



Chemical Approaches to Targeting Drug Resistance in Cancer Stem Cells

COST Action CM1106

1st Working Group Meeting

IPATIMUP

(Institute of Molecular Pathology and Immunology of the University of Porto)

Rua Dr Roberto Frias s/n

Porto, Portugal

21 – 22 February 2013



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From Memorandum of Understanding:

This COST Action aims to unite researchers with expertise in rational drug design and the medicinal chemistry of synthetic and natural compounds with biomedical investigators dedicated to the understanding the mechanisms governing drug resistance in cancer stem cells. Through exchange of information, experience and expertise, researcher mobility and fostering new collaboration between chemistry and biology groups, the Action endeavours to develop new, effective methods for identifying novel compounds and drug candidates that target drug-resistant cancer stem cells.



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The Institute of Molecular Pathology and Immunology of the University of Porto (**IPATIMUP**) is a private non-profit association of public utility, established in 1989 under the aegis of the University of Porto. IPATIMUP is an Associated Laboratory of the Ministry of Science and Higher Education since 2000.

IPATIMUP aims to understand the causes and evolution of human oncologic diseases to go forward in early diagnosis, maximize treatment efficiency, improve the quality of patients' life and reduce cancer incidence in the population.

IPATIMUP strands of activity are: Research on Oncology and Population Genetics, through the interaction of several scientific domains (Medicine, Biology, Genetics, Pharmacy and Biophysics); Development of human resources specialized in Oncology and Oncobiology; Diffusion of Science, thus contributing to the population's scientific culture; Specialized diagnostic and consultation services on cancer issues.

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21st February 2013

- 08h30** **Registration**
- 09h15** **Introduction: Gabriela Almeida** (IPATIMUP, Portugal)
Daniele Passarella (Università degli Studi di Milano, Italy)
- Section I:** **Chairman: James Lorens** (University of Bergen, Norway)
- 09h30** **Christel Herold-Mende** Div. Exp. Neurosurgery, Univ. of Heidelberg, Germany
Aberrant self-renewal and quiescence contribute to glioblastoma aggressiveness
- 09h50** **Panagiota Sotiropoulou** IRIBHM, Université Libre de Bruxelles, Belgium
Differential resistance of distinct types of stem cells to DNA damage
- 10h10** **Magdalena Król** Warsaw University of Life Sciences - WULS, Poland
mRNA and microRNA expression profiling of canine mammary carcinoma cell lines and tumor-associated macrophages grown as a co-culture in vitro
- 10h30** **Richard Clarkson** Cardiff University School of Biosciences, UK – **Invited Expert**
TRAIL mediated targeting of breast cancer stem cells in a model of acquired drug resistance
- 10h50** **Coffee break**
- 11h15** **Welcome: Manuel Sobrinho Simões** (IPATIMUP, Portugal)
- 11h20** **M. Angela Nieto** Instituto de Neurociencias CSIC-UMH , Spain - **Invited Expert**
The reversibility of the EMT in development and cancer progression
- 12h05** **James Lorens** University of Bergen, Norway
Axl receptor signaling is required for EMT-associated stem cell and malignant traits in cancer
- 12h25** **Joana Paredes** IPATIMUP and FMUP, University of Porto, Portugal
P-cadherin: a stem cell marker overexpressed in high-grade breast carcinomas
- 12h45** **Lunch and Poster Session I**
- Section II:** **Chairman: Danijel Kikelj** (University of Ljubljana, Slovenia)
- 14h45** **Antonello Mai** Università “La Sapienza” di Roma, Italy - **Invited expert**
Assessing Sirtuins as Drug Targets in Human Illnesses
- 15h30** **Ruta Navakauskiene** Institute of Biochemistry, Vilnius University, Lithuania
Effects of HDACI, HMTI and HMTI in combination with retinoic acid on granulocytic differentiation of human promyelocytic leukemia cells
- 15h50** **Annalisse Cassar** University of Malta, Malta
Effects of Insect Conditioned Medium in Combination with Chromatin-Modifying Agents on the Terminal Differentiation of Leukaemia
- 16h10** **Coffee break and Poster Session I**
- 16h45 – 18h00** **MC meeting:** *Only for the members of the Management Committee*
- 19h15** **Bus departure from Conference Hotels to Conference Dinner**
- 20h30** **BD sponsored Conference Dinner – Caves Taylor (Port Wine Cellars)**

22nd February 2013

Section III: Chairman: Daniele Passarella (Università degli Studi di Milano, Italy)

- 09h15** **Nadine Martinet** Institute of chemistry, Nice, France - **Invited Expert**
The essential epigenetic Compound library
- 10h00** **Bruno Botta** Università "La Sapienza" di Roma, Italy
Natural lead products inhibit the Hedgehog signaling pathway in medulloblastoma
- 10h20** **Ferenc Hudecz** Eötvös Lorand University, Budapest, Hungary
Oligopeptide bioconjugates: synthesis, characterization and use for antitumour drug targeting
- 10h45** **Coffee break**
- 11h20** **M. Helena Vasconcelos** FFUP and IPATIMUP, Porto, Portugal
A new small molecule with antitumour and chemosensitizing potential as P-gp inhibitor
- 11h40** **Attila Hunyadi** Institute of Pharmacognosy, University of Szeged, Hungary
Activity of ecdysteroid and protoflavone derivatives on MDR cancer cells
- 12h00** **Graça Soveral** Faculty of Pharmacy, University of Lisbon, Portugal
Aquaporin channels as targets for cancer therapy
- 12h20** **Lunch and Poster Session II**

Section IV: Chairman: Maurizio Botta (Università degli Studi di Siena, Italy)

- 14h20** **Carlo Unverzagt** Universität Bayreuth, Bayreuth, Germany
Carbohydrates on and around cancer stem cells
- 14h40** **Juraj Kóňa** Inst. of Chemistry, Slovak Acad. Sciences, Bratislava, Slovak Republic
How to improve predictive accuracy of a QSAR model for the design of inhibitors of sugar processing enzymes with transition metal ion co-factors
- 15h00** **Rosanna Dono** Aix-Marseille University - IBDML, Marseille, France
Forcing cancer stem cells to behave: GLYPICAN4 as potential molecular target to trigger cancer stem cell differentiation
- 15h20** **Presentation of Selected Posters** (5 presentations, 5 min each)
- 15h50** **Coffee break and Poster Session II**
- 16h20** **Round Table Discussions:** Chairmen: Daniele Passarella, James Lorens and Wolfgang Link
High throughput screening for novel CSC targeted molecules
- 18h00** **Closing Remarks**

Poster Session I

P1. Agata Homa (Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences-WULS, Warsaw, Poland) *miRNA expression in canine mammary cancer stem cells*

P2. Ana Čipak Gašparović (Rudjer Boskovic Institute, Zagreb, Croatia) *Oxidative modifications of extracellular matrix change Hedgehog signaling pathway*

P3. Ana Mitanoska (Faculty of Natural and Technical Science, University Goce Delcev - Stip, Stip, Republic of Macedonia) *Developing of inducible cell lines for study gene gain of function*

P4. Andreani Odysseos (EPOS-lasis, R&D and University of Cyprus, Cyprus) *Quantitative phosphoproteomics dissects sensitization of EGFR-resistant colon cancer cells to Tyrosine Kinase Inhibitors through Spliceosome Modulation*

P5. Bernd Groner (Georg Speyer Haus, Institute for Biomedical Research, Frankfurt am Main, Germany) *The functional interplay between activated Stat5 and Stat3 in breast cancer formation and progression and the therapeutic effects of targeted inhibition*

P6. Daniele Lo Re (School of Chemistry, National University of Ireland Galway, Galway, Ireland) *Synthesis of Multi Drug Resistance modulators based on jatrophone framework*

P7. Danijel Kikelj (University of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia) *Dual antithrombotic compounds with antiangiogenic activity as potential approach to targeting cancer stem cells*

P8. Engin Ulukaya (Uludag University, Medical School, Department of Medical Biochemistry, Bursa, Turkey) *Differential Cytotoxic Activity of a Novel Palladium-Based Compound on Prostate Cell Lines, Primary Prostate Epithelial Cells and Prostate Stem Cells*

P9. Flavio Maina (Aix-Marseille University – Developmental Biology Institute of Marseille-Luminy (IBDML) – CNRS UMR7288, Marseille, France) *Abl acts as a “signalling node” for maintenance, propagation, and motility of glioblastoma cells*

P10. Giovanna Damia (Department of Oncology, Istituto di Ricerche Farmacologiche “Mario Negri”, Milan, Italy) *ALDH enzymatic activity and CD133 positivity in ovarian cancer patients*

P11. Ioannis S. Vizirianakis (Laboratory of Pharmacology, Department of Pharmaceutical Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece) *Development of a novel mesoporous carbon drug delivery system: Characterisation and cytocompatibility studies*

P12. Juraj Dobiáš (Comenius University, Faculty of Natural Sciences, Dep. of Organic Chemistry, Bratislava) *TKI Inhibitors in Targeting Cancer Stem Cells – Development of New KDR Inhibitor*

P13. Katarzyna Wnuk-Lipinska (University of Bergen, Bergen, Norway) *Targeting cancer stem cells with an Axl inhibitor*

P14. Lidija Milkovic (Rudjer Boskovic Institute, Zagreb, Croatia) *Cell type-dependent response to chemotherapy treatment with doxorubicin in breast cancer*

P15. Patrícia Oliveira (Institute of Molecular Pathology and Immunology of the University of Porto, IPATIMUP, Porto, Portugal) *Using an in vitro model of Epithelial-Mesenchymal-Epithelial transitions to uncover novel biological mechanisms*

P16. Sabrina Dallavalle (Department of Food, Environmental and Nutritional Sciences, Università di Milano, Milano, Italy) *New captothecin-linked platinum anticancer agents*

P17. Sara Toscano (Dipartimento di Chimica e Tecnologie del Farmaco, Università "La Sapienza" di Roma; IIT Center for Life Nano Sciences, CLNS@Sapienza, Roma, Italy) *Natural lead products inhibit the Hedgehog signaling pathway in medulloblastoma*

P18. Veronika Borutinskaite (Department of Molecular Cell Biology, Institute of Biochemistry, Vilnius University, Vilnius, Lithuania) *Studies of the DNA methyltransferases inhibitor EGCG effects on human leukemia cell proliferation and differentiation*

Poster Session II

P1. Ana Martins (University of Szeged, Szeged, Hungary and Universidade Nova de Lisboa, Lisbon, Portugal) *Novel ecdysteroids as modulators of multi-drug resistance*

P2. Andreia Sousa (Institute of Molecular Pathology and Immunology of the University of Porto, IPATIMUP, Porto, Portugal) *MUC1: uncovering a new target for pancreatic cancer therapy*

P3. Anna Lucia Fallacara (Dipartimento di Biotecnologie, Chimica e Farmacia, University of Siena, Italy) *Design, Synthesis and Biological Evaluation of Pyrazolo[3,4-d]pyrimidines Active in vivo on Bcr-Abl mutant*

P4. Bruno Pereira (Institute of Molecular Pathology and Immunology of the University of Porto, IPATIMUP, Porto, Portugal) *CDX2 regulation by the RNA-binding protein MEX3a: impact on intestinal differentiation and stemness*

P5. Constantinos M. Athanassopoulos (Laboratory of Synthetic Organic Chemistry, Department of Chemistry, University of Patras, Patras, Greece) *Total Synthesis of Acidic Retinoids: A Library of Potential Cancer Stem Cell Regulators*

P6. Daniela P. Freitas (Institute of Molecular Pathology and Immunology of the University of Porto, IPATIMUP, Porto, Portugal) *Drug-induced enrichment of putative cancer stem-like cells for the identification of potential therapeutic targets to overcome their chemoresistance*

P7. Darko Bosnakovski (Faculty of Medical Sciences, University Goce Delcev - Stip, Stip, Republic of Macedonia) *Stem cell based high-throughput screen to identify inhibitors of DUX4*

P8. Dušan Sladić (University of Belgrade – Faculty of Chemistry, Belgrade, Serbia) *Anti-metastatic and anti-angiogenic properties of potential new anti-cancer drugs based on metal complexes of selenosemicarbazones*

P9. Emmanuel N. Pitsinos (Laboratory of Natural Products Synthesis & Bioorganic Chemistry, NCSR “DEMOKRITOS”, Athens, Greece) *Synthesis of Taepeenin D analogues as potential cancer stem cell-targeted agents*

P10. Gilles Hanquet (Laboratoire de Stéréochimie associé au CNRS, ECPM, Université de Strasbourg, Strasbourg, France) *A Straightforward access to an advanced precursor of Triptolide and analogues*

P11. José M. Padrón (BioLab, Instituto Universitario de Bio-Orgánica "Antonio González", Universidad de La Laguna, La Laguna, Tenerife, Spain) *Mechanism of action the novel tubulin destabilizing agent DTA0100*

P12. Katarina Anđelković (University of Belgrade – Faculty of Chemistry, Belgrade, Serbia) *Synthesis, characterization, cytotoxic and antioxidative activity of d-metal complexes with 2,6-diacetylpyridine bis(selenosemicarbazone)*

P13. Maria M. M. Santos (Research Institute for Medicines and Pharmaceutical Sciences, Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal) *Targeting the wt p53: synthesis of a new family of modulators*

P14. Mark P. Farrell (School of Chemistry, National University of Ireland, Galway, Ireland) *Anomerisation of Glycosidic linkages using Titanium(IV) Chloride*

P15. Michael Christodoulou (Dipartimento di Chimica, Università degli Studi di Milano, Milano, Italy) *Camptothecin scaffold modification: synthesis and biology*

P16. Mehdi Ghassemi (Bilkent University, Ankara, Turkey) *In silico prediction and in vitro validation of biomarkers for chemosensitivity to src inhibitors in melanoma cell lines*

P17. Stella Borrelli (Università degli Studi di Milano, Dipartimento di Chimica, Milano, Italy) *Novel nanoassemblies of known anticancer compounds by coupling with squalene moiety*

P18. Wolfgang Link (Regenerative Medicine Program, Department of Biomedical Sciences and Medicine, University of Algarve, Faro, Portugal) *Learning old tricks from viruses to fight cancer*

**ORAL
PRESENTATIONS**

Aberrant Self-Renewal and Quiescence Contribute to Glioblastoma Aggressiveness

Christel Herold-Mende

Division of Experimental Neurosurgery, University of Heidelberg, INF 400, 69120 Heidelberg

Stem-like tumor cells endowed with enhanced self-renewal capacity are believed to drive tumor growth in malignant gliomas. So far a variety of surrogate markers has been proposed to characterize and enrich these cells emphasizing the need of devising new isolation methods based on common functional and phenotypic criteria.

In this study we made use of a collagen-based self-renewal assay, to screen for clonogenic cell subpopulation in malignant gliomas. Cells were profiled using a gene expression chip and tested for tumor formation capacity in an orthotopic mouse model. Label retention was used to detect quiescent tumor cells.

