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EACR- OECI Conference

Precision Medicine for Cancer

1 - 4 March 2015

Neumünster Abbey, Luxembourg

Scientific Organising Committee

Richard Marais (UK) • Simone Niclou (Luxembourg)

Daniel Peeper (the Netherlands)

Programme Book

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OECI Organisation of European Cancer Institutes European Economic Interest Grouping

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LUXEMBOURG INSTITUTE OF HEALTH RESEARCH DEDICATED TO LIFE

Sunday 01 March 2015

Session 1: Personalising precision medicine — Chair: Simone Niclou

14.00 Registration opens (Posters may be put up 14.00 – 16.00)

15.30 – 16.00 Tea, Coffee & Biscuits

16.00 – 16.05 Welcome by the Conference Chairs

16.05 - 16.15
Franck Glod, Head of Unit
Strategic Research Programmes, Fonds National de la Recherche

16.15 – 16.50
Enhancing precision medicine through a Research Information Exchange: The Moffitt Cancer Centre approach
Thomas A. Sellers (USA) - Keynote Speaker
16.50 – 17.00 Questions

17.00 – 17.20
Genetic and Phenotypic Heterogeneity in Glioblastoma: Where Does it End?
Simone P. Niclou (Luxembourg)
17.20 – 17.30 Questions

17.30 – 17.50
Clonal evolution and drug resistance: from cancer avatars to liquid biopsies
Alberto Bardelli (Italy)
17.50 – 18.00 Questions

18.00 – 20.00 Evening Meal (buffet style)

Monday 02 March 2015

Session 2: Tumour heterogeneity — Chair: Richard Marais

9.00 – 9.20
Multilevel molecular analyses in cancer: gauging intra-tumour heterogeneity and elucidating its role in treatment response
Anne-Lise Børresen-Dale (Norway)
9.20 – 9.30 Questions

9.30 – 9.50
Genome driver-based classification of breast cancer and its implications
Carlos Caldas (UK)
9.50 – 10.00 Questions

10.00 – 10.10
Proffered Paper 1
Chronic lymphocytic leukemia exosomes switch stromal cells into cancer-associated fibroblasts to promote leukemogenesis
Jerome Paggetti (Luxembourg)
10.10 – 10.15 Questions

10.15 Trade Exhibition opens

10.15 – 10.45 Coffee Break

Session 3: Responses to precision drugs — Chair: Daniel Peeper

10.45 – 11.05
Cancer Pharmacoepigenetics: Genes and Drugs
Manel Esteller (Spain)
11.05- 11.15 Questions

11.15 – 11.25
Proffered Paper 2
Protein phosphatase 2A activity is a major determinant of therapy response in cancer cells
Jukka Westermarck (Finland)
11.25 – 11.30 Questions

11.30 – 11.50
Deciphering drug response in the era of cancer genomes
Ultan McDermott (UK)
11.50 – 12.00 Questions

12.00 – 12.20
Individualising therapy in children with relapsed malignancies
Stefan Pfister (Germany)
12.20 – 12.30 Questions

12.30 Lunch

13.30 – 14.00 Satellite Symposium I

14.00 – 16.00 Poster Session I (Coffee served at 15.30)

Session 4: Precision medicine in melanoma — Chair: Sergio Quezada

16.00 – 16.20
The biology of melanoma and how to get the best outcomes for patients
Richard Marais (UK)
16.20 – 16.30 Questions

16.30 – 16.40
Proffered Paper 3
A technology platform for personalised medicine in melanoma
Maria Romina Girotti (UK)
16.40 – 16.45 Questions

16.45 – 17.05
In vivo screen for cancer drug target discovery
Daniel Peeper (the Netherlands)
17.05 – 17.15 Questions

17.15 – 17.35
Targeted therapy for the non-BRAF V600 mutant melanoma patient
Jeffrey A. Sosman (USA)
17.35 – 17.45 Questions

17.45 – 18.15 Coffee Break

18.15 – 19.15
Round Table Discussion Forum 1

20.00 Conference Dinner (Abbey Cloisters)

Tuesday 03 March 2015

Session 5: Mining the metabolome for precision drug targets
Chair: Anne-Lise Borresen-Dale

9.00 – 9.20
Therapeutic targeting of tumour metabolism
Susan Critchlow (UK)
9.20 – 9.30 Questions

9:30 – 9.50
Targeting cancer metabolic vulnerabilities
Eyal Gottlieb (UK)
9.50 – 10.00 Questions

10.00 – 10.10
Proffered Paper 4
Targeting hypoxia-induced autophagy improves Natural killer cell mediated anti-tumour immune response
Bassam Janji (Luxembourg)
10.10 – 10.15 Questions

10.15– 10.45 Coffee Break

Session 6: New strategies
Chair: Susan Critchlow

10.45 – 10.55
Proffered Paper 5
XAF1 expression mediated by KIF1Bb and DHX9 interaction leads to neuroblastoma tumour suppression
Zhi Xiong Chen (Singapore)
10.55 - 11.00 Questions

11.00 – 11.10
Proffered Paper 6
Patient derived tumor models for precision medicine: crosstalk between tumour and microenvironment conditions distinct histopathological features of Glioblastoma
Anna Golebiewska (Luxembourg)
11.10 - 11.15 Questions

11.15 – 11.35
Targeting immune-checkpoints in cancer: New mechanistic insights
Sergio Quezada (UK)
11.35 – 11.45 Questions

11.45 – 13.00 Lunch

13.00 – 13.30 Satellite Symposium II

13.30 – 15.30 Poster Session 2 (Coffee served at 15.00)

15.30 – 16.30 Round Table Discussion Forum 2

16.30 Light buffet

17.00 Free Evening to Explore Luxembourg including Guided Tour of the Catacombs (optional)

Wednesday 04 March 2015

Session 7: Resistance to precision medicines Chair: Daniel Peeper

9.00 – 9.20
Strategies to Overcome Resistance to EGFR Targeted Therapies in Lung Cancer
Passi Jänne (USA)
9.20 – 9.30 Questions

9.30 – 9.50
Circulating Tumour cells in Lung Cancer, Biomarkers, Biology and New Mouse Models
Caroline Dive (UK)
9.50 – 10.00 Questions

10.00 - 10.10
Proffered Paper 7
Understanding the Mechanism of Cancer Progression under Hypoxia
Safia Thaminy (Switzerland)
10.10 - 10.15 Questions

10.15 – 10.45 Coffee Break

10.45 Trade Exhibition closes

10.45 – 11.05
Closing Lecture
Title to be confirmed
Anton Berns (Netherlands)
11.05 – 11.15 Questions

11.15 – 11.45 Summary, Award Presentation, Closing Remarks by the Conference Chairs

11.45 – 12.45 Lunch (light buffet)

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The primary objectives of its 70 Members are to reduce fragmentation and to give all European cancer patients the possibility of receiving the best available care. The OECI Members have established themselves in the innovation frontline by tailoring solutions to the individual patient, by gaining in efficiency and efficacy and with the potential to transform the way healthcare is delivered today.

To better achieve its goals, the Organisation works in close collaboration with the European Cancer Patients Coalition.

The OECI goals are achieved by promoting and strengthening the concept of Comprehensive Cancer Centres in Europe in order to improve quality in cancer care and translational research, as well as from an organisational viewpoint. In an effort to efficiently contribute towards an increase in the quality of care, 45% of all OECI Members are already participating in the OECI Accreditation/ Designation programme.

Giving the crucial role to pathology departments in oncology and in lieu of the expected "influx" of new markers and diagnostics, the OECI acts in close cooperation with the European Association for Cancer Research and the European Society of Pathology, in order to better disseminate the innovation process amongst its members and abroad. The EACR-OECI training course series in Luxembourg on "Precise Medicine", and on "Molecular Pathology Approach to Cancer" in Amsterdam, are just two examples of the OECI efforts topromote the dissemination of innovation.

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Speaker abstracts

Enhancing precision medicine through a Research Information Exchange: The Moffitt Cancer Center approach

Thomas Sellers[1]
[1] *Moffitt Cancer Center, Tampa, Florida, USA*

The mainstay of medical research is the randomized controlled clinical trial. That remains the gold standard for testing new interventions. Precision medicine will also require research on how to maximize the effectiveness of existing treatments. For example, predicting response based on tumor markers, germline DNA variation, or lifestyle factors such as use of tobacco or folate intake can be accomplished outside the realm of clinical trials. Similarly, there can be a significant difference between efficacy of a new therapy derived from the subset of cancer patients eligible for, and enrolled in, clinical trials, compared to the effectiveness of therapy delivered to patients who may have significant comorbidities, as is the case in the typical practice or academic medical center. For these reasons, much can be learned from the careful evaluation and analysis of the experience of patient cohorts using observational research methods. In addition, the expenditure on health care is increasing around the world, especially in the United States. There are hopes and expectations that precision medicine will decrease costs by demonstrating improved quality and value. This adds yet another motivation to turn the practice of oncology into a rapid learning system. At the Moffitt Cancer Center, we have an ongoing research protocol, approved by the Institutional Review Board in 2006 that consents patients for use of their medical record, collection of biological specimens for molecular profiling, permission to follow them through their journey with cancer, and to recontact them for future studies. This ambitious effort has resulted in the recruitment of over 100,000 cancer patients and required the creation of an infrastructure that spans patient recruitment, consent, the electronic health record, tissue collection, and data governance. In this talk, the development of the HRI will be described and examples of application demonstrated, including evaluation of detailed care pathways.

