and sub-micromolar IC_{50} values on Kv1.3-Kv1.6 channels. For the best compounds, cytotoxic activity was studied on selected cancer cell lines [3-5].

Promising IC_{50} values against some Nav and Kv channels and relatively low molecular weights of the prepared compounds highlight their potential for further optimization of their inhibitory and anticancer activities.

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WG 3

Natural flavonoids and flavonolignans as modulators of MDR

Viktorova J[1], Rehorova R[1], Valentova K[2], Kren V[2], Macek T[1]

[1] University of Chemistry and Technology, Prague, Czechia [2] Institute of Microbiology of the Czech Academy of Sciences, Czechia

Multiple drug resistance in both somatic cells and microorganisms involves a molecular mechanism of increased expression of transmembrane efflux pumps, whose role is to export the drugs from cell to the outside. Increased expression of such pumps in tumor cells results in reduced drug accumulation. Therefore, profiling of new compounds that are effective and would exhibit a minimal toxicity level is very important.

In this project, we evaluate the effects of 27 natural flavonolignans. These compounds were tested for their potent antioxidant, immunomodulatory, antimicrobial, quorum sensing inhibition and anticancer activity as well as for their ability to inhibit P-glycoprotein. Substances interacting with P-gp may stimulate or inhibit its activity, resulting in the modulation of ATPase activity. The measured results confirmed the ability of the tested compounds to inhibit P-gp transport activity. The biological activity of flavonolignans was monitored from the point of view of their bioactivity, cytotoxicity and effect on cell proliferation. Further measurements will be focused on the evaluation of the selected compounds against the tumor cell line of ovarian carcinoma resistant to adriamycin.

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The role of antioxidant, coenzyme Q10, in suppressing invasion of temozolomide resistant rat glioma

Stojković Burić, S[1],Podolski-Renić, A[1],Dinić, J[1],Stanković, T[1],Jovanović, M[1],Hadžić, S[1],Ayuso, JM[2,3,4],Virumbrales-Muñoz, M[2,3,4], Fernández, LJ[2,3,4],Ochoa, I[2,3,4],Pérez-García, VM[5],Pešić, M[1]

[1]Department of Neurobiology, Institute for Biological Research "Siniša Stanković", University of Belgrade, Despota Stefana 142, Belgrade 11060, Serbia [2]Group of Applied Mechanics and Bioengineering (AMB), Aragón Institute of Engineering Research (I3A), University of Zaragoza, Zaragoza, Aragon 50018, Spain [3]Centro Investigación Biomédica en Red. Bioingenieria, biomateriales y nanomedicina (CIBER-BBN), Zaragoza, Aragon 50018, Spain [4]Aragon Institute of Biomedical Research, Instituto de Salud Carlos III, Madrid, Madrid 28029, Spain [5]Departamento de Matemáticas, E.T.S.I. Industriales and Instituto de Matemática Aplicada a la Ciencia y la Ingeniería (IMACI), Universidad de Castilla-La Mancha, Ciudad Real 13071, Spain

Development of chemoresistance and the invasion of cancer cells into surrounding brain tissue are major obstacles to successful glioma treatment. New therapeutic approaches are warranted to improve the survival of glioma patients. The purpose of this study was to assess the potential of lipophilic antioxidant coenzyme Q10 (CoQ10) to increase sensitivity to temozolomide (TMZ) and suppress glioma cells invasion. Therefore, we have developed TMZ resistant RC6 rat glioma cell line with altered antioxidant capacity and high invasion potential. CoQ10 in combination with TMZ exerted a synergistic effect additionally confirmed in a 3D model of microfluidic devices. Co-treatment with TMZ increased expression of mitochondrial antioxidant enzymes in RC6 cells. The anti-invasive potential was studied by gelatin degradation and 3D spheroid invasion assays. Inhibition of MMP9 gene expression as well as decreased N-cadherin and vimentin protein expression implied that CoQ10 can suppress invasiveness and the epithelial to mesenchymal transition in RC6 cells. Therefore, CoQ10 supplementation could be used with standard glioma treatment due to its potential to inhibit cancer cells invasion through modulation of the antioxidant capacity.