In a panel of glioblastoma cell lines (n=21) we identified several cell lines enriched for cells with enhanced self renewal capacity. These cell lines were capable of matrix-independent growth and formed fast-growing, orthotopic tumors in mice. Employing isolation and replating techniques, we could further show that these cells invariably re-established a cellular hierarchy through a series of asymmetric cell divisions. However, the ratio of symmetric to asymmetric cell divisions seemed to be pathologically increased and was linked to idiosyncratic transcriptomal changes as well as to poor overall survival of corresponding patients. Finally, through label-retention experiments we further identified a subpopulation of quiescent and chemo-resistant cells, which retained the ability to reinitiate growth of secondary cell clones and thus, might play a role in tumor recurrence after therapy.

Conclusions: Altogether, our results suggest tumor quiescence and aberrant proliferations influence the aggressiveness of glioblastoma.

Differential resistance of distinct types of stem cells to DNA damage

Peggy Sotiropoulou

IRIBHM, Université Libre de Bruxelles

The accurate maintenance of genomic integrity is essential for tissue homeostasis and its deregulation leads to cancer and ageing. We used as a model the skin epidermis, which is maintained by distinct pools of stem cells (SCs). Upon irradiation-induced DNA damage, the hair follicle bulge SCs are profoundly resistant to irradiation-induced cell death due to high expression of the anti-apoptotic Bcl2, and more efficient non-homologous end-joining repair mechanism. On the other hand, conditional deletion in the epidermis of *Brca1*, crucial for DNA repair and critical mediator of the choice of the repair pathway, resulted in specific loss of the hair follicle bulge SCs, while the rest types of epidermal SCs remained unaffected.

Enhanced DNA repair and resistance to apoptosis have been shown for several types of cancer SCs, albeit usually in vitro, and for this reason cancer SCs have been accused for tumour relapse after therapy. Using genetic lineage-tracing in squamous cell carcinoma, we showed that benign papillomas exhibit two distinct proliferative cell compartments, mirroring the normal tissue hierarchy, while invasive malignant carcinomas grow with geometric expansion of a single cancer SC population. Further studies following the fate of cancer SCs upon treatment and tumour relapse are needed in order to show the response of cancer SCs to therapy and to design successful anti-cancer treatments.

mRNA and microRNA expression profiling of canine mammary carcinoma cell lines and tumor-associated macrophages grown as a co-culture *in vitro*

**Magdalena Król¹, Karol Pawłowski², Kinga Majchrzak³, Alicja Majewska¹,
Tomasz Motyl¹**

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Solid tumors comprise various cells, including cancer cells, resident stromal cells, migratory haemopoietic cells and other. These cells regulate tumor growth and metastasis. Macrophages constitute probably the most important element of all interactions within the tumor microenvironment. However, the molecular mechanism, that guides tumor environment, still remains unknown. Exploring the underlying molecular mechanisms that orchestrate these phenomena has been the aim of our study.

The co-cultures of five various canine mammary carcinoma cell lines and macrophages were established and maintained for 72 hrs. After that, having sorted the cancer cells, an integrated analysis of genome-wide mRNA and microRNA expression profiles have been assessed.

The analysis showed that the up-regulated genes in the cancer cell lines are involved mainly in: macrophages activation, cell motion, mammary gland development, cell-cell adhesion, and angiogenesis. The presence of macrophages in the cancer environment induces acquisition of the macrophage antigens and phenotype in cancer cells.

The findings of miRNA expression are in accordance with results obtained at mRNA level.

Our analysis identified a number of pathways and processes that are up-regulated in cancer cells by the presence of macrophages. Taken together, this integrated comparative study generated a sketch of the molecular changes that occur during interactions between macrophage and cancer. Because as far as we realize this is the first analysis of changes in miRNA expression in cancer cells due to a presence of macrophages, our study can serve as a valuable source for future studies on a tumor microenvironment, and some of the highlighted genes, miRNAs, pathways or processes may be useful for diagnostic or therapeutic purposes in the future.

This work was supported by grant no N N308012939 from the Ministry of Sciences and Higher Education.

TRAIL mediated targeting of breast cancer stem cells in a model of acquired drug resistance

Luke Piggott¹, Julia Gee², Rhiannon French¹ and **Richard Clarkson**¹

Schools of ¹Biosciences and ²Pharmacy, University of Cardiff, Cardiff, Wales.

TRAIL is a naturally occurring peptide that selectively induces apoptosis in tumour cells. TRAIL analogues/agonists are undergoing clinical trials for a number of cancer types and the current indications are that these agents have relatively poor overall clinical efficacy, but with a minority of patients experiencing marked clinical improvement. The general consensus is therefore that TRAIL could be of benefit if appropriately targeted to responsive disease sub-groups.

It is widely accepted that breast cancers are refractory to TRAIL cytotoxicity and thus breast cancer patients are not regarded as a target group for this new therapeutic. We have investigated the underlying causes of this TRAIL resistance in breast cancer cells and have found that the endogenous TRAIL inhibitor c-FLIP is responsible for this resistance. In cell-line based work, suppression of c-FLIP by RNAi sensitized all sub-types of breast cancer tested to TRAIL killing, and selectively eliminated cancer stem cell-like activity (determined by tumoursphere culture and tumour initiation studies in xenografts) from the tumour cell populations. This has important implications for long-term therapy.

Furthermore we have shown that breast cancer cells that have acquired resistance to anti-hormone agents (eg. Tamoxifen, Faslodex) gain an inherent susceptibility to TRAIL alone, and that this correlates with a reduction in levels and/or subcellular redistribution of c-FLIP in the cytosol of tumour cells. Once again, cancer stem cell-like activity was eliminated from these populations and systemic TRAIL treatment of mice bearing tumours with acquired endocrine resistance displayed a profound and persistent tumour relapse. We are currently investigating whether this therapeutic effect can be recapitulated in ex vivo primary breast tumours derived from patients who have relapsed on endocrine therapy, and whether c-FLIP could be used as a biomarker of this TRAIL-responsive disease group.

These studies suggest that TRAIL might in the future be indicated for ER positive breast cancer patients with relapsed disease. We also propose c-FLIP as a new drug target and potential biomarker for breast cancer.

The reversibility of the EMT in development and cancer Progression

M. Angela Nieto

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The epithelial to mesenchymal transition (EMT) is required in the embryo for the formation of tissues which cells originate far from their final destination. This programme endows cells with migratory and invasive properties. Interestingly, the reverse process, known as mesenchymal to epithelial transition (MET), is also essential in embryogenesis to allow the differentiation of tissues and organs once the embryonic migratory cells reach their final destination. The pathological activation of the EMT programme in the adult promotes tumour progression and organ fibrosis and recent findings indicate that the EMT can also confer stem cell properties. Similar to the situation in embryos, it has been suggested that a reversion of the EMT (MET) might also be necessary for metastatic colonization once malignant cells extravasate and find their niche in distant organs.

The main inducers of the EMT are transcription factors of the Snail, Zeb and Twist families (EMT-TFs). Recently, we have identified a novel EMT inducer expressed in a subset of lateral plate mesodermal (lpm) cells devoid of Snail1 and Snail2, the main EMT-TFs expressed during early mesoderm development. Gain of function experiments in zebrafish embryos show a dramatic phenotype, whereby lpm cells become invasive and violate the embryonic boundaries to enter the extraembryonic tissues. By contrast, loss of function experiments prevent the migration of the mesodermal cells, failing to colonize their normal territory. Silencing experiments in mesenchymal cancer cells induce a full MET. I will discuss that MET is required for cancer cells to metastasize in vivo, concomitant with the acquisition of stem cell properties. These findings have important implications both in terms of the differentiation of embryonic stem cells, reprogramming and metastatic colonization.

Axl receptor signaling is required for EMT-associated stem cell and malignant traits in cancer

James Lorens

Department of Biomedicine, University of Bergen, Bergen, Norway

Epithelial-to-mesenchymal transition (EMT) endows carcinoma cells with migratory, survival and stem cell-like attributes that facilitate therapeutic resistance and metastasis. Expression of the Axl receptor tyrosine kinase in aggressive cancers correlates with EMT, drug resistance, metastasis and poor patient survival. Induction of EMT in immortalized mammary epithelial cells by EMT-transcription factors, TGFbeta or hypoxia upregulates Axl and establishes an autocrine-signaling loop with its ligand, Gas6. Axl receptor signaling is required to maintain EMT-related invasive, drug resistance and cancer stem cell (CSC) traits of malignant breast cancer cells. Targeting Axl signaling with RNAi or pharmacological agents blocks the EMT/CSC gene program and inhibits malignant functions including invasiveness, drug resistance, mammosphere formation, in vivo tumor initiation, and spontaneous metastasis in orthotopic breast cancer models. These results suggest that Axl expression is requisite for the maintenance of cancer stem cell-like traits during malignant progression. The EMT program is characteristic of normal adult mammary epithelial stem/progenitor cells. Congruently, we show that the Axl receptor expressed on mammary epithelial stem/progenitor cells, and Axl signaling is necessary for mammary epithelial multipotent progenitor activity. Thus Axl receptor signaling represents a novel regulatory pathway linking normal mammary stem/progenitor cells and breast cancer stem cells. Clinical Axl inhibitors represent a novel therapeutic avenue to target EMT/CSC traits of aggressive breast cancer.

P-cadherin: a stem cell marker overexpressed in high-grade breast carcinomas

Ana Sofia Ribeiro¹, André Filipe Vieira¹, Bárbara Sousa¹, Ana Rita Nobre¹, André Albergaria^{1,2}, Raquel Seruca^{1,2}, Fernando Schmitt^{1,2}, **Joana Paredes**^{1,2}

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P-cadherin is a cell-cell adhesion molecule, whose expression is highly associated with undifferentiated cells in normal adult epithelial tissues. In fact, its expression has been already reported in human embryonic stem cells and it is presumed to be a marker of stem or progenitor cells of some epithelial tissues [1,2].

In breast cancer, our group has found that P-cadherin is frequently overexpressed in high-grade tumors, being a well-established indicator of poor patient prognosis [3,4,5]. Additionally, it has been shown as an important inducer of cancer cell migration and invasion, with underlying molecular mechanisms involving the signaling mediated by its juxtamembrane domain, the secretion of matrix metalloproteases to the extracellular media, and the cleavage of a P-cadherin soluble form with pro-invasive activity [6,7]. Intracellularly, we found that this protein interferes with the endogenous cadherin/catenin complex, inducing p120-catenin delocalization to the cytoplasm, and the consequent activation of Rac1/Cdc42 and associated alterations in the actin cytoskeleton [8]. Furthermore, our recent data supports the idea that P-cadherin confers stem cell properties to breast cancer cells, surviving in anchorage independent conditions and resisting to radiotherapy [9]. This association between P-cadherin and signalling involved in invasion, stemness and survival were validated by microarrays [8], turning this protein as a putative therapeutic target for invasive carcinomas overexpressing this protein.

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Assessing Sirtuins as Drug Targets in Human Illnesses

Antonello Mai

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NAD⁺-dependent histone deacetylases (sirtuins, SIRT1-7) have emerged as potential therapeutic targets for treatment of human illnesses such as cancer, metabolic, cardiovascular and neurodegenerative diseases. In our lab, several chemically different series of sirtuin inhibitors (SIRTi) have been identified and will be further developed.

The first series comprises some sirtinol analogues, obtained by replacement of the benzamide linkage of the prototype with other bioisosteric groups. Such compounds showed higher apoptosis induction and/or higher cytodifferentiation than sirtinol in human leukemia U937 cells. One of them, salermide, was well tolerated by mice, prompted tumor-specific apoptosis and antiproliferative effects in human cancer cells. Starting from cambinol, a SIRTi reported as highly active against the Burkitt lymphoma, we designed some (thio)barbituric acid analogues (BDF4s) related to cambinol to evaluate as SIRTi and anticancer agents. The BDF4 prototype, MC2141, displayed in U937 cells higher apoptosis induction than cambinol and showed antiproliferative effects against a panel of cancer cells. More recently we devoted our attention to AGK-2, a SIRT2-selective inhibitor useful in a Parkinson disease model. We designed and synthesized some AGK-2 analogues, called acylpyrrolyliden-cyanacetamides (APCs) bearing an acylpyrrole moiety instead of the phenylfuran group of AGK-2, and we started to study the effect of substitution at the pyrrole as well as cyano and/or quinoline level on the SIRT inhibiting activity. MC2494, a prototype of the APC series, gave significant apoptosis induction in a series of cancer cells, while AGK-2 failed in the same conditions. Some our SIRTi belonging to different chemical classes and studied as anticancer agents were tested against colon and glioblastoma cancer stem cells (CSCs), obtaining in some cases excellent antiproliferative activity.

In contrast to the number of SIRT inhibitors, only few SIRT1 activators are known. Here, we rationalized the potential of the previously unexplored dihydropyridine scaffold to develop sirtuin ligands by preparing a series of 1,4-dihydropyridine-based (DHP) derivatives. Assessment of their SIRT1-3 deacetylase activities revealed the importance of the substituent at the N1 position of the dihydropyridine structure on sirtuin activity. Introduction of cyclopropyl, phenyl, or phenylethyl groups at N1 conferred non-selective SIRT1 and SIRT2 inhibition activity, while a benzyl group at N1 conferred potent SIRT1 and -2 activation. Senescence assays performed on hMSC, and mitochondrial function studies conducted with murine C2C12 myoblasts confirmed the compounds' novel and unique SIRT-activating properties. One of DHPs, MC2791, tested against colon carcinoma cell lines, showed high antiproliferative activity. To assess the effect of selected DHPs during angiogenesis and wound healing following skin damage, a series of experiments were performed in mice after cutaneous punch-biopsy. In vitro experiments revealed that SIRT activation stimulated proliferation of endothelial cell, keratinocytes and skin fibroblasts paralleled by the induction of eNOS phosphorylation and nitric oxide (NO) production.

Effects of HDACI, HMTI and HMTI in combination with retinoic acid on granulocytic differentiation of human promyelocytic leukemia cells

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Differentiation therapy is the most promising strategy in the haematology oncology. The human leukaemia cell lines have been used repeatedly as a model to study the control mechanisms of proliferation, differentiation and apoptosis. These exponentially growing neoplastic cells can be induced to differentiate into granulocytes by treatment with chemical agents such as all-trans retinoic acid (RA) or its isomer and other agents. In our work we examine the anti-leukemic effects of novel chemical agents such as HDACI (belinostat, phenylbutyrate etc.), DNMTI (RG108, Zebularine, EGCG etc.), HMTI (3-DZNeplanocin A) that are potential for modulating chromatin and genes of myeloid origin in combination with RA. We observed that combined treatment and/or pre-treatment with epigenetic modifiers (HDACI, DNMTI, HMTI) before the induction of differentiation with all-trans retinoic acid leads to more effective and accelerated granulocytic differentiation of leukemic cells. Differentiation level was assessed by NBT reduction and confirmed by the expression of the early myeloid differentiation marker CD11b. It was also demonstrated that combinations of these agents arrests cells in the G₀/G₁ phase of the cell cycle. Terminal granulocytic differentiation of human promyelocytic leukaemia cells is associated with HDAC, HMT, DNMT, C/EBP genes expression modulation. We also demonstrated that changes in histone modifications (histone H4 acetylation, histone H3K4me3) are associated with induction of granulocytic cell differentiation. These results suggest that combination of used agents might have potential in promyelocytic leukaemia differentiation therapy.