Genetic and Phenotypic Heterogeneity in Glioblastoma: Where Does it End?

Simone P. Niclou[1,2]
[1] *Luxembourg Institute of Health, Luxembourg, LUXEMBOURG,* [2] *University of Bergen, Bergen, NORWAY*

Diffusely infiltrating gliomas including Glioblastomas are cancers of the brain for which no curative treatment is available. The molecular and pathophysiological complexity of these tumors represent a major obstacle for effective treatment. Neuropathology remains the mainstay of clinical diagnosis, but is increasingly complemented by molecular genetic analysis to improve classification and provide prognostic and predictive information. Extensive molecular profiling based on transcriptomic, epigenetic and next generation sequencing studies have provided a better delineation of tumor subtypes and underlying driver mutations. However such data also highlighted the prominent heterogeneity between and within patient tumors. In our work, we aim to correlate genetic heterogeneity with differences in tumor phenotypes at the molecular, cellular and histopathological level. We have also described adaptive responses to treatment which appear to be independent of clonal selection, but rely on inherent tumor cell adaptive capacities. Our current understanding of inter- and intra-tumor heterogeneity will be presented and the challenges for glioblastoma therapies will be discussed.

Clonal evolution and drug resistance: from cancer avatars to liquid biopsies

Alberto Bardelli[1]
[1] *University of Turin and Candiolo Cancer Institute IRCCS, Candiolo, Turin, ITALY*

It is now evident that colorectal cancers (CRC) indistinguishable by light microscopy are molecularly distinct diseases requiring unique therapeutic approaches. Tissue and liquid biopsies can be used to define CRC molecular subtypes and to monitor clonal evolution during therapy. Using these approaches, CRC patients were found to respond selectively to targeted agents interfering with oncogenic nodes of the EGFR signaling pathway. Notably, the patient-specific responses can be recapitulated and paralleled in cellular and mouse clinical proxies (CRC-avatars). The inevitable development of acquired resistance to inhibitors of the EGFR signaling pathway presently limits further clinical advances. Strategies to prevent or overcome resistance are therefore essential to design the next generation of molecularly-driven clinical trials for CRC patients.

Multilevel molecular analyses in cancer: gauging intra-tumor heterogeneity and elucidating its role in treatment response

Anne-Lise Børresen-Dale[1]
[1] *Institute for Cancer Research, Oslo University Hospital , Oslo, NORWAY*

The accumulation of data from high-throughput molecular analyses of tumors at various levels are emerging. Combined analyses of gene regulation at various levels may point to specific biological functions and molecular pathways that are deregulated in breast cancer cancers and reveal novel subgroups of patients for tailored therapy and monitoring. We have generated high-throughput data at several molecular levels derived from fresh frozen samples from healthy breast tissue, primary breast tumors and matched metastases, as well as samples from before during and after treatment, using whole-genome mRNA and miRNA expression, SNP-CGH array, DNA-methylation, high-throughput paired-end sequencing, protein expression using RPPA, and metabolic profiles revealed from HR-MAS MR analyses. We have explored to which extent combining the various profiles derived from each level, can further subdivide the initially discovered expression subclasses and improve prognostic potential. Specific alterations identified from such studies have been analyzed using a combination of immunofluorescence and fluorescence in situ (FISH) technique (“double immunoFISH”) to identify intra-tumor heterogeneity in tumors from neo-adjuvant treated patients prior to and after therapy. Results show that the genomic variability prior to therapy was more diverse in the partial-responders vs. the responders, and the remaining tumor was even more heterogeneous after treatment than prior to treatment. Single-cell analyses has been performed using whole-genome amplification and subsequently next-generation sequencing of 40 single DTCs from seven breast cancer patients. Comparing copy-number profiles of the multiple DTCs per patient to each other, to the corresponding primary tumor and when available to those in the lymph node, suggests a continuous dissemination of single tumor cells throughout the tumors evolution. By demonstrating subclonality in the lymph node metastasis, and their copy number profiles resemblance to primary tumor, we provide novel insight into the metastatic process. This study provides a proof-of-principle for sequencing of DTCs and allows insight into tumor cell dissemination and copy-number evolution in DTCs compared to the primary tumors.

Genome driver-based classification of breast cancer and its implications

Carlos Caldas[1]
[1] Cambridge Research Institute - CRUK, Cambridge, UK

Abstract not available at the time of printing.

Proffered Paper 1

Chronic lymphocytic leukemia exosomes switch stromal cells into cancer-associated fibroblasts to promote leukemogenesis

Jerome Paggetti[5], Franziska Haderk[2], Martina Seiffert[2], Bassam Janji[5], Ute Distler[5], Wim Ammerlaan[4], Yeoun Jin Kim[5], Peter Lichter[2], Eric Solary[3], Guy Berchem[5], Etienne Moussay[5]
[1] Centre Hospitalier de Luxembourg, Luxembourg, LUXEMBOURG, [2] German Cancer Research Center DKFZ, Heidelberg, GERMANY, [3] Gustave Roussy, Villejuif, FRANCE, [4] Integrated BioBank of Luxembourg, Luxembourg, LUXEMBOURG, [5] Luxembourg Institute of Health, Luxembourg, LUXEMBOURG

Chronic lymphocytic leukemia (CLL) is characterized by the accumulation in blood and primary lymphoid organs of mature non-functional B lymphocytes. Although CLL cells survive for long time periods in vivo, cells are undergoing apoptosis relatively quickly in vitro. Apoptosis is strongly reduced by mesenchymal stem cells and endothelial cells, which provide anti-apoptotic stimuli to CLL cells via direct contact and soluble factors.

Exosomes, which are small extracellular vesicles (50-100 nm) originating from endosomes, can efficiently transport nucleic acids and transfer RNA, including microRNA, and proteins to target cells. Exosomes derived from solid tumor cells constitute a new component of intercellular communication and are involved in immune suppression, angiogenesis, and metastasis but the role of leukemia-derived exosomes has been less investigated. The pathogenesis of CLL is stringently associated with a tumor-supportive microenvironment and a dysfunctional immune system. The molecular mechanisms by which malignant cells create a favorable surrounding are barely understood.

First we characterized CLL-derived exosome contents and identified oncogenic miRNAs and proteins. Phenotyping at single-particle level confirmed the presence of exosome- and B cell-specific proteins at the surface of exosomes. Then we showed that CLL-derived exosomes are actively incorporated by endothelial and mesenchymal stem cells ex vivo and in vivo. The transfer of exosome proteins and microRNA induces an inflammatory phenotype in target cells, which mimics the phenotype of cancer-associated fibroblasts. As a result, stromal cells showed enhanced proliferation, migration and secretion of inflammatory cytokines, contributing to a tumor-supportive microenvironment. Exosome uptake by endothelial cells increased angiogenesis ex vivo and in vivo, and co-injection of CLL-derived exosomes and CLL cells in immunodeficient mice promoted tumor growth. These findings demonstrate that CLL-derived exosomes modulate several functions of surrounding stromal cells to promote disease progression. Therefore exosomes constitute new interesting biomarkers and their removal could represent a potential therapeutic advance in CLL.

Cancer PharmacoEpigenetics: Genes and Drugs

Manel Esteller[1]
[1] Bellvitge Biomedical Research Institute (IDIBELL), Barcelona, Spain

For the last twenty-five years an increasing amount of evidence has shown the relevance of epigenetics in cell biology and tissue physiology, being DNA methylation aberrations in cancer the flag-ship for the recognition of its disturbance in human diseases. From the candidate gene approaches, new powerful technologies such as comprehensive DNA methylation microarrays and whole genome bisulfite sequencing has recently emerged that have reinforced the notion of epigenetic disruption in the crossroad of many sickness. From the poster-boy cases of MGMT and GSTP1 hypermethylation in the prediction of alkylating drug response and prostate cancer detection, respectively, to the personalized treatment of leukemia with small molecules targeted to fusion proteins involving histone modifiers such as DOT1L and MLL, the field has walked a long path. The current talk will focus in the epigenetic profiling, basically at the level of DNA methylation and histone modifications, that is starting to provide clinical value in the diagnosis, prognosis and prediction of response to drug therapies, with an emphasys in neoplasia, but without forgetting the novel advances in other human disorders. For cancer, we have already a wide view of the undergoing DNA methylation events that expand beyond classical promoter CpG islands of tumor suppressor genes and we have a growing list of mutated chromatin remodeler genes that contributes to the tumorigenesis process. It is time to apply this knowledge in practical clinical situations like the diagnosis of cancers of unknown primary, the screening of malignancies in high-risk populations or a biomarker selection of the patients that should receive treatment with epigenetic drugs.