Pt(IV) cisplatin carrying epigenetically active ligands shows enhanced anti-cancer activity in vitro and in vivo

Poetsch, I.[1, 2, 3], Klose, M.[1, 3], Kowol, C.[1, 3], Berger, W.[2,3], Keppler, B.[1, 3], Gibson, D.[4], Heffeter, P.[2, 3]

[1] Institute of Inorganic Chemistry, Faculty of Chemistry University of Vienna, Austria [2] Institute of Cancer Research and Comprehensive Cancer Center, Medical University of Vienna, Austria [3] Research Cluster "Translational Cancer Therapy Research", University of Vienna, Austria [4] Institute for Drug Research, Hebrew University of Jerusalem, Israel

Platinum-based cytotoxic compounds represent a substantial part of anticancer therapies. However, therapy with these compounds is characterized by frequent occurrence of resistance and severe adverse effects thereby limiting efficient chemotherapy. To overcome these drawbacks, kinetically inert Pt(IV) prodrugs were developed that are activated by reduction in the hypoxic conditions of malignant tissue. The axial ligands of Pt(IV) prodrugs provide a broad range of possibilities to combine individual drugs in a single prodrug that targets cancer cells. [1]

The synergism of chemotherapy and epigenetically active anticancer compounds has been demonstrated in various studies. [2], [3] Here, we investigated the anti-cancer activity of two Pt(IV) compounds carrying the histone deacetylase (HDAC) inhibitor 4-phenylbutyrate (PhB) as the axial ligand. Preliminary in vitro data showed enhanced cytotoxicity of the cisplatin derivatives ctc-$[Pt(NH_3)_2(PhB)(OH)Cl_2]$ (1) and ctc-$[Pt(NH_3)_2(PhB)_2Cl_2]$ (2) compared to cisplatin. [4] Moreover, compound 2 showed significantly enhanced anti-proliferative activity in mice bearing melanoma or colon tumors. In addition, organ distribution of 2 was measured by ICP-MS 24 h after drug application. In conclusion, we provide evidence that conjugation of 4-phenylbutyrate to Pt(IV) cisplatin results in superior anti-cancer activity in vitro and in vivo. Further studies are planned to understand the mechanisms underlying this enhanced cytotoxic combinatory effect.

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The role of MAP kinase signalling in the paraptotic cell death induced by thiosemicarbazones

Hager, S[1,2], Korbula K[1,2], Grusch, M[1], Pirker, C[1], Nowikovsky K[3], Pape, VFS[5,6], Mohr, T[1], Szakacs, G[1,6], Keppler, BK[2,4], Berger, W[1,2], Kowol, CR[2,4], Heffeter, P[1,2]

[1] Institute of Cancer Research and Comprehensive Cancer Center, Department of Internal Medicine I, Medical University Vienna, Austria [2] Research Cluster "Translational Cancer Therapy Research", Vienna, Austria [3] Department of Internal Medicine I and Comprehensive Cancer Center, Medical University Vienna, Austria [4] Institute of Inorganic Chemistry, Faculty of Chemistry, University of Vienna, Austria [5] Department of Physiology, Faculty of Medicine, Semmelweis University, Budapest, Hungary [6] Institute of Enzymology, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary

Thiosemicarbazones (TSCs), especially α-*N*-heterocyclic TSCs, have long been known for their anticancer activity. Triapine is one of the best known TSC for anticancer therapy. Multiple clinical phase I and II trials revealed that Triapine is mainly effective against hematologic malignancies but not against solid tumors. Underlying reasons may be inappropriate drug delivery into tumor tissue, fast excretion or intrinsic/acquired drug resistance. To improve this situation, novel terminally substituted TSCs, such as DpC (in clinical development), Dp44mT and Me_2NNMe_2 are currently studied with increasing interest as they show highly improved (nanomolar) anticancer activity compared to terminally unsubstituted compounds such as Triapine.