Effects of Insect Conditioned Medium in Combination with Chromatin-Modifying Agents on the Terminal Differentiation of Leukaemia

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Leukaemia is the most common type of cancer that occurs in children worldwide. Despite the intense research carried out in several facilities, acute myeloid leukaemia is still considered the highest cause of cancer death in children to young adults with a long-term survival rate of only approximately 10%. The successful use of retinoic acid in the treatment of acute promyelocytic leukaemia has prompted further research of differentiation therapy to include other leukaemia sub-types. The rationale is to force leukaemia cells into entering the apoptotic pathway by undergoing terminal differentiation rather than use cytotoxic drugs to treat this cancer.

Media conditioned from cells of an insect pupa has already been proven to effect the differentiation of the HL60 cell line. Now we aim to expand these results to other leukemia cell lines with the aid of chromatin-modifying agents.

The organic fraction of the insect conditioned medium was used to treat various Leukaemia cell lines including KG-1a, K562 and NB4-R2, with and without histone deacetylase inhibitors and the DNA demethylator 5'Azacytidine.

Differentiation was assessed through reduction of nitroblue tetrazolium and results were normalized using the MTT assay.

Where the insect conditioned medium did not cause a strong differentiation effect, pretreatment with histone deacetylase inhibitors considerably improved the result. This implies that the insect conditioned medium extract has a possible application in the treatment of different leukaemia sub-types other than acute promyelocytic leukaemia. The next step will be to test the insect conditioned medium, and successful combinations with chromatin-modifying agents, on patient primary cells in the hope of formulating a novel drug that will help in the treatment of leukaemia.

The essential epigenetic Compound library

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During Cost TD 0905 'Epigenetic from bench to bedside' some researchers provided published compounds but also new ones. We used them to build a compound library.

In parallel, biologist offered screening for biological activities: HDAC, sirtuin, PRMT, DNA methylation, HAT (pan screening first and then specific screening for the hits). 10 different screens have been achieved. *In silico* screen is also beginning for HDAC, sirtuin and DNA methylation. Some assays were new.

With the first results, we built EITeen:an Initial training network for epigeneticist. 400 compounds were screened and 40 make the essential library with well characterized standards. An example of a new assay for multidrug resistance will be given.

Natural lead products inhibit the Hedgehog signaling pathway in medulloblastoma

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The medulloblastoma is the most frequent malignant brain tumor of childhood, it belongs to the group of embryonal neuroepithelial tumors and arise from stem cells or early progenitor cells in the cerebellum¹. The Hedgehog pathway (Hh) is an essential embryonic signalling cascade that regulates stem-cell and progenitor-cell differentiation in multiple developmental processes. Smoothed homologue (SMO) is a transmembrane protein that activates the downstream Hedgehog signaling pathway. PTCH1 is an inhibitory cell-surface receptor that constitutively suppresses activation of the hedgehog pathway by inhibiting SMO. Hedgehog ligands bind to and inactivate PTCH1, derepressing SMO and promoting pathway activation². The most promising targets of the pathway are the G-protein coupled receptor SMO, which transduces the Hh signal inside the cell, and the effector protein Gli1 which acts downstream of the SMO and promotes the transcription of Hh target genes. In the literature, some SMO antagonists have been described, some of which are currently under clinical investigation, whereas no records on the discovery of Gli1 antagonists are available³. The aim of this project is to discover and develop small organic molecules and/or natural compounds capable of inhibiting the Hh signaling pathway by antagonizing the SMO receptor and/or the Gli1 protein, thereby providing anticancer activity. According to molecular modeling studies the most promising compounds predicted by virtual screening will be tested *in vitro* on various cell-based assays as, for example, in Hh-responsive cell lines and in stem/progenitor cells. The binding properties on the SMO receptor would be investigated by competition assays with Bodipy-cyclopamine, whereas the direct interaction with Gli1 could be studied by biophysical methods. Biological activity data will be used for refining computational models, while the most interesting active hits will be optimized through the rational design and synthesis. The most potent and less toxic compounds will be finally considered as the lead for further and in depth studies such as pre-clinical trials.

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Oligopeptide bioconjugates: synthesis, characterization and use for antitumour drug targeting

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Oligopeptides containing specific recognition unit (e.g. antibody or T-cell binding site, enzyme substrate) or possessing cell penetrating properties (e.g. oligoarginine, penetratin) are frequently utilized to target cell-surface or intracellular binding structures for delivering covalently attached entities (e.g. fluorophore, drug, enzyme modulators). Therefore the appropriate combination of conjugation strategies to merge two components without losing their functional properties is important. Examples will demonstrate the application of different synthetic approaches for construction of oligopeptide conjugates with different partner molecules (e.g. antitumour drugs, like daunomycin, pemetrexed, ferrocene and vinblastin derivatives). The importance of isomerization during derivatization, the selection of the linkage between the components (thioether, disulphide, acid labile amide, etc.) will be discussed in the context of functional properties. The biomedical application of these bioconjugates as potential biological as well as diagnostic tools for identification of novel biomarkers in tumour, infection or metabolic diseases will be outlined.

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A new small molecule with antitumour and chemosensitizing potential as P-gp inhibitor

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P-glycoprotein (P-gp, a drug efflux pump) is often upregulated in cancer stem cells and in drug resistant cells. P-gp is usually localized in the cytoplasmic membrane but can also be found in the nuclear, mitochondria or in other intracellular membranes, indicating that it might be involved in intracellular mechanisms other than drug efflux (reviewed in Palmeira *et al.*, *Curr Med Chem* 2012). We have also seen that P-gp may be transferred via microvesicles between drug-resistant and drug-sensitive cells (unpublished results), which confirms data from other researchers and indicates the influence of this protein in the drug resistant phenotype of tumour cells.

Previous studies from our group have shown that by downregulating the expression of P-gp in a chronic myeloid leukaemia (CML) cell line which overexpresses P-gp (K562Dox), it was possible to increase cellular sensitivity to molecular targeted drugs such as Imatinib (Lima *et al.*, *Cancer Therapy*, 2007). We have also proved that concomitant downregulation of P-gp and XIAP (an antiapoptotic protein) in a drug resistant cell line increased sensitivity to that drug (Seca *et al.*, *Hematology*, 2011). In addition, given our interest in P-gp, we have started collaboration with CEQUIMED-UP and University of Madeira and we have worked towards identifying P-gp inhibitors (Palmeira *et al.*, *Curr Pharm Design*, 2012) which would have concomitant antitumour activity. This collaborative work allowed to find some novel small-molecules with dual activity (anti-Pgp activity and antitumour) (Palmeira *et al.*, *Biochem Pharmacol*, 2012). Nevertheless, at least for some of them, modulation of the activity of other drug efflux pumps, such as MRP and BCRP, was also observed. In addition, interference with CYP34 which was predicted by *in silico* studies, was also confirmed *in vitro* (Palmeira *et al.*, *J Pharm Pharm Sci*, 2012).

These results indicated that some of these molecules may have potential as anticancer drugs. Indeed, we will present unpublished data which indicates that one of the synthesised molecules has a potent antitumour potential, inducing autophagy and apoptosis in melanoma and in non-small cell lung cancer (NSCLC) cell lines. Importantly, this small molecule was found not to be toxic to human lymphocytes (at the GI₅₀ concentration determined for the tumour cell lines) and was shown to significantly reduce the growth of human NSCLC cancer cells in xenograph models in nude mice.

These molecules may be of interest to target cancer stem cells, particularly in the cases with P-gp overexpression.

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Activity of ecdysteroid and protoflavone derivatives on MDR cancer cells

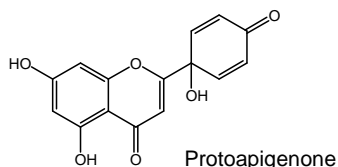
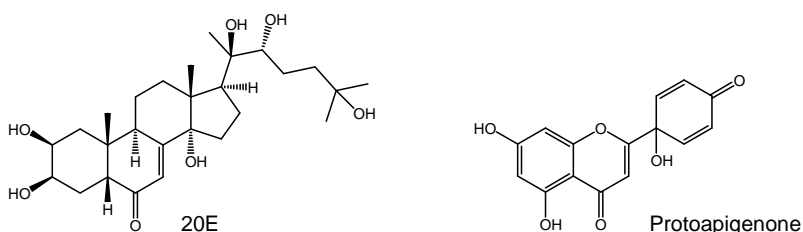
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Ecdysteroids are insect molting hormone analogs also present in several plant species including dietary ones such as spinach, where principal role of these compounds is apparently defensive against non-adapted herbivores. Ecdysteroid can also exert various beneficial metabolic effects in mammals via rather unclear mechanisms; non-hormonal anabolic activity of these compounds resulted in the marketing of a wide range of ecdysteroid containing food supplements worldwide.

Protoflavones are oxidated flavonoid derivatives with an unusual non-aromatic B-ring. These flavonoids are potent anticancer agents both *in vitro* and *in vivo*: they were shown to promote oxidative stress and exert apoptosis in various cancer cell lines and tumor xenografts. Most recently, some of these compounds were found to inhibit ATR-dependent signaling, a crucial regulator of DNA-damage response.

Structures of 20-hydroxyecdysone (20E), the most abundant phytoecdysteroid, and protoapigenone, a natural protoflavone are shown below.



A brief summary is to be presented on our latest developments in these two topics. Our group has recently discovered, that less polar ecdysteroid derivatives such as for example 20E 2,3;20,22-diacetonide, are potent modulators of the ABCB1 efflux pump mediated multi-drug resistance, while the classical, polar ecdysteroids rather increase resistance. Testing around 60 compounds allowed us to conclude fundamental SAR. According to protoflavonoids, a set of around 33 derivatives have been prepared by semi- or totalsynthetic approach and tested against various cancer cell lines, including MDR ones. Compounds with a 6-methyl substitution were found to exert a mild yet statistically significant selectivity against a transfected mouse lymphoma cell line expressing the human ABCB1 transporter, based on which SAR is currently being explored with different substituents at C-6.

Aquaporin channels as targets for cancer therapy

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Aquaporins (AQPs) belong to a highly conserved group of membrane proteins involved in the transport of water and small solutes such as glycerol through the plasma membranes of many cell types and with a variety of important physiological roles. Overexpression of cell membrane AQPs has been detected in tumor cells of different origins, particularly aggressive tumors, being associated with tumor formation, angiogenesis, cell migration and proliferation, and suggesting that AQPs might be a novel target of diagnostic and prognostic value.

We recently reported on the potent and selective inhibition of AQP3 permeability by a water-soluble gold(III) coordination compound (Auphen), using human red blood cells as well as PC12 cells transfected with either AQP1 or AQP3 [1]. Here we present the effect that this selective inhibitor produces on cell proliferation of cell lines that considerably express AQP3 and compare it with cells with low/or none AQP3 expression. Our results indicate a potential therapeutic effect of Auphen over tumorigenesis in tissues with large expression of AQP3 as the skin, suggesting that epidermoid carcinomas and other skin cancer types could be susceptible to Auphen treatment. In addition, through molecular modeling and site-directed mutagenesis we were able to identify the cys40 residue as crucial for Auphen binding.

In order to gain further insight into the basic structure-activity relationships, fundamental for the design of new AQP3 inhibitors, we investigated other gold-based compounds as possible AQP3 inhibitors. We describe here the selective and potent inhibitory effect (in the nanoM range) of a series of Au(III) complexes bearing nitrogen donor ligands on AQP3 which, together with their high water solubility, makes them suitable candidates for future *in vivo* studies.

Since AQPs have been identified in stem cells [2], investigation on their expression and modulation would provide valuable insights into the knowledge of stem cell biology and technology.

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Carbohydrates on and around cancer stem cells

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Carbohydrates are present on the surface of any mammalian cell as well as in the extracellular matrix surrounding these cells. During the development of cells in their particular environment this composition of glycoconjugates changes dynamically, leading to a continuous turnover. The typical glycoconjugates presented by cells are glycolipids, glycoproteins and proteoglycans, which display a high degree of variability and heterogeneity. The malignancy of cells is always accompanied by changes of the carbohydrates presented on the surface as well as the carbohydrate binding capacity of surface proteins with lectin-like functions. This allows to characterize and to purify cells, including all types of stem cells, by binding to either lectins or to carbohydrates.

There are a number of glycosylated stem cell markers, which are routinely detected by antibodies directed towards the protein part. We are interested in the glycosylation status of cancer stem cell markers and other glycoproteins in relation to the "stemness" of the particular tumor cells. The upregulated presence of α 2,6-linked sialic acid in tumor cells leads to increased formation of metastases. On the molecular level enhanced α 2,6-sialylation reduces the interaction with pro-apoptotic galectins as well as Fas and TNFR1.^[1] It was shown that α 2,3-sialylated N-glycans were present on the tumor stem cell marker CD133 of glioma-initiating cells and that removal of sialic acid reduced the stability of the marker glycoprotein on the cell surface.^[2] The presence of externally added heparin-like glycosaminoglycans was found to drive resting stem cells into particular differentiation pathways.^[3]

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How to improve predictive accuracy of a QSAR model for the design of inhibitors of sugar processing enzymes with transition metal ion co-factors

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A computational prediction of a binding free energy (ΔG_{bind}) between an inhibitor and a sugar-processing enzyme is often complicated by the presence of a transition metal ion at the active site of an enzyme. The accuracy of ΔG_{bind} depends on a correct assessment of interaction energy terms between the inhibitor and the transition metal ion as well as amino acid residues at the active site. We proposed 3D-QSAR model with interaction energy descriptors calculated at the quantum mechanics level (LIE-3D-DFT). Our novel methodology is based on decomposition of 3D-descriptors to small structural fragments. The 3D-QSAR model was built and tested for a library of human Golgi α -mannosidase II (hGMII) inhibitors. hGMII is a glycoside hydrolase (GH38) with zinc ion co-factor and is a target for inhibition of growth and metastasis of cancer cells. We found significantly improved predicted power of the LIE-3D-DFT model compared with empirical models based only on docking score or force field functions. Our LIE-3D-DFT model was able accurately to predict active compounds with an average error of 1.5 kcal/mol for ΔG_{bind} , and it satisfactorily separated potent inhibitors from non-active compounds.