Proffered Paper 2

Protein phosphatase 2A activity is a major determinant of therapy response in cancer cells

Otto Kauko[2], Susumu Omanishi[2], Daniel Laajala[2], Evgeny Kulesskiy[1], Mikael Jumppanen[2], Petteri Hintsanen[1], Bhagwan Yadav[1], Veronika Suni[2], Pekka Haapaniemi[2], Garry Corthals[2], Tero Aittokallio[1], Krister Wennerberg[1], Jukka Westermarck[2]
[1] Institute for Molecular Medicine Finland, University of Helsinki, Helsinki, FINLAND, [2] Turku Centre for Biotechnology, University of Turku and Åbo Akademi University, Turku, FINLAND

Protein phosphatase 2A (PP2A) dephosphorylates majority of Ser/Thr phosphorylated proteins. Consequently, PP2A is an antagonist of multiple oncogenic pathways and PP2A reactivation may provide an alternative route to target these pathways. Importantly, because PP2A reactivation would result in simultaneous dephosphorylation of both collateral and downstream effectors of kinase pathways, it might circumvent commonly encountered kinase inhibitor resistance mechanisms. However, the role of PP2A biology in determining therapy response in cancer cells

has not been systematically evaluated as yet. To systematically study the role of PP2A in cancer therapy response we used RNAi targeting against PP2A inhibitor proteins (PP2A reactivation) and PP2A structural subunits (PP2A inhibition), together with high throughput drug sensitivity screen covering 300 clinical cancer drugs and investigational compounds. Changes in phosphorylation status of PP2A targets in response to RNAi perturbation, was studied by LC-MS/MS-based label-free quantitative phosphoproteomics analysis.

Importantly, we show that cancer cell drug sensitivity across 300 compounds correlates with PP2A activity profile so that PP2A inhibition made cells on average resistant to therapies, whereas their sensitization by PP2A inhibitor protein siRNAs correlated with changes in phosphoprotein regulation. In particular, cancer cell response to kinase inhibitors followed very closely PP2A activity regulation and inhibition of all PP2A inhibitor proteins resulted in increased average sensitivity to 105 kinase inhibitors. Furthermore, we show that PP2A reactivation results in convergent phosphorylation patterns with targeting of kinase pathways by RAS inhibition. Among the kinase inhibitors with highest correlation to PP2A activity we identified multiple inhibitors of EGFR, VEGFR, Trk, Aurora, JAK2, and FLT3 kinases. We also identify novel PP2A regulated phosphorylation sites in target proteins of these kinases. These data reveal the unprecedented importance of PP2A activity for kinase inhibitor responses, and underscore the value of developing PP2A reactivating compounds for combination therapies.

Deciphering drug response in the era of cancer genomes

Ultan McDermott[1]
[1] Sanger Institute, Cambridge, UK

Over the last decade we have witnessed the convergence of two powerful experimental designs towards a common goal of defining the molecular subtypes that underpin the likelihood of a cancer patient responding to treatment in the clinic. The first of these â€˜experimentsâ€™ has been the systematic sequencing of large numbers of cancer genomes through the International Cancer Genome Consortium and The Cancer Genome Atlas. This endeavour is beginning to yield a complete catalogue of the cancer genes that are critical for tumourigenesis and amongst which we will find tomorrowâ€™s biomarkers and drug targets. The second â€˜experimentâ€™ has been the use of large-scale biological models such as cancer cell lines to correlate mutations in cancer genes with drug sensitivity, such that one could begin to develop rationale clinical trials to begin to test these hypotheses. It is at this intersection of cancer genome sequencing and biological models that there exists the opportunity to completely transform how we stratify cancer patients in the clinic for treatment.

Tumor classification based on DNA methylation fingerprints

David Jones[1], David Capper[1, 2], Martin Sill[1], Volker Hovestadt[1], Andreas von Deimling[1, 2], Stefan Pfister[1, 2]
[1] German Cancer Research Centre, Heidelberg, [2] German Cancer Research Center and University Hospital, Heidelberg

Recent revolutionary advances in genomics technologies have fostered a large variety of new discoveries in the field of (pediatric) neurooncology, but at the same time pose the option & challenge of applying these new methods in a clinical setting.
We have very good evidence that doing so is feasible and may help clinical decision making in multiple ways. The most important step before treatment even starts is establishing the correct diagnosis. In contrast to many other tumor entities, morphology is still the mainstay of diagnostics for brain tumors. Adding other layers of information by using complementary molecular information e.g., DNA methylation profiling yields in a much higher diagnostic accuracy. Our experience after profiling >7000 brain tumors (amongst which ~500 were prospective diagnostic cases) yielded in a change in diagnosis in ~10% and a gain in additional information (e.g., tumor subgroup) in another 30-35% of cases. Since the test can be done from minute amounts of formalin-fixed and paraffin-embedded material and shows very little variability across different labs, DNA methylation fingerprinting appears to be a particularly feasible, useful and powerful new tool to aid tumor classification.
The explanation for this close association of DNA methylation fingerprints with molecular subgroups is most likely that genome-wide promoter methylation patterns comprise a very accurate memory of the cell of origin and are very stable upon transformation and even tumor evolution.
Collectively, our findings in more than 70 subgroups of brain tumors including brain metastases from non-CNS tumors strongly suggest that this principle could also exploited in other tumor entities.

The biology of melanoma and how to get the best outcomes for patients using targeted therapies

Richard Marais[1]
[1] CRUK Manchester Institute, Manchester, UK

BRAF is a protein kinase that is mutated in about 40% of melanomas. The small G-protein NRAS, an upstream activator of BRAF, is mutated in a further 20% of cases. These proteins are components of the RAS/RAF/MEK/ERK signalling pathway, which regulates cell growth and survival. Drugs that inhibit BRAF are effective in patients whose tumours have BRAF mutations, and drugs that inhibit MEK are effective in patients whose tumours have BRAF or NRAS mutations. However, most patients develop resistance to these drugs after a relatively short period of disease control. Resistance is mediated by many mechanisms, but in most cases it is driven by reactivation of this signalling pathway. In about 25% of cases resistance is mediated by acquisition of mutations in NRAS, and we have also shown that in a high proportion of cases it can be mediated by hyper-activation of the EGF receptor (EGFR). We have shown that EGFR signals through SRC family kinases (SFK) and NRAS signals through CRAF, a close relative of BRAF, and in parallel, we developed panRAF (BRAF+CRAF) inhibitors that also inhibit SFK. We tested these compounds in cell lines and patient-derived-xenografts (PDX) from patients who had developed resistance to BRAF inhibitors or BRAF/MEK inhibitor combinations. We show that our panRAF inhibitors are active in melanoma when resistance is associated with acquisition of mutation in NRAS, or when it is associated with hyper-activation of SFK. We posit that these drugs work in NRAS mutant tumours because they inhibit both BRAF and CRAF, and in tumours where resistance is mediated by upregulation of receptor tyrosine kinases because they inhibit SFK. These data show that in-depth knowledge of tumour genetics and biology can provide the necessary insight to implement personalised medicine for melanoma patients and thereby improve responses to targeted therapies.

Proffered Paper 3

A technology platform for personalised medicine in melanoma

Maria Romina Girotti[1], Dominic Rothwell[1], Amaya Viros[1], Amit Kumar Mandal[1], Gabriela Gremel[1], Simon Furney[1], Malin Pedersen[2], Jane Rogan[1], Jacqueline Swan[1], Alberto Fusi[3], Ged Brady[1], Paul Lorigan[3], Caroline Dive[1], Richard Marais[1]
[1] Cancer Research UK Manchester Institute, Manchester , UK, [2] The Institute of Cancer Research, London, UK, [3] The University of Manchester & The Christie NHS Foundation Trust, Manchester

UKWe developed patient derived xenografts (PDX), performed whole exome sequencing (WES) and analysed circulating tumour DNA (ctDNA) to complement clinical management of four melanoma patients. Synchronous treatment of patients and mice showed that PDX responses mirrored those of the patients' tumour to BRAF inhibitors. PDXs were used to test second-line treatments in relapsed patients and to validate WES-based therapies in tumours that did not have a BRAF mutation. Longitudinal analysis of ctDNA was predictive of responses to targeted and immunotherapies, and could be used to determine mechanisms of resistance. Thus, we describe a powerful combination of techniques for personalised medicine in melanoma and will present the challenges and limitations of implementing these novel technologies in patient management.