Therefore, the aim of this study was to investigate the differences in activity and cell death induction of Triapine and the nanomolar active TSC Me_2NNMe_2. Interestingly, the appearance of vacuoles in the endoplasmic reticulum as well as mitochondrial dilation was found to be characteristic for Me_2NNMe_2. These morphological changes as well as the independence of cell death from caspases suggested induction of an alternate cell death pathway, called paraptosis, which is often associated with MAPK pathway deregulation. Subsequent analyses of pharmacologic and siRNA-mediated inhibition of MEK as well as whole genome gene expression data showed the importance of especially MEK1/2 in the signalling cascade preceding Me_2NNMe_2-induced paraptosis.

In conclusion, the terminally substituted nanomolar active TSC Me_2NNMe_2 induces paraptosis involving the MAPK pathway. This is of interest, as paraptosis is caspase-independent and, therefore, promising for anticancer therapy against apoptosis-resistant tumors.

Impact of linker modification on the in vitro and in vivo anticancer activity of novel albumin-targeting platinum(IV) drugs

Schueffl, H[1], Mayr, J[2], Groza, D[1], Galvez, L[3], Lahnsteiner, M[2], Koellensperger, G[3], Berger, W[1,4], Keppler, BK[2,4], Kowol, CR[2,4], Heffeter, P[1,4]

[1] Medical University of Vienna, Institute of Cancer Research, Vienna, Austria [2] University of Vienna, Institute of Inorganic Chemistry, Vienna, Austria [3] University of Vienna, Institute of Analytical Chemistry, Vienna, Austria [4] Research Cluster "Translational Cancer Therapy Research", Vienna, Austria

Cancer treatment is often associated with serious side effects due to lack of tumor specificity. To enhance the drug delivery to the malignant tissue, albumin is a promising drug carrier. Thus, one research focus of the Research Cluster "Translational Cancer Therapy Research" in Vienna is the development of the platinum(IV) derivatives containing a maleimide moiety, which is reacting with the thiol group of albumin. Upon tumor-specific drug delivery the compounds are activated by reduction leading to the release of clinically approved platinum drugs (e.g. oxaliplatin). Aim of this study was to investigate the impact of different linkers on the anticancer activity of oxaliplatin-containing platinum(IV) complexes in cell culture as well as in vivo and to test the albumin uptake ability of the cancer cells. To this end, as a first step the albumin uptake kinetic of several murine cancer cell models was established by flow cytometry (using FITC-labeled albumin). Long- and short-term anticancer activity was determined in vitro by clonogenic and MTT assay, respectively. To assess the in vivo anticancer activity Balb/c mice with subcutaneous CT-26 tumors were used. These tests revealed that while the cell culture behavior of the compounds was rather similar, distinct differences in the in vivo anticancer activity were observed. Overall, this indicates that the linker system has to be carefully selected and evaluated in order to find the ideal drug candidate.

WG 4

Evaluation of anticancer compounds activity and toxicity in zebrafish model

Dinić, J[1], Stepanović, M[1], Divac Rankov, A[2], Podolski-Renić, A[1], Stanković, T[1], Dragoj, M[1], Jovanović, M[1], Lazić, K[1], Dimas, K[3], Botta, M[4], Pešić, M[1].