Forcing cancer stem cells to behave: GLYPICAN4 as potential molecular target to trigger cancer stem cell differentiation

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The goal of our research is to uncover new cellular and molecular mechanisms governing the balance between self-renewal and differentiation of stem cells (SCs). The local microenvironment, or “SC niche”, holds the key to control this SC fate choice by providing a number of instructive cues such as cytokines and growth factors. Crucial for proper implementation of these signals is the capability of SCs to perceive them at the precise time point and with the right strength. Identifying the critical mediators of SC:microenvironment crosstalk is a basic topic in SC research as it can permit a high degree of control over SC state and fate. We have recently demonstrated that different SC types express a new cell surface marker: the heparan sulphate proteoglycan Glypican4. We have demonstrated that Glypican4, which acts as gatekeepers of environmental signals to modulate their perception by target cells, is specifically required to maintain the self-renewal of mouse embryonic and neural SCs. Mechanistically, Glypican4 regulates self-renewal of ES cells by modulating Wnt/b-catenin signalling activities. Thus, our findings establish that Glypican4 acts at the interface of extrinsic and intrinsic signal regulation to fine tune SC fate. Another issue we have tackled in these studies is the serious problem that embryonic or induced pluripotent SCs develop teratomas when transplanted to adult tissues. We found that it is possible to uncouple cell differentiation properties of pluripotent cells from tumorigenic potential by manipulating Glypican4 activity levels. In particular, pluripotent cells lacking Glypican4 lose their intrinsic tumorigenic properties after implantation into nude mice, while maintain the pluripotent differentiation program both in vitro and in vivo. Gene expression profile analysis has also revealed that loss-of-Glypican4 activity leads to the over expression of tumour suppressor genes in embryonic SCs. As Glypican4 is a cell surface protein our findings identify Glypican4 as a suitable target for designing therapeutic strategies aiming at impairing the teratoma potential of pluripotent SCs and increasing their differentiation efficiency. Glypican4 is over-expressed in human cancers such as colon cancer and glioblastoma where cancer SCs have been described. Interestingly, signalling pathways active in normal SCs regulates the self-renewal and differentiation programs of cancer SCs. In collaboration with members of this cost action we are interested in assessing the expression profile of Glypican4 in different cancer SC types and in evaluating the possibility that Glypican4 modulation triggers cancer SC differentiation. We anticipate that Gpc4 could permit the development of new therapeutic strategies targeting cancer SCs and involving both cytotoxic and differentiation triggering agents.

POSTER SESSION I

P1.

miRNA expression in canine mammary cancer stem cells

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Stem cells possess the potential to generate identical daughter stem cells as well as more restricted progenitor cells. Stem cells have much longer life span and high proliferative potential. Therefore they are more predisposed to accumulate genetic and epigenetic mutations. That is why cancer is considered as a disease of stem cells.

The aim of our study was to assess miRNA expression of canine mammary cancer stem cells. Three canine mammary cancer cell lines (CMT-U27, CMT-U309 and P114) were stained using Anti-Sca1 (Stem cell antigen 1) antibodies. The FACS analysis showed 0,3-1% of Sca1+ cells in each of the cell line. The cells were sorted (using FACS Aria II) as Sca1+ and Sca1- and subjected to further analysis of miRNA expression (using Agilent custom miRNA microarray).

The analysis revealed that 189 of 323 miRNAs showed significantly increased expression in Sca1+ cells, whereas only 1 miRNA (miR-27a) showed significantly decreased expression. PANTER analysis revealed that miR-27a targeted genes are mainly involved in inflammation mediated by chemokine and cytokine signaling pathway, Wnt signaling pathway, GnRH receptor pathway and EGF receptor signaling pathway.

These genes were mainly involved in metabolic process, cellular process, cell communication and developmental process.

The results of our study showed significant epigenetic regulation of cancer stem cells transition to differentiated cancer cells.

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P2.

Oxidative modifications of extracellular matrix change Hedgehog signaling pathway

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Although tumor heterogeneity has been described since the first description of cancer, only recently recognized, the subpopulation of cancer stem cells (CSCs) are recognized as the ones responsible for cancer progression. Similar to the tumors, perhaps even more pronounced, breast CSC destiny is highly dependent of thier niche, and their fate, activation or quiescence, is determined by microenvironment. Microenvironemt was shown be instructive to tumor cells by modulating their tumorigenicity. Another factor contributing to variations in cell reaction to (chemo)therapeutics is oxidative stress. Oxidative stress, a state of increased reactive oxygen species (ROS) production, affects all cell systems. ROS are very reactive therefore cusing numerous consequences, like genetic instability, one of the major characteristic of cancer, but also affecting lipids causing peroxidation. End products of lipid peroxidation are reactive aldehydes, among which, 4-hydroxy-2-nonenal (HNE), is involved in different signaling pathways influencing cell's fate (e.g., differentiation, proliferation or apoptosis). Here, we investigated the influence of collagen modified by HNE on SUM159 cell differentiation and involvement of Hedgehog pathways in observed changes in cell differentiation.

P3.

Developing of inducible cell lines for study gene gain of function

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Many biological problems are amenable to comparative gain of function experimental approaches. Conventional approach by introducing expression vector at random integration sites are confounded by expression variation. Besides in mouse embryonic stem cells, gene targeting by homologous recombination is not possible in most cell lines. We developed novel expression system based on conditional Tet-on regulation with selectable Cre/Lox cassette exchange in a single-copy integration vector. We demonstrated method for generation of cell lines with highly efficient inducible cassette exchange target loci. This system can be introduced in cell lines despite of their origin, differentiation stage or proliferation ability. Our system is ideal for comparative gain of function studies, such as comparing phenotypic effects of various genes, or evaluating mutants of a given gene. The conditional nature of the expression system facilitates work with genes with toxic phenotypes that would affect generation of stable cell lines. The most important is to generate clonal cell line in which there is single inducible locus, which is expressed robustly, it is not silenced, and it is not leaky. In this presentation we will show successful generation of various inducible cell lines, including human embryonic stem cell line, and we will discuss the possibility of using our system for modifying various cancer cell lines.

P4.

Quantitative phosphoproteomics dissects sensitization of EGFR-resistant colon cancer cells to Tyrosine Kinase Inhibitors through Spliceosome Modulation

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Growth factors such as epidermal growth factor (EGF) and basic fibroblast growth factor (b-FGF) promote cancer stem cell (CSC) proliferation in many solid tumours. EGF signalling is essential for formation and maintenance of colon CSCs while CSCs marker CD133 expression is upregulated in CRC tumors that have a hyperactivated Ras-Raf-MEK-ERK pathway and is related to mutations in K-Ras. Intrinsic and acquired resistance to EGFR Tyrosine Kinase Inhibitors (TKIs) is increasingly well-recognized and is epitomised by, (i) genetic and epigenetic variations of genes in the EGFR oncogenic cascade, (ii) critical downstream effectors RAS/RAF/MEK/ERK and PI3K/AKT/mTOR, (iii) parallel pathways, including VEGFR, (iv) expression of the EGFRvIII variant, deprived of the extracellular ligand binding domain and (v) nuclear translocation and constitutive activation of pEGFR.

By employing label-free phosphoproteomics, an unprecedented tool in dissecting molecular mechanisms of combined therapies in clinical molecular oncology and defining biomarkers of efficacy, we have shown that sensitivity of cancer cells to TKI cannot always be predicted by the activation status of the target. Compromised sensitivity during CRC progression could be reverted by an “off-target” functional nutrient, γ -Tocotrienol. The combination of γ -Tocotrienol with quinazoline-based TKIs has been effective for both EGFR+ve and EGFR-ve tumors despite K-Ras mutation status, thus offering therapeutic solutions for primary, metastatic and recurrent tumors. In fact, EGFR-pathway activation did not seem to be the main determinant in making our cell model sensitive to TKIs. Pathways and phosphoprotein clusters with emerging role in malignant transformation and progression such as the spliceosome cluster and the endocytosis pathways disclose novel therapeutic targets and associated biomarkers of response, disclosing new opportunities and challenges for synthetic chemistry in overcoming resistance in colon cancer stem cells.

P5.

The functional interplay between activated Stat5 and Stat3 in breast cancer formation and progression and the therapeutic effects of targeted inhibition

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Cancer cells with stem cell-like characteristics, also called cancer stem cells (CSCs), are thought to be instrumental in tumor initiation, progression and recurrence. Recent studies have shown that cells undergoing epithelial-mesenchymal transition (EMT), a step thought to be an early event in metastatic dissemination, share gene expression profiles with CSCs. Establishment and growth of disseminating tumor cells at the site of distant metastases is accompanied by their re-differentiation and the transition from a mesenchymal to a more epithelial phenotype (MET). We are studying the functional interplay between two related transcription factors, signal transducer and activator of transcription 5 (Stat5) and Stat3 in the genetic and epigenetic regulation of non-metastatic and metastatic breast cancer cells. Simultaneous activity of both Stats is observed in a high percentage of human luminal breast cancers. In both normal and malignant cells, Stat5 activity regulates cell proliferation, differentiation and survival. Stat5 activation in tumors is associated with favorable prognosis, due to a more differentiated phenotype, the expression of the estrogen receptor and response to antiestrogen therapy. During involution, i.e. the postlactational regression of the mammary gland, P-Stat3 regulates lysosomal-mediated cell death.

To gain insights into the functions of Stat3 and Stat5 in oncogenesis, we established a mouse model which allows the investigation of genetic and epigenetic contributions to the initiation and progression of mammary tumors. In this model we used genetic manipulation of mammary stem cells (MaSCs) derived from primary tissue and the reconstitution of functional epithelium through transplantation into cleared fat pads to generate transgenic glands. The persistent activation of Stat5 during post-lactational involution resulted in the induction of non-metastatic adenocarcinomas, resembling human luminal-A breast cancer subtype. These tumors could be serially transplanted in BALB/c wild-type recipient mice and contain a small fraction of Lin-CD24^{low}CD44^{high} tumor initiating cells. Both primary and secondary tumors show simultaneous activity of Stat5 and Stat3 and express estrogen and progesterone receptors (ER+PR+) and their downstream target genes like amphiregulin, RANKL and Wnt4. We performed microarray gene expression analysis of tumor tissues and P-Stat5 transgenic grafts to identify potential Stat target genes, which might be involved in Stat induced oncogenesis. We are also studying the contribution of Stat5 and Stat3 to the regulation of metastatic processes and regulation of EMT and MET. For this purpose we employ newly discovered specific inhibitors of these transcription factors. Our tumor model allows the identification of new genetic and epigenetic determinants in the etiology of breast cancer and metastasis formation, and the evaluation of the therapeutic potential of Stat3 and Stat5 directed drugs.

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“TGFβ signaling serves dual purposes during the dissemination and metastasis formation of breast tumor cells” Vida Vafaizadeh, Ulrike Graab, Tahmineh Darvishi, Rita Salomé Gonçalves Machado and Bernd Groner *Horm Mol Biol Clin Invest* 10, 227–239 (2012)

P6.

Synthesis of Multi Drug Resistance modulators based on jatrophone framework

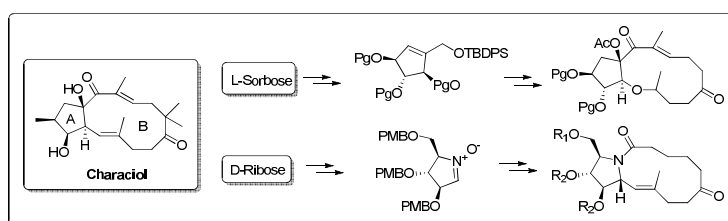
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The occurrence of resistance to anticancer agents is a major obstacle for successful cancer chemotherapy. The emergence of resistance to anticancer drugs, in particular multidrug resistance (MDR) has made many of the available anticancer drugs ineffective.¹ MDR, is a complex multifactorial phenomenon that can result from a number of biochemical mechanisms. The enhanced activity of various members of the family of adenosine triphosphate binding cassette (ABC)-transporters was associated with different types of MDR.² ABCB1, also known as P-glycoprotein, is a membrane protein member of the ATP-binding cassette (ABC) transporters superfamily. These membrane-embedded transport proteins decrease the intracellular drug accumulation, by an ATP-dependent efflux. This reduces the cytotoxicity of the anticancer agent and enables the tumour cells to survive. MDR problem could be overcome using potent and selective Pgp inhibitor.³ Since MDR is a major obstacle in clinical management of human cancers, it is important to design alternative therapy strategies that can be used in the treatment of drug-resistant phenotype. Until now, potential candidates failed clinical trials due to poor selectivity. The first generation of chemosensitizers were ineffective at non-toxic concentrations, while second generation chemosensitizers often failed because of simultaneous Pgp and CYP3A4 inhibition.⁴ Jatrophone and lathyrane diterpene are a class of natural compounds, which were extracted from plants of the genus of Euphorbia. The phytopharmacological properties of the genus *Euphorbia* are well documented. A broad range of biological properties have been reported for constituents of the plant extract.⁵ In particular, jatrophone and lathyrane have been found to be potent and specific P-glycoprotein modulators.

A scalable and solid synthesis of ring A is necessary in order to prepare a small library of jatrophone analogue. L-Sorbose, is a cheap and readily available natural sugar. Despite the similarity with the well exploited D-Fructose, only few methods were developed for the easy modification of this interesting sugar.⁶ Our results on this subject will be communicated.



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P7.

Dual antithrombotic compounds with antiangiogenic activity as potential approach to targeting cancer stem cells

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Our recent efforts to combine a pharmacophore responsible for thrombin inhibition and a pharmacophore common to fibrinogen receptor antagonists into a low molecular weight peptidomimetic compound resulted in a series of 1,4-benzoxazine and 1,4-benzodioxine compounds [1,2] which were identified as potent inhibitors of angiogenesis as revealed by inhibition of proliferation of four cell lines, inhibition of endothelial cell migration, inhibition of tube formation and CAM assay. Although thrombin inhibition remains the most probable explanation for their inhibition of angiogenesis, docking experiments and VEGFR2 kinase assay indicate that other targets such as VEGFR2 might be involved [1]. Their interaction with growth factor/receptor tyrosine kinase signaling pathways and antiangiogenic activity is a potential which might be exploited in targeting cancer stem cells. This avenue will be explored as part of COST Action CM1106 cooperation.

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P8.

Differential Cytotoxic Activity of a Novel Palladium-Based Compound on Prostate Cell Lines, Primary Prostate Epithelial Cells and Prostate Stem Cells

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The outcome for patients with advanced metastatic and recurrent prostate cancer is still poor. Therefore, new chemotherapeutics are required, especially for killing cancer stem cells that are thought to be responsible for disease recurrence. In this study, we screened the effect of a novel palladium-based anticancer agent (Pd complex) against six different prostate cancer cell lines, and primary cultures from seven Gleason 6/7 prostate cancer, three Gleason 8/9 prostate cancer and four benign prostate hyperplasia patient samples, as well as cancer stem cells selected from primary cultures. MTT and ATP viability assays were used to assess cell growth and flow cytometry to assess cell cycle status. In addition, immunofluorescence was used to detect γ H2AX nuclear foci, indicative of DNA damage, and Western blotting to assess the induction of apoptosis and autophagy. The Pd complex showed a powerful growth-inhibitory effect against both cell lines and primary cultures. More importantly, it successfully reduced the viability of cancer stem cells as first reported in this study. The Pd complex induced DNA damage and differentially induced evidence of cell death as well as autophagy. In conclusion, this novel agent may be promising for use against the bulk of the tumour cell population as well as the prostate cancer stem cells, which are thought to be responsible for the resistance of metastatic prostate cancer to chemotherapy. Significantly, this study also indicates that the combined use of the Pd complex with an autophagy modulator may be a more promising approach to treat prostate cancer.