Systematic genetic perturbation to reveal oncogenic dependencies in vitro and in vivo

Daniel S. Peeper[1]
[1] *Netherlands Cancer Institute, Amsterdam*

Melanoma is the most aggressive type of skin cancer and its incidence is steadily increasing. Melanomas tend to spread rapidly, which is associated with a grim prognosis. Until recently, most advanced stage melanomas were refractory to the available therapeutic options, but there are recent developments offering better perspectives. For example, new therapeutic approaches have become available, which target genetic vulnerabilities within the melanomas. A primary example of such a dependency is the common $BRAF^{V600E}$ mutation, which is essential for proliferation and survival of melanoma cells. In the clinic, the mutant BRAF oncogene product can be targeted by specific inhibitors, including vemurafenib, which cause unprecedented melanoma regression. However, relapse eventually occurs around six months due to a variety of resistance mechanisms, both MAP kinase-dependent and -independent. Therefore, in spite of these new perspectives, there is a dire need to identify additional targets amenable to therapeutic intervention, to be used in combination with vemurafenib or other specific inhibitors to overcome or prevent drug resistance and achieve more durable responses. To achieve this, we set out to identify melanoma factors that are required for proliferation and survival specifically in an in vivo setting. Thus, we performed negative selection RNAi screens parallel in vitro and in vivo and focused on the hits that were preferentially depleted in tumors relative to the corresponding cells in culture. The results from these screens will be discussed.

Targeted Therapy for the Non-$BRAF^{V600}$ mutant melanoma patient

Jeffrey A. Sosman[1], Igor Puzanov[1], Douglas B Jonson[1], Katie Hutchinson[1], and Kim Dahlman[1]
[1] *Vanderbilt University Medical Center, Nashville, Tennessee*

The presence of the $BRAF^{V600}$ mutation in melanoma has had a great impact on therapy allowing 40% of patients to be offered and respond to targeted therapy with BRAF and MEK (i) inhibitors. However, targeted therapy options in patients without $BRAF^{V600}$ mutations have not been well defined. We have taken several tactics to approach treatment in this large cohort that make up 60 % of melanoma patients. NRAS mutant melanoma make up about 1/3 of these patients and the other 2/3 are a heterogeneous population with only about 11% that are melanomas with NF1 loss of function due to genetic alterations. So our approach has centered on finding ways to better target NRAS mutant melanoma and better define subsets in the NRAS,-BRAF- cohort that may be responsive to specific therapies. First, preliminary data based on preclinical experiments have shown a greatly enhanced anti-tumor response when CDK4/6i were added to MEKi. This finding along with TCGA data that demonstrated a high frequency of alterations in the genes regulating cell cycle led to two phase I/II clinical trials combining MEK i (binimetinib or trametinib) with CDK4/6i (LEE001 or palbociclib) Early results in the trial combining binimetinib and LEE001 have demonstrated clinically significant objective responses in 7/21 (33%) patients with most of the other patients demonstrating tumor reduction. Some patients had failed intense immunotherapy and others had rapid relief of clinical symptoms. Both of these regimens are completing phase I components of the trial to determine a recommended phase II dose and schedule.

On the other hand, we have found the presence of atypical BRAF mutations (non-V600) and BRAF fusions both that activate the MAP kinase pathway in about 10% of the BRAF-,NRAS- population. Furthermore, in vivo studies demonstrate the sensitivity of these genetic alterations to MEKi. This has been also observed in patients receiving MEKi therapy with underlying atypical BRAF mutations. Based on these findings we have initiated a trial of MEKi (trametinib) in patients with atypical BRAF mutant melanoma and those with BRAF fusions. This trial has just been opened in multiple centres across the United States. Certainly efforts to target NFI mutated or deleted melanomas whose changes lead to loss of function are critical to clinically define. We believe MEKi based therapy will provide a template to define other combination regimens of inhibitors that will make progress expanding the options for targeted therapy in those patients without $BRAF^{V600}$ mutant melanoma.

Therapeutic targeting of tumour metabolism

Susan Critchlow[1]
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One of the emerging areas of cancer biology that has been recognised as a 'hallmark of cancer' is the reprogramming of tumour metabolism to fuel cell growth and proliferation. Several metabolic pathways have been implicated in the metabolic reprogramming observed in tumours including increased flux through the glycolysis, glutamine and pentose phosphate pathways, increased rates of lipid synthesis and maintenance of redox balance. Furthermore, activation of oncogenes and/or inactivation of tumour suppressor genes results in specific metabolic reprogramming that drives addiction to a specific metabolic pathway. Targeting tumour metabolism pathways to identify new cancer therapies has received renewed interest.
The metabolic phenotype of many tumours switches from oxidative phosphorylation (used by most normal epithelial cells) to aerobic (Warburg Effect) or anaerobic glycolysis. This significantly increases rates of glucose consumption and lactate production, enabling tumours to meet their energy and biosynthetic demands even under conditions of low nutrients and O_2. However the end-product of glycolysis, lactate, is a metabolic 'dead-end', which if allowed to accumulate in the tumour cell can cause feedback inhibition of glycolysis, intra-cellular acidification and inhibition of cell growth. Therefore pharmacological inhibition of lactate transport represents a promising therapeutic strategy to target a range of human cancers. Monocarboxylate transporters (MCT1-4) catalyse proton-linked transport of monocarboxylates across the plasma membrane with MCT1 and MCT4 being the key tumour-associated lactate transporters. We will describe the development of selective inhibitors of MCT1 and MCT4 and show that inhibition of lactate transport offers a novel mechanism for targeting the metabolic phenotype of tumours.

Targeting cancer's metabolic vulnerabilities

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Due to the nature of tumorigenesis, cancer cells constantly encounter environments in which nutrient and oxygen availability is severely compromised. In order to survive these harsh conditions, cancer cell transformation is often coupled with large changes in metabolism to satisfy the demands for energy and biomass imposed by continued cellular proliferation. This metabolic adaptation often involves increases in the consumption and metabolism of extracellular resources. However, during instances of nutrient stress cancer cells can further modify or shift their metabolism to confront these new challenges. siRNA screens identified acetyl-coA synthetase 2 (ACSS2), an enzyme which converts acetate into acetyl-coA, as an essential enzyme for cellular growth under metabolic stressed conditions of breast and prostate cancer cells. In accordance, these same conditions markedly induced the expression of ACSS2. Data mining and disease linkage revealed that ACSS2 exhibited a high level of genomic copy number gain in invasive breast carcinomas. Characterization of acetate metabolism confirmed it was consumed by cancer cells under hypoxia in an ACSS2-dependent manner and was used to synthesize lipids and TCA-cycle intermediates. These mechanistic effects were reinforced by phenotypic studies showing that ACSS2 silencing inhibited growth in 2D cultures, 3D spheroids and tumour xenografts. In summary, our data revealed a previously unappreciated role for acetate as a nutritional source for the growth and survival of cancer cells under metabolically stressful conditions.

Proffered Paper 4

Targeting hypoxia-induced autophagy improves Natural killer cell mediated anti-tumour immune response

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Hypoxia, a common feature of the tumor microenvironment, is associated with tumor resistance to anti-cancer therapies. Recently, it has been reported that hypoxic tumor microenvironment also plays a significant role in shielding tumor cells from immune attack either by promoting immune suppression, or by inducing many other oncogenic events in cancer cells, allowing tumors to escape immune surveillance. Natural killer (NK) cells are effectors of the innate immune system, able to kill cancer cells through the release of the cytotoxic protease granzyme B. It has been reported that hypoxic tumor microenvironment interferes with the antitumor function of NK cells by mechanisms that are not fully understood. Here, we provided evidence that hypoxia decreases breast cancer cell susceptibility to NK-mediated lysis by a mechanism involving the activation of autophagy in cancer cells. Targeting autophagy was sufficient to restore NK-mediated tumor cell killing under hypoxia. Furthermore, we showed that the resistance of hypoxic tumor cells to NK cell attack was neither related to a defect in their recognition by NK cells, nor to a defect in the cytolytic function of NK cells toward hypoxic cells. We demonstrated that autophagy activation degrades NK-derived granzyme B in the lysosomes of hypoxic cells making them less sensitive to NK-mediated killing. Inhibition of autophagy restored granzyme B levels and reverted the resistance of hypoxic cells in vitro. Our results highlight autophagy as a critical factor in modulating NK-mediated anti-tumor immune response. We have validated this concept in vivo by showing that targeting autophagy significantly improved NK-mediated tumor shrinking in breast and melanoma models. This study provides a cutting-edge advance in our understanding of how hypoxia-induced autophagy impairs NK-mediated lysis and paves the way for formulating more effective NK-based antitumor therapy by combining autophagy inhibitors.

Proffered Paper 5

XAF1 expression mediated by KIF1Bb and DHX9 interaction leads to neuroblastoma tumour suppression

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Background: Developmental apoptosis of precursors is crucial in determining the final number of terminally differentiated neurons. During neural development, cells undergo apoptosis as NGF becomes limiting. Aberrant developmental apoptosis is implicated in pediatric sympathetic nervous system tumors. When NGF becomes limiting, a developmental apoptotic pathway is activated which requires KIF1Bb. KIF1Bb is necessary and sufficient for neuronal apoptosis during NGF withdrawal. KIF1Bb maps to 1p36.2, a frequently deleted region in neural crest-derived tumors including neuroblastomas.