[1]Department of Neurobiology, Institute for Biological Research "Siniša Stanković", University of Belgrade, Serbia [2]Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Serbia [3]Department of Pharmacology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa, Greece [4]Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Italy

Zebrafish (*Danio rerio*) is an excellent model for studying toxicity and biological activities of novel compounds with anticancer potential. This model is widely utilized in biological research as it is comparable to human counterpart both molecularly and pathologically. As an *in vivo* system for toxicology, zebrafish has numerous advantages such as rapid and *ex utero* development, transparent embryos in early stages, high fecundity allowing high-throughput screening and cost effectiveness. Furthermore, evaluation of known toxic compounds in zebrafish revealed 63–100% predictability making zebrafish a very useful tool for studying toxic effects [1, 2]. In addition, embryonic zebrafish cancer models can be used for studying pathways and processes relevant to human malignancy including tumor-induced angiogenesis, tumor invasiveness, proliferation and migration. These models can be generated using transgenesis, gene inactivation, xenotransplantation, and cancerogenic induction. Herein, we present the results obtained in zebrafish toxicity studies of siramesine, a sigma receptor agonist with anticancer potential. Concentration dependent increase in lethality, induced by siramesine treatment, was observed in zebrafish embryos at 24 h post fertilization (hpf), 48 hpf and 72 hpf. Various concentration dependent toxic effects on embryo development were also observed, as well as decreased hatching rate in embryos treated with 5 μM and 10 μM siramesine at 72 hpf. Results obtained in zebrafish cancer model generated via xenotransplantation are also presented. This model was utilized to study the effect of Src tyrosine kinase inhibitor pro-LDS10 on the invasiveness of microinjected human glioblastoma cell line U87. Treatment with 5 μM pro-LDS10 resulted in significant reduction of U87 migratory potential at 4 days post injection.

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In vitro and *in vivo* approaches for non-clinical safety assessment: emphasis on hepatotoxicity

Aluani Denitsa and Tzankova Virginia

Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy, Medical University – Sofia, Bulgaria

Liver toxicity is one of the leading cause of drug withdrawals from the market. Iproniazid (monoamine oxidase inhibitor), troglitazone (anti-diabetic drug), and bromfenac and Benoxaprofen (non-steroid anti-inflammatory drug, NSAID) are in the long list of drugs, withdrawn from the market, because of idiosyncratic liver injury. However, at present liver safety does not form part of the core battery of pre-clinical tests required for initial safety pharmacology from regulatory bodies. EMEA have published draft guidance on the non-clinical assessment of hepatotoxic potential, but no regulations are set in place yet. Currently, liver toxicity screening during both the pre-clinical in vitro and in vivo testing and clinical phases of the development process forms the basis of hepatic safety testing. Here, we present some methodologies applicable to the early assessment of potential intrinsic hepatotoxicity of new drug molecules. The presentation will focus on *in vitro* and *in vivo* methods for evaluation of liver toxicity of newly developed nano-sized drug-delivery systems. An important goal of research into this field is to establish adequate *in vitro* / *in vivo* models that are valid and able to predict drug induced liver toxicity during lead optimization, before any hepatotoxic molecule under development unnecessarily progresses into clinical studies.

IN VITRO AND IN VIVO SAFETY PROFILING OF NOVEL COMPOUNDS WITH RESPECT TO RENAL FUNCTION

Luca, A[1], Alexa-Stratulat, T[1], Vochița, G[2], Mihai, CT[2]

[1] "Grigore T. Popa" Medicine and Pharmacy University, Romania [2] Institute of Biological Research Iași, branch of NIRDBS, Romania

Advancements in oncology treatment along with an increase in the number of drugs administered in association, especially in multi-drug resistant tumour management lead to cumulative side-effects. Moreover, new compounds or novel associations require rigorous safety profiling in both in vitro [1] and in vivo testing [2]. Besides cardio toxicity and hepatic toxicity questions arise about the effects on the renal function [3,4] of every new compound. The development of new compounds with anti-tumour proprieties is more difficult due to time consuming evaluation methods through the in vitro in vivo toxicity cycle testing. Addressing this issue is the need for more reliable assays which could predict more accurate the safety profile of a new substance with specific markers for the site of toxicity evaluation. In the following paper we propose to discuss the main techniques used to assess the safety in general and especially renal safety of new possible therapeutic agents with promising use in clinical oncology.

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