P9.

Abl acts as a “signalling node” for maintenance, propagation, and motility of glioblastoma cells

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The fate and position of cells during nervous system development and adult neurogenesis is finely regulated by environmental signals. These signals in adult brain ensure maintenance of a pool of stem cells needed for normal brain physiology and for regeneration processes. Alteration of cell signalling response triggers pathological programs leading to tumorigenesis. The identification of key signalling mediators is crucial to maximize effectiveness of molecularly targeted anticancer therapies.

We are interested to explore how the plasticity of cells during developmental and oncogenic processes allows them to respond and adapt to environmental signals. We found that the non-receptor tyrosine kinase Abl is expressed and constitutively activated in different human glioblastoma (GBM) cells. This is consistent with previous studies showing that Abl is one over the 12 proteins discriminating high- versus low-grade gliomas. We hypothesized that Abl may integrate environmental outcomes by functioning as a “switch modulator”. Such “switch modulator” property of Abl is highlighted by a number of apparently contradicting results showing that Abl can act either as a signalling “inhibitor” or as a signalling “promoter” of a given biological response, depending on the cellular context and on the incoming environmental stimuli.

We found that impairment of Abl signalling causes acquisition of mesenchymal-like characteristics, altering cell polarity and motility. Intriguingly, Abl inhibition interferes with tumour sphere formation and anchorage-independent growth of GBM cells in vitro. Xenograft experiments show that Abl inactivation suppresses the tumorigenic capacity of GBM cells in nude mice. We have performed biochemical and qRT-PCR studies to highlight the underlined molecular mechanism and found that Abl signalling inhibition provokes a drastic change in the activation of multiple pathways. We also found expression changes for genes related to epithelial and mesenchymal state (e.g. CDH1, Snail1), to stemness (e.g. CD133, nestin), and to the SHH pathway (e.g. SHH, SMO). Altogether, these biological and biochemical outcomes point to Abl as a “signalling node” that is capable of modulating the biological properties of GBM cells. Intriguingly, we found that Abl signalling modulates also self-renewal of human primary GBM stem cells. It has been shown that expansion and maintenance of Bcr-Abl positive leukemic stem cells is dependent on SHH pathway activation. Thus, it is tempting to speculate that Abl signalling impacts the maintenance of normal and cancer stem cells, possibly according to the environmental signals.

P10.

ALDH enzymatic activity and CD133 positivity in ovarian cancer patients

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Ovarian cancer ranks fifth in both cancer incidence and mortality in Western women and has a high mortality rate. Despite the fact that more than 70% of the patients respond to front line therapy (surgery followed by a platinum based chemotherapy) most of them will eventually relapse and die from chemo-resistant disease. A number of evidence suggests that the cancer stem cell model also applies to ovarian tumor, even if no consensus on which markers define the ovarian cancer stem cell has reached yet. Among other CD133 and Aldehyde Dehydrogenase (ALDH) have been evocated as possible markers associated with ovarian cancer stem cells. The aim of the present work was to study the expression of these markers in 9 stabilized ovarian cancer cell lines and in 108 fresh tumour ovarian samples. We found that among the total patients analyzed, 13% of them was completely negative for ALDH activity and 26% was negative for CD133 staining. Similar data were obtained in cancer cell lines. Both markers were variably expressed within the samples and when both studied in the same tumor sample, no statistically significant correlation between ALDH enzymatic activity and CD133 expression was found. No statistical significant correlation was found also between the percentage values of positive ALDH and CD133 cells and the number of serial passages patient's cultures underwent, suggesting that these markers do not confer by themselves a self-renewal growth advantage to the cultures. In some cases, cells positive and negative for these markers were sorted and studied for their ability to grow in nude mice. We correlated the expression of CD133 and ALDH in our patient cohort, but no correlation with response to therapy, progression free survival and overall survival was found, suggesting that neither ALDH enzymatic activity and CD133 expression provide additional predictive/prognostic information in ovarian cancer patients.

P11.

Development of a novel mesoporous carbon drug delivery system: Characterisation and cytocompatibility studies

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The development of novel drug delivery systems to enable and ensure improved pharmacokinetic and pharmacodynamic profiles of medicines is of crucial importance in modern pharmacology and therapeutics. The use of ordered mesoporous carbons (OMCs) in drug delivery practices has received considerable attention due to the properties of these materials such as high-surface areas, uniformity, tunable pore sizes ranging from 3 to 10 nm, high degree of structural ordering, facile synthesis, high thermal, chemical and mechanical stability. To this end, mesoporous carbon materials (CMK-3) were synthesized and studied for their ability to incorporate poorly water soluble drugs. Namely, the non-steroidal anti-inflammatory drugs ibuprofen and indomethacin were encapsulated and further characterised by means of Differential Scanning Calorimetry (DSC), X-ray Powder Diffraction (XRPD), Fourier transform Infrared (FT-IR) and X-ray Photon Electron Spectroscopy (XPS). In both cases, drugs were in an amorphous state in the pores of the carbon carrier as being evident from the physicochemical characterisation studies. In particular, in acidic environment (pH 1.2) a faster release profile for indomethacin has been observed compared to ibuprofen formulation. Both formulations reached a plateau within 2 hours. Based on these data, cytotoxicity assays were conducted on the Caco-2 cell culture model to further evaluate the pharmacological profile of CMK-3. Importantly, the material has exhibited no significant toxicity as observed by the kinetics of cell proliferation capacity and the assessment of cell death in culture. Moreover, uptake and subcellular localisation of the material was evident very early upon exposure of Caco-2 cells and further explored in a dose- and time-dependent manner through optical and fluorescence microscopy. Overall, the CMK-3 material has shown promising technological and pharmacological profiles as a novel potential drug delivery system.

P12.

TKI Inhibitors in Targeting Cancer Stem Cells – Development of New KDR Inhibitor

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Many of tyrosine kinase inhibitors (TKI) are already involved in cancer treatment. There are some data in the recent literature that TKI are influencing also highly resistant tumor stem cells. There is substantial evidence that many human cancers are driven by a small subpopulation of cells that display stem cell properties. **Cancer stem cells (CSC) are chemoresistant and implicated in tumor recurrence, metastasis and high patient mortality.** Substances impairing CSC activity could be invaluable as novel cancer therapeutics. [1] Glioblastoma multiforme (GBM) including a small subpopulation of brain tumor stem-like cells (BTSC) are likely responsible for the therapy resistant and pervasive nature of GBM. Current therapies have only limited effect on targeting and eliminating BTSC. The current attempts to implement **tyrosine kinase inhibitors (TKIs) as novel mechanism-based molecularly-targeting therapies against GBM** have been reviewed recently. [2] The possible role of **EGFR as a regulator of "stemness" was investigated** in HNSCC cells. **Activation of EGFR by the addition of EGF ligand resulted in the induction of CD44, BMI-1, Oct-4, NANOG, CXCR4, SDF-1 and increased tumorsphere formation,** a characteristic ability of cancer stem cells. Conversely, **treatment with the EGFR kinase inhibitor, Gefinitib (Iressa), resulted in decreased expression of the aforementioned genes, and loss of tumorsphere-forming ability.** Cancer stem cells, when treated with Gefitinib, **possessed a lower capacity to invade and became more sensitive to cisplatin-induced death in vitro.** These results suggest that **EGFR plays critical roles in the survival, maintenance, and function of cancer stem cells.** Drugs that target EGFR might be an effective treatment for HNSCC. [3] The **receptor tyrosine kinase (RTK) family mediates the multiple oncogenic growth factor receptor signaling and contributes to the pathogenesis of glioblastoma.** The expression of stem cell marker in glioblastoma tissue has prognostic significance. **Notch receptor expression** is moderately upregulated and **correlated with that of VEGFR2, VEGFR3, and PDGFR β .** [4] **Bevacizumab (humanized VEGF antibody) inhibits brain CSC growth. Nimotuzumab, a monoclonal antibody against the epidermal growth factor receptor (EGFR), reduces CSC-like subpopulations in mouse models of brain tumors.** [5] **Anti-angiogenesis agents and cancer stem-like cells (CSC) are opening new avenues for targeted cancer therapy. Endothelial cells are a key component of the CSC niche.** One approach may be direct **targeting of tumor endothelial cell fate could inhibit angiogenesis but also the self-replication of CSC, which relies on signals from surrounding endothelial cells in the tumor microenvironment.** The **Notch pathway is central to controlling cell fate both during angiogenesis and in CSC** from several tumors. [6] **VEGFR2 is regarded as an endothelial cell protein,** evidence suggests that VEGFRs **may be expressed also by cancer cells.** Glioblastoma multiforme (GBM) is characterized by florid vascularization and aberrantly elevated VEGF. Antiangiogenic therapy with **bevacizumab reduces GBM tumor growth. VEGFR2 is preferentially expressed on the cell surface of the CD133+ human glioma stem-like cells**

(GSCs), whose viability, self-renewal, and tumorigenicity rely, at least in part, on signaling through the VEGF-VEGFR2-Neuropilin-1 (NRP1) axis. A direct inhibition of VEGFR2 kinase may block the highly dynamic VEGF-VEGFR2-NRP1 pathway and inspire a GBM treatment strategy. [7]

On the poster a development of new KDR inhibitor will be depicted.

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P13.

Targeting cancer stem cells with an Axl inhibitor

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Cancer stem cells (CSC) are defined as the tumorigenic subpopulation of cancer cells. CSC are resistant to current therapies, drive tumor post-therapy recurrence and are responsible for tumor seeding ability during metastatic spread. The receptor tyrosine kinase Axl is associated with metastasis and poor overall survival in breast cancer. Axl is activated by epithelial-to-mesenchymal transition and correlates with markers of the CSC phenotype. Hence, targeting Axl may affect CSC activities in breast cancer. We assessed the effect of Axl inhibitors on CSC functions in in vitro and in vivo models systems. RNA interference, antibody and small molecule Axl kinase inhibitors effectively blocked CSC activity in in vitro mammosphere forming assays with breast cancer cells. Pretreatment with the chemotherapeutic docetaxel enriches chemoresistant CSC populations that form tumors when implanted subcutaneously into the flank of syngeneic Balb/C mice. Docetaxel and Axl inhibitor combination treatments blocked tumor initiation activity in vivo. These results suggest that Axl inhibitors can target CSC activity in breast cancer.

P14.

Cell type-dependent response to chemotherapy treatment with doxorubicin in breast cancer

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Breast cancer still represents leading cause of mortality and morbidity in women nevertheless of the great improvements in early diagnostics and cancer therapy made in the last few decades. Even though these improvements led to better understanding of the underlying molecular mechanisms, resistance to classical chemotherapeutics is still a tremendous challenge for breast cancer therapy. About 30% of all breast cancer patients who are successfully treated at early stages are suffering a relapse accompanied by metastasis and chemoresistance to classical drugs. Among the wide variety of drugs used in breast cancer chemotherapy treatments, doxorubicin is frequently used either as single-agent or in combination with other drugs. Since breast cancer is heterogeneous disease it can be categorised into three basic therapeutic groups: hormone (oestrogen (ER) and progesterone (PR)) positive with the best prognosis, HER2 positive and triple-negative (ER-, PR-, HER2-) with the worst prognosis. These subtypes were shown to respond differently to chemotherapy. On the other hand we must not neglect the role of the microenvironment, especially extracellular matrix (ECM) and its modifications, on the cancer treatment and its prevention or progression. Therefore we have investigated the effect of doxorubicin treatment and 4-hydroxynonenal-modified microenvironment, in the 3 different breast cancer cell lines: MCF-7 (ER+, Pr+), SkBr3 (Her2+) and SUM 159 (triple negative). Our results of cell proliferation and intracellular reactive oxygen species (ROS) production have revealed difference in cell type responses.

P15.

Using an *in vitro* model of Epithelial-Mesenchymal-Epithelial transitions to uncover novel biological mechanisms

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Epithelial-mesenchymal transition (EMT) and mesenchymal-epithelial transition (MET) are fundamental mechanisms controlling multiple events during embryonic development and cancer. Cancerous cells undergoing EMT, exhibit a mesenchymal-like phenotype with concomitant polarity loss, increased invasion and apoptosis resistance, which unable detachment from the primary environment. Nevertheless, the establishment of cancerous cells at novel locations is only possible for cells that are successful in undergoing the reverse process MET. Although EMT inducers and cellular outcomes have been largely studied, the molecular players and features of cells that revert through MET are far from being recognized.

In the present work, we produced a dynamic EMT/MET model aiming at analyzing the whole transcriptome variation and defining the biological pathways that determine these transitions. We induced EMT/MET in a normal mouse mammary cell line (EpH4) by adding/removing TGF- β 1. DNase treated total RNA extracted at distinct EMT/MET-timepoints was subjected to whole transcriptome sequencing (RNAseq). Bioinformatic analysis was performed using in house pipelines and commercially available software. Validation was performed using qRT-PCR, Western Blot and immunofluorescence of selected differentially expressed targets.

We confirmed the occurrence of EMT and MET by analysing the differential transcription of classical epithelial and mesenchymal markers. Bioinformatic analysis of RNAseq data uncovered thousands of genes differentially expressed in correlation with differential activation of several pathways. For example, and in addition to the TGF- β signalling pathway, we have observed differential activation of the *Wnt-signalling pathway*, *BMP signalling pathway*, *Toll-like receptor-signalling pathway*, *Human Embryonic Stem Cell Pluripotency pathway*, etc. We have also uncovered novel biological mechanisms underlying EMT/MET such as functional inactivation of E-cadherin by impaired glycosylation (1). In addition, the dynamicity of our EMT/MET model allowed us to uncover novel epigenetic mechanisms: for example, we explored the differential expression of a recently annotated differentiation-related gene, *Dies1*, which became overexpressed in cells after undergoing MET in concomitance with its promoter demethylation.

In conclusion, we were able to establish a dynamic *in vitro* model of EMT/MET, which allows uncovering and studying novel biological and genetic mechanisms using a non-biased genome wide approach. In addition, this model could also be used to test novel inhibitors designed specifically against the identified differentially expressed genes/pathways.

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P16.