Methods: Immunoprecipitation/mass spectrometry screen, mutagenesis studies, apoptosis assays, overexpression or silencing studies, RNA-SEQ, immunohistochemistry, patient studies and mouse models are the key methods used.

Results: We identified that KIF1Bb-induced apoptosis requires RNA/DNA helicase DHX9. KIF1Bb interacts with DHX9 to promote translocation of cytoplasmic DHX9 into the nucleus, resulting in transcription of apoptotic XAF1. Transcription-impaired or nuclear localization-impaired DHX9 is unable to potentiate KIF1Bb-induced cell death. Knockdown of DHX9 also protects from KIF1Bb-induced cell death whereas KIF1Bb loss-of-function domains or patient-associated point mutants are unable to translocate cytoplasmic DHX9 into the nucleus, impairing XAF1 expression. Furthermore, XAF1 silencing protects from KIF1Bb-induced apoptosis whereas XAF1 overexpression is necessary and sufficient to induce apoptosis in a variety of neuroblastoma cell lines. Conditional knockout of KIF1Bb in the superior cervical ganglia neurons of mouse pups also specifically ablates XAF1 expression, suggesting that KIF1Bb and XAF1 act along the same pathway. These results are being further validated in mouse xenografts harboring inducible XAF1-overexpressing or –silenced neuroblastoma cells in conjunction with tissue microarray studies on pre-treatment or post-treatment neuroblastoma patients who are 1p-intact or 1p-deleted.

Conclusion: Recent literature strongly pointed to KIF1Bb as a bonafide tumor suppressor. Our findings provide a mechanistic understanding of this role, whereby KIF1Bb interacts with DHX9 to mediate the expression of XAF1, which is necessary and sufficient to induce apoptosis in neuroblastomas.

Proffered Paper 6

Patient derived tumor models for precision medicine: crosstalk between tumour and microenvironment conditions distinct histopathological features of Glioblastoma

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The molecular and pathophysiological complexity of Glioblastoma (GBM) represents a major obstacle for effective therapies. Like all solid tumors, GBM do not develop autonomously, but evolve in a unique brain microenvironment, that contributes to the temporal and regional heterogeneity of these malignant tumors. It has long been difficult to reproduce the complex histopathological features of GBM in animal models. Over the last years we and others have established patient derived xenograft (PDX) models based on organotypic GBM spheroids that recapitulate genetic and histological characteristics of human GBM, a prerequisite for personalized translational research. Here we show that such models reproducibly generate tumors in the mouse brain that can be classified in three distinct histological phenotypes: 1. a highly ‘invasive’ phenotype with normal brain vessels, 2. a highly ‘angiogenic’ phenotype displaying microvascular proliferation and 3. an ‘intermediate’ phenotype combining features of invasion and vessel pathology. Angiogenic and intermediate tumor phenotypes display increased hypoxia, dependence on glycolysis and release of angiogenesis-associated factors. Interestingly the phenotypic differences establish early during tumor development suggesting an instructive role of tumor cells on the surrounding microenvironment. On the other hand we also find that such ‘instructed’ stromal cells impact on tumor growth, indicating a reciprocal and reinforcing crosstalk between tumor and stroma. By large scale gene expression profiling of isolated tumor and endothelial cells, we have identified the key molecular drivers underlying this crosstalk. Insight into the molecular and neuropathological complexity of GBM xenografts allows to use PDX models for personalized translational research including testing differential drug responses.

Targeting immune-checkpoints in cancer: New mechanistic insights

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The continual interplay between the immune system and cancerous cells is thought to result in the establishment of a dynamic state of equilibrium. This equilibrium depends on the balance between subsets of effector and regulatory lymphocytes. Whereas the overall mechanisms underpinning the establishment and maintenance of the intra-tumour balance between Teff and Treg cells remain unknown, in many solid cancers it is characterized by the dominant infiltration of regulatory T cells over effector T cells resulting in a low Teff/Treg ratio. Furthermore, different subtypes of regulatory cells and inhibitory molecules such as CTLA-4 tightly control the few effector T lymphocytes that manage to infiltrate the tumour. The outcome of this balance is critical to survival, and while in a few cases the equilibrium resolves in the elimination of the tumour by the immune system, in many other cases the tumour manages to escape immune control.
Remarkably, antibodies against CTLA-4, a key immune modulatory receptor expressed on T cells, efficiently modify this balance, driving effector T cell expansion and increasing the ratio of Teff/Treg within the tumour. Whilst the high Teff/Treg ratio driven by anti-CTLA-4 directly correlates with tumour destruction in mice and humans, the mechanisms underpinning this phenomenon remain unknown.
By focusing in the study of effector and regulatory tumour-reactive CD4+ T cells my group is interested in the mechanism underpinning the activity of different immune-modulatory antibodies within the tumour microenvironment, and the potential positive and negative impact that the tumour microenvironment may have in the recruitment, survival and function of different T cell subsets. In this context and using a murine model of melanoma we have recently demonstrated that both, the change in the Teff/Treg balance as well as tumour rejection, depend on the selective depletion of tumour-infiltrating Treg cells expressing high levels of surface CTLA-4. Regulatory T cell depletion is mediated by ADCC and completely depends on the expression of FcgRIV on tumour infiltrating CD11b+ myeloid cells. These results reveal novel and unexpected mechanistic insight into the activity of anti-CTLA-4-based cancer immunotherapy, and illustrate the importance of specific features of the tumour microenvironment on the final outcome of antibody-based immune-modulatory therapies.

Strategies to Overcome Resistance to EGFR Targeted Therapies in Lung Cancer

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Abstract not available at the time of printing.

Circulating Tumour cells in Lung Cancer, Biomarkers, Biology and New Mouse Models

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There is increasing agreement that minimally invasive biomarkers to monitor patients receiving targeted therapies have huge potential benefit. The rarity and heterogeneity of CTCs make them a technically demanding source of biomarkers but one that holds great promise as the technical challenges are met. Marker independent technology platforms are in development which will better assess CTC subpopulations but none so far have been fully validated for clinical trial use and treatment decision making. CTC enumeration with CellSearch (EpCam and cytokeratin positive CTCs) has prognostic significance in many epithelial tumours, and in diseases with prevalent CTCs such as small cell lung cancer (SCLC), the dynamic range of CTC number allows pharmacodynamic evaluation. Biomarkers based on multiplex protein analysis and FISH are also evaluable in CTCs if sufficient can be detected. Developments in single CTC isolation and genomic profiling are increasingly reported by allowing insight to the biology of invasive tumour cells including cellular co-expression of cancer specific mutations. I will present our progress in DNA profiling of single SCLC CTCs, and how this approach may inform on heterogeneity, and via serial sampling, on mechanisms of drug resistance.
Most recently, we developed lung cancer patient CTC derived mouse models (termed CDX). SCLC CDX models faithfully recapitulate patient drug responses and will be useful to test novel therapeutics. CDX are generated at patient presentation and for those patients who first respond and then relapse with drug resistant disease, the relapse blood sample can be used to generate a paired CDX. Alongside single CTC profiling, the CDX approach will allow a comprehensive analysis of acquired drug resistance to chemotherapy, the discovery of new drug targets and testing of targeted therapies and drug combinations. To evaluate heterogeneity in CDX, we have developed a protocol for viable disaggregation of CDX tumours coupled with removal of dead cells and contaminant mouse cells. We are currently using this protocol to ask how many CDX derived cells are required to regrow a CDX and whether regrowth is from a distinct sub-population which may correspond to human SCLC ‘cells with stem-like characteristics. Finally, I will present a case report on a patient whose blood sample contained no CellSearch CTCs yet generated a CDX model. CTC filtration of this patient’s blood revealed a high proportion of mesenchymal CTCs consistent with Epithelial to Mesenchymal Transition (EMT).

Proffered Paper 7

Understanding the Mechanism of Cancer Progression under Hypoxia

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Although extensive studies have been conducted to understand breast cancer progression, we do not precisely know how hypoxia, a pivotal microenvironmental factor, is involved in this process. Addressing this question, more precisely identifying the critical genes involved, is of crucial importance to provide alternatives and more efficient anti□cancer therapies.

In this study, we used as a model breast cancer cell lines with distinct aggressiveness properties and developed an innovative strategy based on a system□wide quantitative proteomics in combination with a high-throughput migration screen and protein network analysis.

We found that the less aggressive cell line temporally regulated cell migration under hypoxia and the underlying mechanism involved the PTPRG and TACSTD2 driver genes. In a large□scale migration screen, we identified a novel migration component enriched in lysosomal genes that suggested a mechanism of cell migration based on the cellular trafficking of proteins. We dissected the underlying mechanism by phosphoproteomics analysis and found that the regulation profile of stathmin (STMN1) at serine 16 remarkably discriminated the poorly invasive from the highly invasive cell line. Unexpectedly, stathmin knockdown dramatically enhanced cell migration of poorly invasive cells only under hypoxia.