New camptothecin-linked platinum anticancer agents

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Presently, platinum-based coordination complexes are among the most widely used antitumor agents in the clinic. The effectiveness of cisplatin lies in its ability to covalently bind DNA, leading to important changes in the helical structure. In spite of the efficacy of platinum-based treatment regimens, long-term cure is difficult to obtain. The major drawbacks include a) severe and sometimes life-threatening toxic side effects; b) activation of drug resistance mechanisms by tumor cells; c) inadequate intratumor concentration of the drug and tumor microenvironmental interactions; d) relatively poor pharmacokinetic profiles and e) increased DNA repair capacity. As part of our program aimed to advance DNA as a drug target, we were interested in devising novel platinum containing dual compounds that interacted in an effective way with DNA and in addition accomplished the requirements of solubility in the body fluids and improved cellular uptake. In this context, a promising approach seemed to be the conjugation of platinum to analogues of the natural antitumor compound camptothecin (CPT).

In this poster we report the design, modeling, synthesis and biological activity evaluation of new hybrid agents formed by CPT derivatives and diaminedichloro-platinum (II) complex. The compounds showed growth inhibitory activity against a panel of human tumor cell lines, including sublines resistant to topotecan and platinum compounds. The derivatives were active in all the tested cell lines, and the most active one was able to overcome cisplatin resistance in the osteosarcoma U2OS/Pt cell line. Platinum-containing camptothecins produced Platinum-DNA adducts and topoisomerase I-mediated DNA damage with cleavage pattern and persistence similar to SN38, the active principle of irinotecan. The results support the interpretation that the diaminedichloro-platinum (II) complex conjugated to a functionalized camptothecin resulted in a new class of effective antitumor compounds.

P17.

Natural lead products inhibit the Hedgehog signaling pathway in medulloblastoma

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The medulloblastoma is the most frequent malignant brain tumor of childhood, it belongs to the group of embryonal neuroepithelial tumors and arise from stem cells or early progenitor cells in the cerebellum¹. The Hedgehog pathway (Hh) is an essential embryonic signalling cascade that regulates stem-cell and progenitor-cell differentiation in multiple developmental processes. Smoothed homologue (SMO) is a transmembrane protein that activates the downstream Hedgehog signaling pathway. PTCH1 is an inhibitory cell-surface receptor that constitutively suppresses activation of the hedgehog pathway by inhibiting SMO. Hedgehog ligands bind to and inactivate PTCH1, derepressing SMO and promoting pathway activation². The most promising targets of the pathway are the G-protein coupled receptor SMO, which transduces the Hh signal inside the cell, and the effector protein Gli1 which acts downstream of the SMO and promotes the transcription of Hh target genes. In the literature, some SMO antagonists have been described, some of which are currently under clinical investigation, whereas no records on the discovery of Gli1 antagonists are available³. The aim of this project is to discover and develop small organic molecules and/or natural compounds capable of inhibiting the Hh signaling pathway by antagonizing the SMO receptor and/or the Gli1 protein, thereby providing anticancer activity. According to molecular modeling studies the most promising compounds predicted by virtual screening will be tested *in vitro* on various cell-based assays as, for example, in Hh-responsive cell lines and in stem/progenitor cells. The binding properties on the SMO receptor would be investigated by competition assays with Bodipy-cyclopamine, whereas the direct interaction with Gli1 could be studied by biophysical methods. Biological activity data will be used for refining computational models, while the most interesting active hits will be optimized through the rational design and synthesis. The most potent and less toxic compounds will be finally considered as the lead for further and in depth studies such as pre-clinical trials.

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[3] *Cancer Science* **2011**, 102, 1756;

P18.

Studies of the DNA methyltransferases inhibitor EGCG effects on human leukemia cell proliferation and differentiation

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EGCG [(–)epigallocatechin-3-gallate], a green tea-derived polyphenol, has been shown to suppress cancer cell proliferation, and interfere with the several signaling pathways and induce apoptosis. EGCG was reported to cause cell-cycle arrest in various mouse, rat and human cell lines through regulation of cell-cycle related oncogenes and proteins, particular cyclin D1 and its cyclin-dependent kinases (cdk4 and cdk6).

The present investigation was designed to characterize the effects of EGCG alone and its combination with retinoic acid on NB4 cell growth and apoptosis. In this work it was found out that natural DNA methyltransferases inhibitor EGCG reduces cell proliferation, increases cell death – apoptosis and decreases the level of DNA methyltransferases gene expression (DNMT3a, 3b) in NB4 leukemia cells.

NB4 cells were treated with 40 μ M EGCG alone, in combination with 1 μ M RA or with 1 μ M RA alone. EGCG significantly inhibited the growth of NB4 cells at 40 μ M concentration alone or in combination with 1 μ M RA in a time-dependent manner. To examine the differentiating activity of EGCG we evaluate expression of the early differentiation marker CD11b in treated NB4 cells. EGCG alone did not affect differentiation of NB4 cells, but increased proportion of cells in the subG1 phase (undergoing apoptosis) up to 17% at 24 h and double augmentation at 48 h (42%).

For cell cycle analysis, NB4 cells were treated with 40 μ M EGCG or 1 μ M RA alone and in combination (1 μ M RA and 40 μ M EGCG) for 72 h. Cell populations in cell cycle phases were determined by flow cytometry. There was no additive effect on cell accumulation in the phases at 24-h treatments with EGCG or its combination with RA. Cell cycle analysis revealed that EGCG caused a decrease in the proportion of NB4 cells in the S (12 %) and G2/M (9.2%) and an increase in the G0/G1 phase at 72 h point up to 78%, compared with control (non treated) cells. The combination treatment with EGCG and RA for 72 h caused an increase in the G0/G1 phase up to 85% and decrease in the S (10%) and G2/M (5%) phases.

To evaluate the changes in global methylation status after treatment of NB4 cells with EGCG, we labeled control NB4 cells and cells treated with 40 μ M EGCG for 72 h with anti-5-Methyl Cytosine antibody and prepared flow cytometry analysis. We detected that EGCG alone decreased global methylation level in NB4 cells.

In this study we examined the DNMT1 and DNMT3a, 3b gene expression in 40 μ M EGCG-treated NB4 cells undergoing apoptosis or RA-treated cells undergoing growth inhibition. The results showed that EGCG alone or in combination with RA slightly decreased the expression of DNMT3a gene in NB4 cells, but had no effect on DNMT1 and DNMT3b gene expression.

To sum up, these results show that DNMT inhibitor EGCG is able to restore important functions of the cell by remodeling epigenetic changes and could be a potential agent in cancer treatment.

POSTER SESSION II

P1.

Novel ecdysteroids as modulators of multi-drug resistance

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Ecdysteroids, herbal analogs of the insect molting hormone, have several beneficial effects in mammals among which is a mild anabolic effect with no interference with their steroid hormonal system. In our recent studies on over 60 ecdysteroids, some of these compounds were found to modulate ABCB1 transporter mediated resistance to doxorubicin. Moreover, Structure activity relationship (SAR) studies showed that apolar ecdysteroids were more effective: for example, ecdysteroid diacetone derivatives such as 20-hydroxyecdysone 2,3;20,22-diacetone (**1**) presented strong synergism with doxorubicin.

In order to investigate the role of the substituents on the dioxalane rings at positions 2,3 and 20,22, new compounds have been synthesized from the plant derived 20-hydroxyecdysone. For that purpose formation of dioxalane rings was achieved using different reagents, such as methyl-ethyl ketone, butyraldehyde, valeraldehyde or 3-pentanone, resulting in over 10 new compounds. Moreover, three further diacetone derivatives of other natural ecdysteroids were obtained as side-products of the scale-up synthesis of **1**.

The compounds were tested for their capacity to modulate efflux mediated by the ABCB1 transporter following the intracellular accumulation of the dye rhodamine 123, using flow cytometry, in L5178 mouse T-cell lymphoma cell line (non MDR) and its sub-cell line transfected with pHa *MDR1/A* retrovirus, overexpressing the human ABCB1 efflux pump (MDR cell line). The same MDR cell line was used to test the capacity of the compounds to modulate resistance of the cells to doxorubicin. These combination studies were done using the checkerboard microplate method and the MTT colorimetric assay, and the results were evaluated by using the CompuSyn software.

The activity of the compounds formed using methyl-ethyl ketone studied is in accordance with the previous SAR studies, with synergistic activity comparable to that of compound **1**, with exception to one that contained only a methyl group as substituent of the 2,3 dioxalane ring. Activity studies on several other compounds, as well as further extensions of the SAR study, are still in progress.

Acknowledgements

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P2.

MUC1: uncovering a new target for pancreatic cancer therapy

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MUC1, a membrane-associated glycoprotein, is overexpressed in more than 80% of pancreatic tumors being correlated with carcinogenesis and poor prognosis. MUC1 participates in several oncogenic signaling pathways and was recently reported as being involved in resistance to standard chemotherapeutic agents and as a relevant marker of pluripotency in human embryonic stem cells. Recent studies revealed that a subpopulation of tumor cells with the potential to self-renew and differentiate - Cancer Stem Cells (CSC) - is responsible for initiation, progression, metastization and tumor recurrence.

The objective of this study was to investigate the possible involvement of MUC1 in CSC biology in pancreatic cancer, one of the most fatal neoplasias.

We investigated MUC1 expression in pancreatic CSC and we characterized MUC1 involvement in oncogenic signaling pathways of CSC.

We isolated subpopulations of putative cancer stem cells from pancreatic cancer cell line HPAF II using a stem cell surface marker CD133 (prominin-I) and assessed the tumorigenic potential of this small subpopulation, MUC1 expression levels and possible alterations in associated oncogenic signaling pathways.

The present work shows that pancreatic CSCs have a different expression profile regarding MUC1 and associated signaling partners. MUC1 seems to be a relevant player in pancreatic CSC biology that can contribute to the tumorigenic potential of this subpopulation and thus might constitute an elective target for alternative therapeutic strategies.

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P3.

Design, Synthesis and Biological Evaluation of Pyrazolo[3,4-*d*]pyrimidines Active in vivo on Bcr-Abl mutant

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Starting from our in house library of pyrazolo[3,4-*d*]pyrimidine, a cross-docking simulation was conducted on c-Abl T315I mutant to select new derivatives for biological investigations. Among the selected compounds (4a-e), derivative 4b showed a high activity against the Abl T315I mutant ($K_i = 36$ nM). Binding free energy calculation (MM-GBSA), molecular interaction field (MIF) analysis and free energy perturbation (FEP) studies highlighted the importance of the bromine atom in para position of the N1 side chain phenyl ring for the interaction with the hydrophobic pocket I in the T315I mutant. A series of 4-Br derivatives were thus synthesized and biologically evaluated in cell-free assays (c-Src, c-Abl wt, c-Abl T315Imut) and in 32D cell lines expressing the wild type p210-Bcr-Abl and the T315I mutant. Compound **4j** was identified as the most promising one showing a profitable balance of different ADME properties, high activity in cell-free assays and an interesting sub-micromolar potency against T315I Bcr-Abl expressing cells. In addition, liposome encapsulated **4j** was tested on 32D p210 and 32D T315I cell lines at concentrations of 0.1 and 1 μ M in comparison with the DMSO dissolved **4j**. Liposomal formulation increases the solubility of pyrazolo[3,4-*d*]pyrimidine preserving a good activity on leukemic T315I cells and avoiding the use of DMSO as solubilizing agent. In vivo studies on mice inoculated with 32D-T315I cells showed a significant reduction (more than 50%) in tumor volumes after 17 days of treatment with **4j** respect to placebo treated mice.

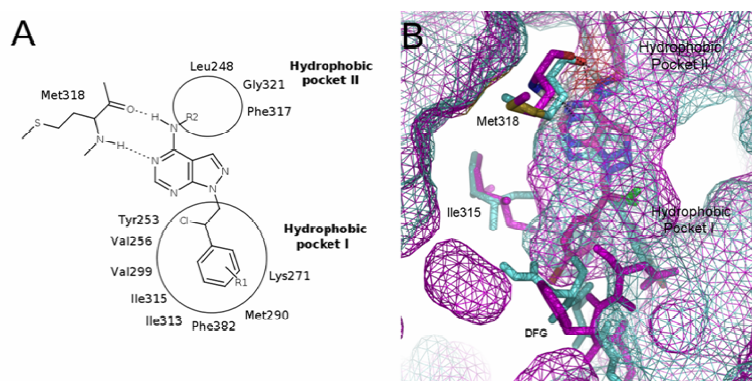


Figure 1. A) Schematic representation of the binding mode of pyrazolo[3,4-*d*]pyrimidines within the T315I Abl mutant binding site B) Binding mode of compound **4b** within 2Z60 (magenta) and 3DK7 (cyano) crystal structures.

Slight differences can be observed in the orientation of the ligand within the hydrophobic pocket I of the two structures.

We are looking for a research group which could be able to test our compounds on CSC lines.

P4.

CDX2 regulation by THE RNA-binding protein MEX3a: impact on intestinal differentiation and stemness

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The homeobox transcription factor CDX2 plays a crucial role in intestinal cell fate specification, both during normal development and in tumorigenic processes involving intestinal reprogramming. The CDX2 regulatory network is intricate but it has not yet been fully uncovered. Through genome-wide screening of a three-dimensional culture system, the RNA-binding protein MEX3A was identified as putatively involved in CDX2 regulation, so its biological relevance was addressed by setting up cell-based assays together with expression studies in murine intestine. We demonstrate here that MEX3A has a translational repressive function by controlling CDX2 levels in gastric and colorectal cellular models. This is dependent on the interaction with a specific binding determinant present in *CDX2* mRNA 3' untranslated region. We have further determined that MEX3A impairs intestinal differentiation and cellular polarization, affects cell cycle progression and promotes increased expression of intestinal stem cell markers, namely *LGR5*, *BMI1* and *MSI1*. Finally, we show that MEX3A is expressed in mouse intestine, supporting an *in vivo* context for interaction with CDX2 and modulation of stem cell properties. Therefore, we describe a novel CDX2 post-transcriptional regulatory mechanism, through the RNA-binding protein MEX3A, with a major impact in intestinal differentiation, polarity and stemness, likely contributing to intestinal homeostasis and carcinogenesis.

P5.