Combined, these results led us to propose a model of cancer progression based on the hypoxia□dependent trafficking of vesicles and their release in the extracellular space. This study represents the first integrative□level attempt to understand the mechanism of cell migration under hypoxia and suggests that hypoxia might inhibit the early stages of cancer progression, however promotes progression at later stages.

Closing Lecture

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Abstract not available at the time of printing.

Poster abstracts

1

Patient-derived prostate cancer models for personalized medicine

Taija af Hällström[3], Päivi Östling[3], Khalid Saeed[3], Vesa Rahkama[3], Samuli Eldfors[3], Dmitry Bychkov[3], John Patrick Mpindi[3], Bhagwan Yadav[3], Tero Aittokallio[3], Tuomas Mirtti[1,3], Antti Rannikko[2,3]
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Prostate cancer (Pca) is the most common noncutaneous malignancy and the second leading cause of cancer mortality in men. Despite the development of new next generation anti-androgens many initial responders develop resistance in a relatively short time. Thus, novel therapies are needed for the treatment of Pca. The aim of this project is to develop new patient-derived Pca models and pioneer the application of high-throughput drug sensitivity and resistance testing (DSRT) together with molecular and protein expression profiling to identify novel treatment possibilities for Pca.

We have established conditionally reprogrammed cells (CRC) from prostate patient-derived benign and cancer tissue as described by Liu *et al*., 2012. The CRC protocol allows us to initiate and expand cultures directly from the patient for 3 months and beyond, and to generate the needed amount of CRCs for genomic, transcriptomic and protein expression profiling and DSRT in 2-3 weeks from a small piece of tissue. CRC protocol is based on co-culture of freshly isolated primary cells with Rho/Rock kinase inhibitor (Y-27632) and irradiated 3T3-feeder cells. We have initiated CRCs from 7 Pca patients (6 hormone naïve and 1 castration resistant Pca sample; CRPC). However, only CRPC material gave rise to cancer CRCs. CRCs retain their epithelial phenotype and represent transit amplifying cells with simultaneous expression of basal and luminal cell markers. Cancer CRCs retain chromosomal changes typical to Pca patients. The DSRT platform has been piloted on CRCs with a library of 306 FDA-approved and investigational oncology drugs for high-throughput screens testing drugs in five different concentrations using cell viability as read-out (Pemovska et al., 2013). The ultimate goal is to carry out DSRT while the patient is undergoing treatment in the clinic, to assess the possibility of providing options for n-of-1 type trial, if the disease progresses beyond current treatment options.

2

Distribution and types of EGFR Mutations in Pakistani Lung Adenocarcinoma for Predicting Response to Targeted Therapy

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Objective

To screen for EGFR mutations in tumor tissue of NSCLC patients by Cobas EGFR mutation test.

Introduction

In lung cancer, the discovery of acquired genetic alteration in EGFR has changed the way it is currently being diagnosed and treated. EGFR mutation screening has become imperative for the selection of metastatic NSCLC patients eligible for targeted treatment. This report presents distribution of EGFR mutations in 191 NSCLC patients.

Methods

EGFR mutation in tumor samples was screened by multiplex real time PCR (Roche Diagnostics, USA) according to the manufacturer's instructions. Briefly, DNA from FFPE tissue, obtained from Histopathology sections, was extracted and amplified with primers and probes specific to 43 different EGFR mutations in Cobas z 480 instrument. The assay can detect 43 mutations in four exons (18-21) of EGFR gene, including several point mutations, deletions and insertions.

Results

Out of 191 patients, 122 were male and 68 were females; male to female ratio was 2:1. The mean age of the patients was 62 years and age distribution was 23 and 85 years. On the basis of immuno histopathological finding tumors were categorized into two groups; well to poorly differentiated adenocarcinoma 145(76%) and metastatic adenocarcinoma 46 (24%). EGFR mutation Del 19 was detected in 26 patients, its short in-frame deletion in exon 19, clustered around the amino acid resideus747-750(most common variant delL746-A750, delL747-T751insS, and delL747-P753insS). Whereas L858R point mutation (substitution of amino acid leucine to arginine) was found in 20 patients. In one patients compound mutation, two point mutations together [S768I and G719X] and another one insertion (amino acid residues inserted) on Exon 20 was observed. EGFR mutations were almost equally distributed in both gender in male (52%) and female (48%) patients respectably, in published literature female preponderance was significant.

Conclusion

Our study showed Del 19 and L858R were the most frequent mutations in Pakistani lung cancer patients. In additions, 25% of the patients were found eligible for targeted therapy.

3

Synergistic induction of apoptosis by mapatumumab and anthracyclines in human bladder cancer cells

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Each year significant number new cases of bladder cancer are diagnosed globally, making bladder cancer the most frequent cancer throughout the world. The chemotherapeutic drug resistance of bladder cancer cells remains a major obstacle to successful treatment, and more effective therapy is needed to prevent recurrence and reduce risk of tumor progression. Tumor necrosis

factor-related apoptosis-inducing ligand (TRAIL) triggers apoptosis in a variety of tumor cells by engaging the death receptors 4(DR4) and 5(DR5). Mapatumumab, a human agonistic monoclonal antibody specific for tumor necrosis factor (TNF)-related apoptosis-inducing ligand receptor-1 (TRAIL-R1)/DR4, is a promising molecular targeted therapeutic agent.

In this study we investigated the effect of chemotherapeutic drugs on DR4-mediated apoptosis in human bladder cancer cells.

Cytotoxicity was determined by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay. Synergy was assessed by isobolographic analysis.

Treatment of human bladder cancer T24 cells with mapatumumab in combination with mytomycin C, vinblastine, or gemcitabine did not overcome resistance to these agents. However, treatment with mapatumumab in combination with epirubicin (EPI) has a synergistic cytotoxic effect. Synergy was also obtained in KU7, and RT112 human bladder cancer cells. Synergistic effect was also observed with mapatumumab in combination with pirarubicin (THP). The synergy obtained in cytotoxicity with mapatumumab and EPI was also achieved in apoptosis. EPI remarkably increased DR4 expression in bladder cancer cells at both the mRNA and protein levels. Furthermore, the combination-induced cytotoxicity was significantly suppressed by the DR4:Fc chimeric protein. The combination of EPI and mapatumumab significantly activated caspase cascade, including caspase-8, caspase-9, and caspase-3, which are the downstream molecules of death receptors.

These findings indicate that EPI sensitizes bladder cancer cells to DR4-mediated apoptosis through induction of DR4 and activation of caspases, suggesting that the combination therapy of EPI and mapatumumab might be effective for bladder cancer therapy.

4

Prediction of responsible factors in regulating expression of IL-28RA and the SNP involved

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IL-28RA and IL10R collectively constructs a fully functional heterodimeric receptor for type III interferons (IFNs). IL-28RA is also called the private chain for type III IFNs because it has not been found to be involved in any other pathway and is specific to few cell types only, hence making type III IFNs specific in their function. The regulation of the expression of IL-28RA at its molecular level is not fully known yet and needs to be scrutinized at primary levels. We have used various bioinformatics softwares like AliBaba2.1, TESS, Transfac, P-Match and MatrixCatch 2.7 in this study and found AP1-2, STAT 1-6, P-53, LyF-1 (lymphoid transcription factor), c-Jun, PU.1, CREB (cAMP response element-binding), PLAG (pleotropic adenoma gene), MYOD (myoblast determination protein 1), NOFL, KLFS to be preferred predicted transcription factors. Interlinking among different ISGs are also not very clear and induction of one type of interferon can affect the efficacy of the other, we found that interferon lambda 4 induction can increase the expression of IL-28RA, similar interferon lambda 3 but opposite is the case with type I interferons.

5

Actin cytoskeleton remodeling: a potential mechanism for tumor cells to escape from natural killer cell-mediated death

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Natural killer cells (NKs) are cytolytic lymphocytes able to recognize and kill tumorigenic cells without pre-activation. Target cell recognition by NKs leads to the formation of an immunological synapse whose primary function is to direct polarized secretion of cytolytic granules toward their targets. The formation and activity of the immune synapse requires temporal and spatial modifications of the actin cytoskeleton in NKs while the role of these modifications is relatively well described. In contrast, the cytoskeletal remodeling occurring in tumor cells during NK attack have been overlooked. To investigate the implication of actin reorganization in breast tumor cell resistance to NK-mediated cell death, we use MCF-7 breast cancer cells that are susceptible to NK-mediated lysis and a resistant MCF-7 derived clone named 1001. We here provide some evidence that changes in actin cytoskeleton organization and dynamics in tumor cells can modulate their susceptibility to NK-mediated cell death. Indeed, actin-depolymerizing drugs such as Latrunculin B and cytochalasin D could restore conjugates formation between NKs and 1001. Using spinning disk confocal microscopy, we hope to provide a clearer view of the cytoskeletal modifications taking place in tumor cells during their recognition and killing by NKs. Collectively, our data suggest that the formation of a functional immunological synapse requires an appropriate configuration of tumor cells derived-actin filaments and that tumor cells can escape NK-mediated cell death by remodeling their actin cytoskeleton.