Total Synthesis of Acidic Retinoids: A Library of Potential Cancer Stem Cell Regulators

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Retinoids, a large family of natural and synthetic compounds structurally related to vitamin A, play an important role in a variety of biological functions including vision, development, reproduction and cell differentiation and have been applied successfully to the management of severe skin disorders and more recently to cancer prevention and therapy.¹ Retinoid signaling pathways, mainly induced by *all-trans* retinoic acid (ATRA), are involved in numerous processes in cells, particularly those mediating differentiation and apoptosis. Retinoids exert their effects through their interaction with the nuclear retinoic acid receptors (RARs) and retinoid X receptors (RXRs), which act as ligand-activated transcription regulators for specific genes.² Moreover, ATRA concentration in the cytoplasm is very important for the cell as shortage or excess of causing malfunctions of the retinoid-mediated signaling pathways and is controlled by the so-called cellular retinoid-binding proteins. These proteins, CRABP I and CRABP II enhance the metabolism of ATRA to inactive derivatives³ or mediate channeling of ATRA from the binding protein to its receptors, respectively.²

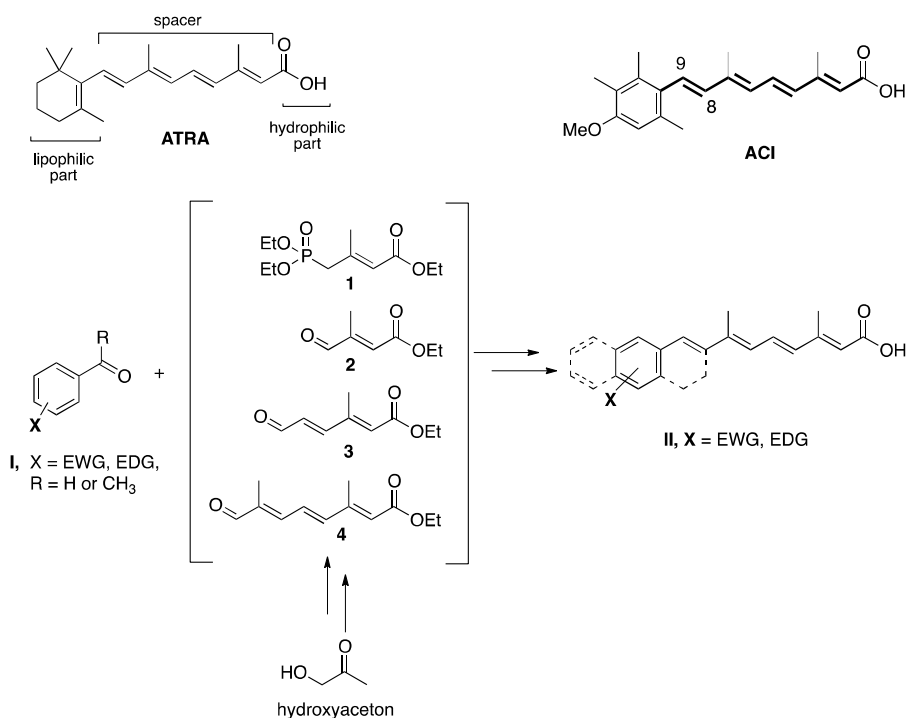
Strategies to induce cancer cell differentiation have been applied to select malignancies. ATRA is the most common differentiating agent in clinical practice and has been successfully used to treat acute promyelocytic leukemia, a stem cell malignancy. Like somatic stem cells, cancer stem cells (CSCs) exhibit self-renewal capacity and differentiation potential, albeit an aberrant and incomplete differentiation potential. Since the initial isolation of CSCs in leukemia, their existence in a wide variety of other cancers has been successfully demonstrated. It is necessary to understand mechanisms, by which differentiating agents influence tumor-initiating CSCs. Ying *et al.* have investigated the cellular and molecular responses of glioblastoma stem-like cells to ATRA. From several studies aiming to reveal the cellular mechanism for this response, it appeared that Notch signaling was the most prominent of these ATRA-responsive pathways.¹ Structurally, retinoids incorporate a lipophilic part (aromatic or non-aromatic), which is connected to a carboxylic group (the hydrophilic part of the retinoid) through a conjugated tetraene chain (Fig. 1). In an effort to improve the therapeutic index of this compound and its selectivity towards several biological targets, a variety of ATRA or acitretin (ACI)⁴ analogs have been synthesized from our research group. Acitretin in particular, a second generation monoaromatic retinoid, is the drug of choice for the systemic treatment of certain types of psoriasis.¹ The mechanism by which acitretin acts seems not to be through binding with the retinoid receptors (it has low affinity for RARs) but through the displacement of ATRA from CRABPs (it has high affinity for CRABPs) and therefore by increasing the ATRA occupancy of the nuclear receptors.⁵

We have synthesized a series of ACI analogs (II), suitable for structure activity relationship studies, and studied initially their potential antiproliferative activity against breast cancer MCF-7 cells. These analogs were designed to present variable electron density, lipophilicity and steric bulk in the aromatic ring as well as variable dihedral angle between the aromatic ring and the spacer.^{4a}

These analogs were readily obtained using commercially available aromatic aldehydes and

methyl ketones (**1**), as starting materials, and linear or convergent method-logies and the Wittig or the Horner-Wadsworth-Emmons reaction for the assembly of the polyene chain. Key-building blocks in the assembly of the tetraene chain were the phosphonate ester *E*-**1**, the *E*-aldehyde **2**, the *E,E*-aldehyde **3** and the *E,E,E*-aldehyde-**4**, all prepared stereoselectively through new methodologies, using hydroxyacetone as starting material.

Given ATRA's importance in the differentiation of CSCs and the library of our synthetic molecules we are currently interested to study (through collaboration with other StemChem members) the differentiation-inducing effect of these acitretin-type retinoids on CSCs, in order to discover and/or develop novel CSC regulators based on retinoids.



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P6.

Drug-induced enrichment of putative cancer stem-like cells for the identification of potential therapeutic targets to overcome their chemoresistance

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Tumour drug resistance is a major issue in the management of cancer patients. It is currently believed that a small sub-population of tumour cells, evidencing stem-like properties (cancer stem-like cells - CSLCs), are partially responsible for tumour drug resistance. The study of this sub-population of cells is therefore extremely important as it may lead to the identification of potential therapeutic targets to improve cancer therapy and patient survival.

Attempting to mimic *in vitro* the enrichment of CSLCs following chemotherapy that may occur in cancer patients, we have incubated lung and pancreatic cancer cell lines with chemotherapeutic agents at sub-lethal concentrations and studied the resulting cell phenotype alterations.

Lung (NCI-H460, A549) and pancreatic (S2013) cancer cell lines were incubated with cisplatin or doxorubicin followed by a drug-free recovery period. Drug treatment led to a transient alteration in cell morphology in lung cancer cell lines, whereby cells acquired a more mesenchymal-like structure. In NCI-H460 cells, cisplatin exposure led to increased resistance towards the selecting drug but also to doxorubicin and gemcitabine. Enrichment of cells expressing the putative stem cell marker ABCG2, and to a much lesser extent CD133, was also observed. Increased expression of the apoptosis-related proteins Bcl-XL and XIAP and of the drug efflux pump P-glycoprotein was verified in the cisplatin-selected population, when compared to the parental NCI-H460 cell line. In the pancreatic cancer cell line (S2013), cisplatin or doxorubicin treatment led to a significant enrichment of cells expressing the stem cell marker CD133. However, only the population of cisplatin-enriched S2013 cells had increased drug resistance when compared to the parental cell line, possibly due to the verified overexpression of anti-apoptotic Bcl2 protein.

The approach of incubating cells with sub-lethal doses of chemotherapeutic drugs has, so far, led to a verified enrichment in cells expressing cancer stem cell markers (CD133 and ABCG2) and possessing increased drug-resistance, possibly due to the overexpression of genes involved in drug efflux and apoptosis. Therefore, this approach could serve as an additional method to enrich for cancer stem like cells, and to identify, and later validate (*e.g.* by siRNA), molecular targets for overcoming chemoresistance in these cells.

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P7.

Stem cell based high-throughput screen to identify inhibitors of DUX4

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First step towards developing a targeted therapy for any diseases is to generate model system for studying the mechanism and for testing various therapeutic approaches. Fascioscapulohumeral muscular dystrophy (FSHD) is diseases which rather affect muscle stem cells than mature myofibers. Most likely the pathological effect is in prenatal and postnatal stage, during muscle formation and muscle regeneration. To address the both stages we engineered various stem cell line suitable to study gain of function during early embryogenesis (human and mouse ES cells) and myogenesis in adults (C2C12). In the cell lines we introduced a single gene, DUX4, which is believed to trigger the molecular cascade of FSHD pathology. We took the advantage of conditional cell toxicity of DUX4 and we developed a small molecule screening platform for identifying inhibitors of DUX4. Assay based on rapid cell death within 24 hours induced by high levels of DUX4 was used for high throughput screen of 44,000 chemicals. The assay was proved to be easy control by titration of doxycycline, adaptable to miniaturized formats, robust, rapid, with low false positive rates and high signal to noise ratios. We identified more than 1280 compounds with significant rescue ability. To narrow down to direct DUX4 inhibitors, we have conducted serial follow up assays, including secondary screens to eliminate compounds which interfere with the rtTA/TRE inducible gene expression system, to distinguish common anti oxidants and/or anti stressors, to confirm reversion of toxicity in different non myogenic cell types. Several classes of compounds reverted toxicity indirectly, including antioxidants.

By developing HTS platform and established cell based systems for follow up studies we established the base for the most crucial topic and urgent needs of FSHD patients: specific and direct pharmacological therapy.

P8.

Anti-metastatic and anti-angiogenic properties of potential new anti-cancer drugs based on metal complexes of selenosemicarbazones

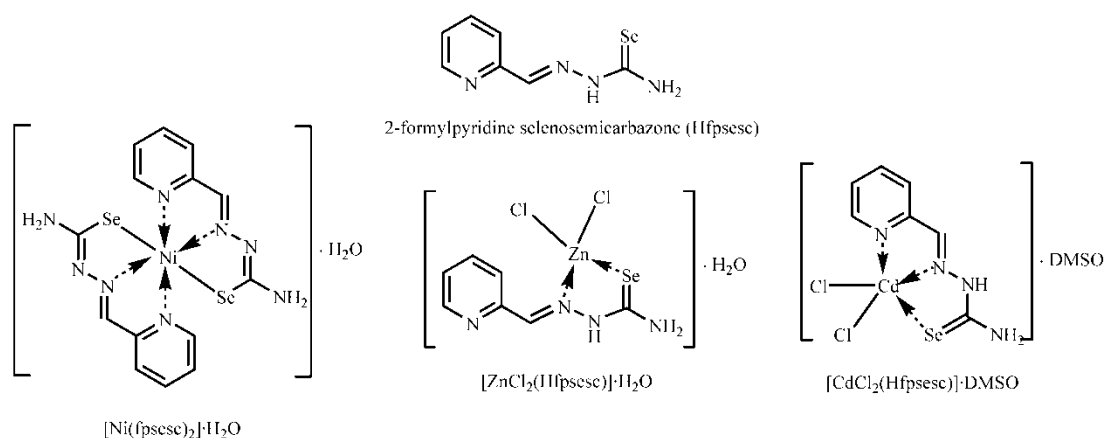
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Matrix metalloproteinases 2 and 9 (MMP-2 and MMP-9) are reliable markers of tumor cell invasion and migration potential. Malignant tumors are characterized by the increased matrix metalloproteinases expression compared to benign tumor, causing more effective degradation of the extracellular matrix resulting in increased invasion and migration of tumor cells. Metal complexes with 2-formylpyridine selenosemicarbazone (Scheme 1) significantly decreased the proteolytic activity of MMP-2 and MMP-9 in metastatic MDA-MB-361 (human breast cancer) cell line. The ligand, Zn(II) and Ni(II) complexes caused a slight decrease of MMP-2 protein secretion and activity in HeLa (human cervix carcinoma) cells, while a significant increase of levels of MMP-2 and MMP-9 secretion and activity was observed upon action of Cd(II) complex or cisplatin on the same cell line. In malignant cells, the complexes inhibited intracellular accumulation of reactive oxygen species, known for pro-angiogenic properties via VEGF (Vascular Endothelial Growth Factor) signaling, but no reduction in total cellular amount of VEGF was found. Also, tubulogenesis test showed anti-angiogenic effect of the complexes in treated endothelial cells. The data indicate that the complexes might have multiple mechanisms of angiogenesis inhibition. In addition, they could modulate metastatic phenotype of tumor cells. Our study provides experimental evidence that metal complexes with 2-formylpyridine selenosemicarbazone inhibited tumor invasion, human vascular endothelial cells migration and tube formation. Ni(II) complex revealed to be the most potent.



Scheme 1.

Acknowledgments. This work was supported by the grant (OI 172055) from Ministry of Education, Science and Technological Development of the Republic of Serbia.

P9.

Synthesis of Taepeenin D analogues as potential cancer stem cell-targeted agents

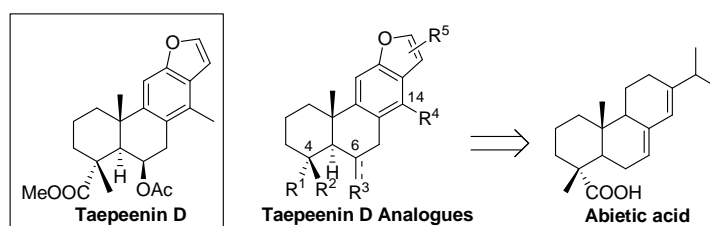
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Signaling pathways, such as the Hedgehog (Hh) pathway, hold the key for unlocking the potential applications of stem cells and are also considered attractive targets for anti-cancer intervention.¹ Although, several small molecule Hh inhibitors are undergoing clinical trials, there is a need to identify new inhibitors capable of overcoming acquired resistance.² In this context, a natural product, Taepeenin D, has been recently identified to interfere with Gli function, which is the last step in Hh signaling.³



Several analogues of Taepeenin D have been targeted for synthesis in order to:

- i) Establish Structure-Activity-Relationships for Taepeenin D, and
- ii) Pave the way for a total synthesis of the natural product itself.

Their preparation, starting from the readily available Abietic acid, will be presented.

Collaboration with other members of this Action is sought in order to evaluate the biological activity of the compounds prepared.

Acknowledgments. This work is part of COST Action CM1106.

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P10.

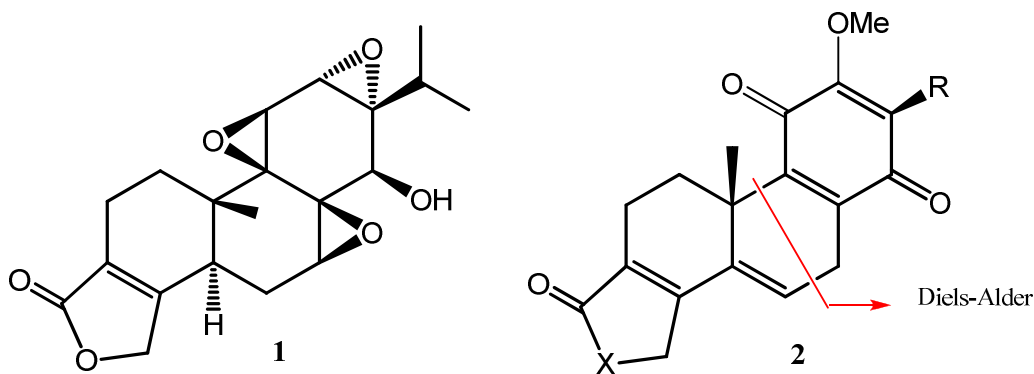
A Straightforward access to an advanced precursor of Triptolide and analogues

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Triptolide **1** is a bioactive ingredient in traditional Chinese medicine that exhibits diverse biological properties, including anticancer properties^{1,2}. One hydrosoluble derivative, minnelide, has been recently developed as a drug against tumor cells in pancreatic cancer.³ The recent identification of GD2 (a glycosphingolipid) as a marker for breast CSCs has opened a way towards the inhibition of CSC-associated tumor growth, chemotherapy resistance and tumor metastasis.⁴ GD3S expression is critical for the expression of GD2, and suppression of its biosynthesis using triptolide against GD3S hampered mammosphere formation and tumor initiation.² Furthermore, as GD2 is an immune suppressant, it would be needed by cancer stem cells to counter immune cells during metastases and inhibition of GD2 expression in cancer cells may enhance the inherent ability of immune cells to kill cancer cells.

In this context, we have developed a straightforward access to an enantiopure triptolide precursor **2** as well as structurally closed analogues (R=alkyls, carbinols, X= O, N, C) using a diastereoselective Diels-Alder cycloaddition of an enantiomerically pure sulfinylquinone⁵ and highly functionalized semicyclic dienes.⁶



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P11.

Mechanism of action the novel tubulin destabilizing agent DTA0100

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The development of new antineoplastic drugs remains a key to progress in cancer treatment. Nowadays, it is widely accepted that in the finding of new biologically active compounds it is essential the use of suitable screening methodologies that can address the mechanism of action at early stages of drug discovery. In addition, identifying the correct target of new bioactive molecules is critical to prevent further drug failures. In the scenario of drug discovery, numerous in vitro testing initiatives had been established. Thus far, no general methodology is established and literature on this hot topic is scarce. In this context, we proposed a strategy based in a Phenotypic Drug Discovery (PDD) approach [1]. Within our program directed at the discovery of new antitumor agents, we have drawn our attention to compounds that disturb the cell cycle. Our strategy relies on the use of a set of biological experiments organized in a modular fashion.

Herein, we exemplified this strategy with a family of propargylic enol ether derivatives [2]. The study of the antiproliferative activity allowed us to define a structure-activity relationship (SAR), being DTA0100 identified as the lead compound. The stereochemistry of DTA0100 is not a critical factor for its biological activity. At a cellular level, DTA0100 induces cellular morphological changes such as the occurrence of multinucleated cells as a consequence of a prolonged mitotic arrest. At a cell signaling pathway level, DTA0100 induces the activation of the mitotic spindle checkpoint. At a protein level, DTA0100 produces a significant reduction of the polymer mass of microtubules affecting the spindle pole formation. We have identified tubulin as the main molecular target of DTA0100. The interaction between DTA0100 and tubulin is located at or near the colchicine binding domain. DTA0100 behaves as a microtubule destabilizing agent.

Using different assays in sequential stages and in stepwise manner, our studies allowed us to understand the bioactivity of this family of compounds and led us to identify tubulin as the main molecular target. This discovery has been carried out using both cell free conditions and living cells. The rational use of chemical techniques in synergy with biological methods in this study led us to understand the bioactivity at the cellular level of a lead selected from a small library of compounds. Moreover, both areas are the foundation that provides a robust and reliable methodology for the identification of target candidates. In this context, bioactive small molecules can be effectively discovered by using a modular approach based on phenotypic changes induced by new chemical entities.

Acknowledgements

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P12.

Synthesis, characterization, cytotoxic and antioxidative activity of d-metal complexes with 2,6-diacetylpyridine bis(selenosemicarbazone)

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A novel ligand 2,6-diacetylpyridine bis(selenosemicarbazone) was synthesized and coordinated with Zn(II), Cd(II) and Ni(II). With Zn(II) and Cd(II), the ligand in dianionic form was coordinated as a quinquedentate in a trigonal bipyramidal geometry. With Ni(II) during coordination the ligand underwent elimination of hydrogen selenide and the product was coordinated as a quadridentate forming a square planar complex, the structure of which was determined by X-ray analysis. The antioxidant activity of the compounds was found to decrease in the order: Cd-complex > Zn-complex > Ni-complex > ligand. The cytotoxic activity of the synthesized compounds was performed on the panel of nine cell lines, including seven tumor cell lines (lung cancer A549, epithelial breast cancer MDA-MB-361, MDA-453, as well as highly metastatic breast cancer MCF-7 cell line, cervical cancer HeLa, melanoma cells FemX, and colorectal cancer cells LS) and two normal (endothelial EA.hy 926 and lung fibroblast MRC-5) cell lines. Cells were treated with the ligand, the complexes, corresponding salts, and cisplatin as a reference compound for 24 h. Cd complex was active to all cell lines, the most susceptible being FemX melanoma cells with IC₅₀ of 1.58 μM. Zn complex was moderately active to almost all cell lines while Ni complex was inactive.

Acknowledgments. This work was supported by the grant (OI 172055) from Ministry of Education, Science and Technological Development of the Republic of Serbia.

P13.

Targeting the wt p53: synthesis of a new family of modulators

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Apoptosis is a tightly regulated multi-step pathway that is responsible for cell death not only during development, but also in adult multicellular organisms, in which it partly controls cell numbers. Deregulation of apoptosis can result in severe pathological syndromes. For example, cancer can be caused in part by a prolonged lifespan of transformed cells that would be normally eliminated. In the last years our research group has been involved in the design and synthesis of new small molecules that act as apoptosis modulators (Figure) [1-4].

In particular, more recently, we became interested in the synthesis of p53 family modulators. In fact, in half of all human cancers, the wild-type p53 tumour suppression protein is inactivated due to the overexpression of endogenous negative regulators such as MDM2. As a consequence, inhibiting the p53-MDM2 protein-protein interaction to reactivate the p53 function represents a promising pharmacological approach for treatment of these cancers. Herein, our recent results on the synthesis of a library of potential new p53 modulators will be disclosed.

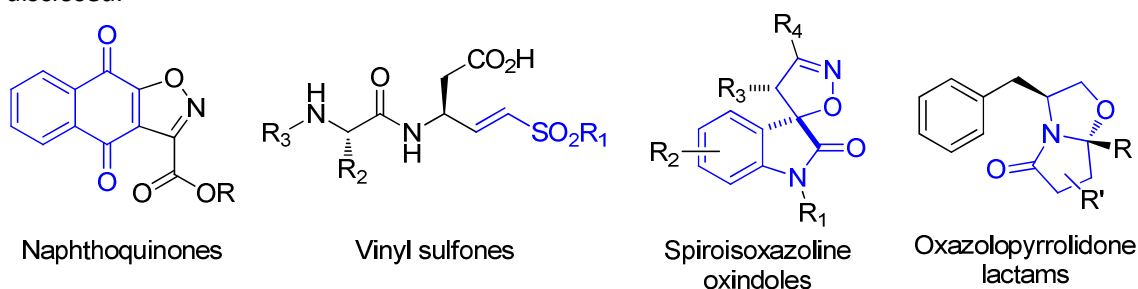


Figure – Libraries of small molecules synthesized at Santos's group.

Acknowledgments

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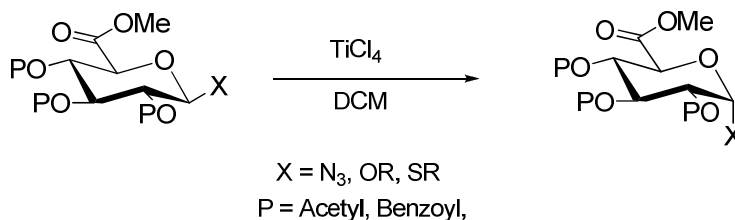
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P14.

Anomerisation of Glycosidic linkages using Titanium(IV) Chloride

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1,2-Cis glycosidic linkages are commonly found in naturally occurring molecules (e.g. KRN7000, heparan sulphate) but are impart difficult to prepare by synthetic means. Thus far the utilization of non-participating protecting groups, the anomeric effect, solvent assisted glycosidations and various glycosyl donors have lead to respectable anomeric ratios in particular examples, but as of yet, a widely applicable and highly stereoselective method for synthesis of such linkages based on the above mentioned methodologies has not been achieved¹.

The Muphy group have previously shown the use of chelation induced anomerisation of 1,2-Trans glycosidic linkages in the presence of acyl protecting groups as highly stereoselective and convenient route to the corresponding α -anomers²⁻⁴. Leading on from this we shall elaborate the utilization of this approach towards to the synthesis of various 1,2-Cis glycosidic linkages.

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P15.

Camptothecin scaffold modification: synthesis and biology

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Camptothecin is a natural occurring pentacyclic quinoline alkaloid that was isolated from *Camptotheca acuminata* and is characterized by remarkable antitumor and antileukemia activity.^[1] It acts as a selective poison of the nuclear enzyme topoisomerase I by forming a ternary complex with topoisomerase I and DNA.^[2,3] The introduction of methylthiol group at position 7 of camptothecin was carried out in four steps. This preparation also yielded the corresponding disulfide, which behaves as a prodrug due to its reactivity with glutathione. Assessment of their antiproliferative activities, investigations of their mechanism of action, and molecular modeling analysis indicated that the 7-modified camptothecin derivatives maintained the biological activity and drug-target interactions of the parent compound.^[4]

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P16.

***In silico* prediction and *in vitro* validation of biomarkers for chemosensitivity to src inhibitors in melanoma cell lines**

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The overall aim of this project is to identify mRNA based biomarkers by which melanoma cell lines can be classified according to their response to src inhibitors.

For this purpose, by analyzing data from melanoma cell lines within two drug screening databases [1,2], we identified a gene signature consisting of 132 genes which could distinguish Saracatinib sensitive and resistant cells. A simultaneous analysis was performed to identify a "melanocyte signature" using two gene expression datasets, E-MTAB-783 and GSE36133; and yet a third one, based on genes functioning in invasion of melanoma [3]. Remarkably, clustering analysis of our samples performed individually for each of these three gene lists yielded the same two distinct clusters with almost the same samples within a given cluster. Thus, among our melanoma cell lines, low expressors of classical melanocyte markers such as Melan-A and Tyrosinase were classified as being Saracatinib sensitive. The same samples showed upregulation of genes involved in invasion suggesting that there is an oncogenic addiction of src protein in invasive melanoma cells. Results from ongoing *in vitro* experiments to validate predicted sensitivity profiles of the melanoma cell line cohort using both Saracatinib as well as two other scr inhibitors, Dasatinib and 10a, will be reported. Future experiments will include testing src activity in src inhibitor sensitive and resistant cells and determining the minimum number of genes by which sensitivity can be predicted by q-pcr.

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P17.

Novel nanoassemblies of known anticancer compounds by coupling with squalene moiety

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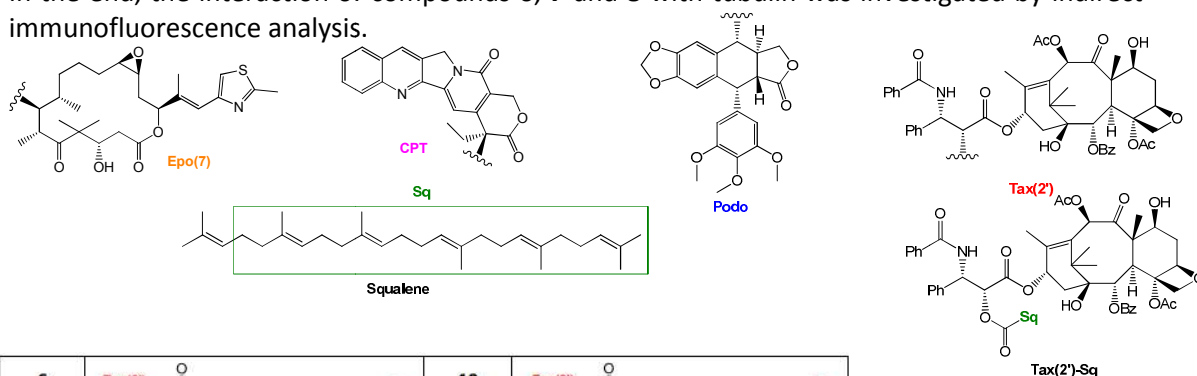
On the base of our experience in the preparation of bivalent compounds in which two drug units are linked via a labile spacer able to release the active compounds¹, we decided to evaluate the possibility of linking two different compounds (a drug and a squalene tail) by a linker containing a disulfide bond. In fact, the presence of the squalene moiety could allow the formation of nanoassemblies in water, facilitating the uptake of the conjugates by cells²: in fact, nanoparticles represent an interesting opportunity, being able to mask the therapeutic agent from its biological environment³. On the other hand, this kind of compounds may undergo a disulfide exchange reaction in the intracellular environment, thus releasing the active drugs.

On this purpose, we prepared a small collection of bioconjugates (Table 1) that demonstrated to be able to self-assemble in water. The nanoassemblies have been characterized for what concerns the mean diameter, the Z potential and the polydispersity index. Compounds **6** and Tax(2')-Sq were also submitted to an Atomic Force Microscopy (AFM) analysis.

We also tested the chemical stability of the disulfide bond in the presence of glutathione (GSH) and, after a mass spectrometry experiment, we found that our compounds are able to release the active drugs. A similar experiment carried on nanoparticles revealed that also in its case the active drug has been released.

A preliminary evaluation of the cytotoxicity of our compounds has been effected against MCF-7 cell line, and some of them showed a good activity.

In the end, the interaction of compounds **6**, **7** and **8** with tubulin was investigated by indirect immunofluorescence analysis.



6 (SB094)		10 (SB087)	
7 (SB090)		11 (SB085)	
8 (SB093)		12 (SB095)	
9 (SB097)		13 (SB098)	

Table 1: Tax(2') = Taxol, Podo = Podophyllotoxin, Epo(7) = Epothilone A, CPT = Camptothecin

COMPOUND	IC50 (μ M)
TAXOL	0.0001
SB094	0.0001
SB087	0.6
TAX(2')-Sq	5
SB090	0.5
SB085	100
SB093	nd

Table 2: Cytotoxic activity of squalene conjugates

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P18.

Learning old tricks from viruses to fight cancer

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The function of many proteins involved in self-renew and tumorigenesis rely on their nucleocytoplasmic transport including FOXO proteins, p53, Stat3 c-Myc and Sox-2. Therefore specific inhibition of nucleocytoplasmic transport has been considered as an attractive anticancer strategy. In order to inhibit this fundamental cellular process we propose to study the molecular strategies used by several viruses to prevent the antiviral response of the host cell by blocking the nuclear import of cellular proteins. We have performed a systematic survey of available data on viral proteins that interfere with the cellular machinery nucleocytoplasmic transport. Viruses capable of interfering with nucleocytoplasmic transport include Venezuelan Equine Encephalitis, Rotavirus Poliovirus, Rhinovirus, Ebola Virus, Influenza Virus, Foot-and-mouth Disease Virus and Vesicular Stomatitis Virus. Specific proteins from these viruses have been shown to interact with cellular proteins such as Nup62, Nup98 Nup153, Nup214, eIF4E, Rae1 and p65 (RelA). Amino acid sequence information of the corresponding viral proteins can be used to design short peptides or peptidomimetics to be analyzed for their capacity to interfere with the nucleocytoplasmic transport of cancer relevant cellular proteins.

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