6

Formal Analysis of Normal and Induced Apoptosis Pathway for Identification of Therapeutic targets against Cancer

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Cancer related deaths are increasing at an alarming rate worldwide. It is an immensely complex disease and diverse factors are being studied at gene level to explore their role in biological regulatory pathways involved in initiation and progression of cancer. Apoptosis, a well-coordinated programmed cell death process, remain the focus of current research in Cancer Systems Biology. Recent studies have shown the potential of apoptosis in drug development process. Here, we performed an analysis of qualitative model of apoptosis pathway using two formal verification approaches; Model Checking and Process Hitting Framework. First, we modeled the apoptosis pathway

using Process Hitting Framework to identify stable states and logical parameters (possibly incomplete). Secondly, we constructed a model of apoptosis Biological Regulatory Network (BRN) using Rene' Thomas qualitative modeling framework. In this step, we used Model Checking to identify qualitative behaviors such as oscillations, stable steady states, the normal and divergent trajectories (leading to Cancer) along with complete logical parameterization.

Finally, we carried out hybrid modeling to infer the delay parameters and constraints using a hybrid model checker. The study demonstrated certain points and behaviors which may be targeted for development of therapeutic interventions against them.

7

Identification of strong synergistic activity between PIK3 and mTOR inhibitors in uveal melanoma models

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Uveal Melanoma (UM) is the most frequent primary ocular tumor. About a half of the patients develop metastatic disease, for which no treatment has proved effective. We have developed a fast pipeline to screen 2 drug combinations for synergy. We applied this method to a panel of UM cell lines representative of the molecular background of the disease. We tested 7 targeted agents for which promising preclinical results have been reported, assessing all the possible 2-drugs combinations. We selected the most synergistic associations for further in vitro evaluation. Among them the most promising is the association of mTOR inhibitor Everolimus and PI3K inhibitor GDC0941, which resulted in a strong increase of apoptosis compared to monotherapies in several UM cell lines and in disease stabilization in a Uveal Melanoma patient derived Xenograft model.

8

Optimising Anticancer Potential of Lapachol and Derivatized Conjugates from *Kigelia Africana*

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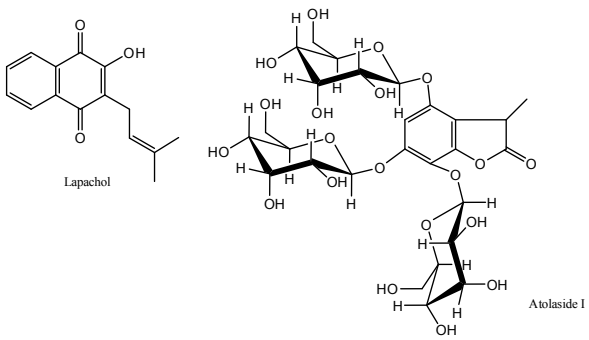
Phytochemical study of the polar extract of the root of *Kigelia africana* afforded compounds which include Lapachol, flavone glycosides and other chromone glycosides called Tolasides **I** and **II**. The structures of the compounds were determined based on various combinations of spectra data which include mass spectrometries and NMR spectroscopies. Lapachol, the major compound in the extract was derivatized to enhance the selectivity to cancer cells. Virtual molecular structure activity relationship studies (SARs) was carried out on all the compounds using molinspiration and toxpredict softwares. The *in vitro* cytotoxic activities of the compounds were evaluated on cell lines using MTT [3-(4,5-dimethylthiazol–2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay. Healthy human prostate (PC-3) and human cervical cancer (Hela) and mouse fibroblast (3T3) cells were maintained and treated with various concentrations (1-100 µM) of compounds to establish their potentials to inhibit cell proliferation. Lapachol, a naphthoquinone was most cytotoxic to prostate cancer cell line (IC_{50}= 20.51 µg/mL) and Hela cancer cell lines (IC_{50}= 19.04 µg/mL). The selectivity of the lapachol was low as the response to normal cell lines (IC_{50}= 6.15µg/mL) was high. However, the derivatives did not produce an increased activity against the prostate cancer cells thereby indicating the loss of activity due to release of the phenolic proton. However, virtual screen indicated that the semi-synthetic derivatives are potential drug candidates as they recorded high bioactivities due to enzyme, protease and kinase inhibition. Lapachol appears to be the major contributor to the anticancer potential of the root extracts. However, consumption of the polar extracts could predispose users to various degrees of fatalities as a result of the low selectivity index. Molecular docking also indicated a possible pathway for future research in pursuit of lapachol-derived anticancer drug candidates.

Keywords: *Kigelia africana*, glycosides, lapachol, anticancer, cytotoxic



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Constitutive Activation of Oncogenic PDGFRα-mutant Proteins occurring in GIST Patients induces Receptor Mislocalisation and alters PDGFRα Signalling Characteristics.

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Gastrointestinal stromal tumours (GIST) are mainly characterised by the presence of activating mutations in either of the two receptor tyrosine kinases c-KIT or platelet-derived growth factor receptor-α (PDGFRα). Most mechanistic studies dealing with GIST mutations have focused on c-KIT and far less is known about the signalling characteristics of the mutated PDGFRα proteins. Here, we study the signalling capacities and corresponding transcriptional responses of the different PDGFRα proteins under comparable genomic conditions. We demonstrate that the constitutive signalling via the oncogenic PDGFRα mutants favours a mislocalisation of the receptors and that this modifies the signalling characteristics of the mutated receptors. We show that signalling via the oncogenic PDGFRα mutants is not solely characterised

by a constitutive activation of the conventional PDGFRα signalling pathways. In contrast to wild-type PDGFRα signal transduction, the activation of STAT factors (STAT1, STAT3 and STAT5) is an integral part of signalling mediated via mutated PDGF-receptors. Furthermore, this unconventional STAT activation by mutated PDGFRα is already initiated in the endoplasmic reticulum whereas the conventional signalling pathways rather require cell surface expression of the receptor. Finally, we demonstrate that the activation of STAT factors also translates into a biologic response as highlighted by the induction of STAT target genes. In conclusion we show that the overall oncogenic response is the result of different signatures emanating from different cellular compartments. Furthermore, STAT mediated responses are an integral part of mutated PDGFRα signalling.

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Identification of common therapeutic targets in NF2 brain tumours

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Neurofibromatosis 2 (NF2) is an autosomal dominant inherited condition that predisposes individuals to develop multiple nervous system tumours, primarily schwannomas, meningiomas and ependymomas. It is characterised by loss of the tumour suppressor protein Merlin, caused by bi-allelic mutations of the encoding gene, nf2. NF2 mutations also cause a variety of spontaneous tumours. They are usually unresponsive to classic chemotherapeutic agents leaving frequent surgeries and radiotherapy as the only treatment options. No drugs are currently approved for the treatment of NF2, highlighting the urgent need for novel therapeutic options. Phosphorylation is a key regulatory mechanism leading to cellular signalling alterations, so we utilized proteomics techniques to analyse the phosphoproteome of meningioma and schwannoma cell lines and primary cells to identify common targets.

Total proteins and phosphoproteins have been isolated from the human meningeal cell line (HMC) to compare with the total and the phospho-proteome of a benign human meningioma cell line (BEN-MEN-1) and three primary meningioma-derived cells. The same was performed on primary Schwann cells and on three primary Schwannoma-derived cells. After LC-MS/MS mass spectrometry analysis we identified >3000 proteins in either case. Statistical analysis was performed applying stringent criteria and corrections; subsequently, the statistically significant candidates were further examined by the functional analysis tools Ingenuity pathway analysis (IPA®) and Metacore. Proteins which showed a significant change in the degree of phosphorylation were additionally normalized to the corresponding total protein amount. The most promising candidates were validated by Western blot analysis and immunofluorescence studies.

We identified phosphoproteins significantly up- and down-regulated in NF2 -/- tumour cells compared to the normal control. Our study will provide the foundations for the development of novel therapeutic strategies designed to specifically switch off tumorigenic signalling events that lead to tumour progression.

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Analysis of genetic determinants intrinsic to the tumor cells related to the heterogeneous response to chemotherapy in a mouse model of ERBB2 breast cancer

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** *Equal contribution as senior authors.*

An essential aspect of breast cancer is its different evolution among patients with the same histopathological disease. Moreover, cancer is a tissue growing in the context of a complex organism, thus it can be identified two main sources of variability responsible for the disease behavior: intrinsic and extrinsic factors which act, respectively, mainly inside the tumor cells and outside them at local or systemic levels.

Our aim is to identify intrinsic factors to the tumor cells responsible for the different responses of breast cancer to chemotherapy with Doxorubicin and Docetaxel. For this purpose, we collected tumors developed in a cohort of genetically heterogeneous mice from a backcross between a resistant strain to breast cancer (C57BL/6) and a susceptible one (FVB) which overexpress the *cNeu/ErbB2* protooncogene controlled by the MMTV promoter. The backcross mice were genotyped by SNP analysis. To identify tumor intrinsic factors controlling the response to chemotherapy, we transplanted 125 tumors collected from the backcross mice into singenic F1-C57/FVB mice to remove variability coming from the host compartments. Each tumor was transplanted into two F1 recipient mice; each one was treated with Doxorubicin or Docetaxel, and we studied tumor response to treatment. Linkage analysis permits us to identify QTL (Quantitative Trait Loci) controlling susceptibility to mammary cancer and evolution of the disease in the backcross population, and the specific intrinsic QTL associated with different chemotherapy responses in the F1 mice. Moreover, we are studying molecular and signalling pathways that control chemotherapy responses and the QTL associated with them. The identification of breast cancer susceptibility genes and their pathways associated with different response to chemotherapy will be important for the prediction of human breast cancer evolution during therapy, and to learn about the mechanisms involved in resistance to chemotherapy, thus it would help to develop new preventive and therapeutic strategies.

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DNA damage tolerance of Smurf2-deficeint cells is associated with hyperactivated MAP kinase signaling

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Cancer is a complex disease derived from a single cell in which genomic integrity has been compromised, and the genetic alterations amplified and propagated through the cell replication cycles. In these cells maintenance of cell homeostasis relies heavily on gene posttranslational functions

Protein ubiquitination is one of the major mechanisms controlling posttranslational functions and the “destiny” of the gene products - proteins. In this evolutionary conserved cascade, E3 protein ubiquitin ligases (E3s) are those which define the specificity of the ubiquitination cascade. Many E3s are encoded by tumor suppressor genes and oncogenes, and are critically integrated into pathogenesis of a wide spectrum of cancers.

Two major types of E3s have been identified in eukaryotic cells: RING-finger and HECT-domain E3s. Of these two groups, HECT-type E3s remain largely unexplored. Significant gaps thus remain in our knowledge of their role in cancer initiation, progression, and/or tumor cell sensitivity to anticancer therapies.

Recently, the anticancer roles of HECT type E3s have become highly relevant as we discovered prominent tumor-suppressor functions of one member of this family - Smurf2. We showed that Smurf2 deactivation leads to perturbations in chromatin structure, DNA damage response and gene expression, compromising genomic integrity and, ultimately, leading to tumorigenesis (Blank M et al., Nature Med 2012). One of the interesting observations in that study was the ability of Smurf2-deficient cells to withstand DNA damage more efficiently than their wild type counterparts. Here we report that the tolerance of Smurf2-deficient cells to genotoxic stress is associated with hyperactivated MAP kinase signaling.

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Base excision repair is associated to cisplatin resistance in G12C KRAS mutant NSCLC cells

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KRAS mutations in NSCLC are supposed to be associated to a poor prognosis and poor response to chemotherapy but this feature lacks a mechanistic evidence so far. In tumors, KRAS is mutated mostly at codons 12, but this process has as result a pool of mutations differing in the base replacement and the amino acid substitution. We hypothesized that different KRAS mutations in NSCLC patients may differently impact on drug sensitivity. We generated isogenic NSCLC cell clones expressing different KRAS mutations to determine the response to cisplatin, the election drug for the first line treatment of NSCLC in the clinic. Cells expressing the KRAS(G12C) mutation were less sensitive to the treatment both in vitro and in vivo to cisplatin. Systematic analyses of drug uptake, DNA adduct formation and DNA damage repair systems involved in cisplatin adducts removal revealed that cells expressing KRAS(G12C) mutation might stimulate Base Excision Repair to remove platinum mono-adducts from DNA even before the formation of interstrand cross-links.

The presented results suggest a different response to cisplatin depending on the KRAS mutation and might provide the proof of principle for further studies on the role of the KRAS mutational status as a predictive marker of NSCLC response to treatment.

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Expression of Bcl-2 and IGF-1R genes in chronic myeloid leukemia - molecular prediction of disease risk and imatinib therapy outcome

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Background: Chronic myeloid leukemia (CML) is characterized by the Philadelphia chromosome, a translocation which produces the Bcr-Abl fusion gene. Treatment with imatinib eliminates CML cells mainly by apoptosis, but resistance to treatment frequently occurs. Impaired cell death related to changes in Bcl-2 expression might contribute to chemotherapy resistance and synergism with Bcr-Abl in inducing blast crisis. IGF-1R is highly expressed in some leukemias and has been linked to poor prognosis. The aim was to determine whether Bcl-2 and IGF-1R are risk factors for CML and/or predictive factors for imatinib therapy.

Material and methods: An age and gender matched case-control study in a Serbian population of 57 CML patients and 63 healthy control subjects of Caucasian descent was performed. Patients were treated with imatinib and stratified into responders (complete Bcr-Abl molecular response) and non-responders, in a one year assessment period. Response to imatinib was also investigated *in vitro* using K562 cells treated with imatinib. RNA was isolated from blood leukocytes or cells and used for cDNA synthesis. Gene expression levels were evaluated (pre- and post-treatment for patients and K562) by real-time PCR. Data was analyzed using the delta-delta-Ct method, with statistical significance $p<0.05$.

Results: *Bcl-2* and *IGF-1R* gene expressions were significantly higher in CML patients than controls ($p=0.014$, and $p=0.0003$, respectively). K562 cells and patients who achieved complete molecular response after imatinib treatment showed significantly reduced expression of *IGF-1R* ($p=0.0286$, and $p=0.0187$, respectively), while no significant reduction of *Bcl-2* was observed.

Conclusions: High expression of *Bcl-2* and *IGF-1R* genes is correlated with CML occurence in Serbia. Successful imatinib treatment coincided with significantly reduced *IGF-1R* expression *in vitro* and *in vivo*. Blocking of IGF-1R signalling pathways might be a good strategy for enhancement of imatinib’s antitumor effects and avoidance of resistance.

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Hypoxia and acidosis modulate effects of combined differentiation-inducing treatment of tumor cell lines

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It is well known that changes in tissue microenvironment are closely related to tumorigenesis. Insufficient vascularisation connected with hypoxia and acidosis is typical feature of tumor microenvironment. These regions may affect cell metabolism and they are often associated with resistance of tumor cells to the conventional treatment. Our previous findings showed that inhibitors of lipoxygenases (LOX) and cyclooxygenases (COX) are able to enhance the differentiating action of all-*trans* retinoic acid (ATRA) in various types of tumor cells. Based on these findings, we investigated effect of hypoxia and low pH on the combined treatment of selected tumor cell lines with ATRA and caffeic acid (5-LOX inhibitor) or celecoxib (COX-2 inhibitor). Saos-2 osteosarcoma cell line and SH-SY5Y neuroblastoma cell line were used in this study. For experimental cultivation conditions, DMEM medium adjusted to pH 6.8 or hypoxic chamber with 1% O_2 atmosphere were used. We examined cell viability by MTT assay, cell cycle by flow cytometry and expression of relevant differentiation markers by RT-PCR. The most interesting results showed that experimental conditions have different influence on the same treatment in both cell lines. In Saos-2 cells, low pH did not affects the combined treatment while hypoxia decreased the effects of the treatment. In contrast, hypoxia slightly enhanced effects of the treatment in SH-SY5Y cell line, while low pH decreased the antiproliferative effect. Furthermore, our results show a remarkable decrease in cell viability under both modified cultivation conditions if compared with standard conditions. We also detected changes in expression of relevant differentiation markers using RT-PCR but these changes were not unequivocal and they should be further analyzed in detail.

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Differential expression profile of plasmamiRNAs for estrogen and progesterone receptor positive and negative breast cancer

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MicroRNAs (miRNAs) are stable, short RNA molecules that have the potential to become biomarkers for diagnosis and prognosis. The present study focused on identifying the specific signature for two subtypes of breast cancer, estrogen and progesterone receptor positive (DPBC) and negative (TNBC). miScript miRNA PCR Array Human Breast Cancer was used to evaluated the differential expression of plasma miRNA in TNBC, DPBC and healthy controls. Studying the miRNA signature associated with breast cancer, we were able to identify a panel of miRNAs (four up-regulated and four down-regulated) capable to discriminate among these two subgroups. miR-204 was observed to be specific for DPBC. Overexpression of let-7b, let-7c, miR-19a, miR-19b was related with a lower Nottingham stage, while miR-328 correlated with tumor stage. By integrating the altered miRNAs in the Ingenuity Pathways Analysis software for TNBC, we observed the signaling pathways that are controlled by these potential biomarkers.

Identified miRNAs are involved in the modulation of apoptosis and cell cycle regulation (targeting p53 gene and its effectors), carcinogenesis and invasion. Moreover, p53 was observed to be an important interacting gene for the statistically significant miRNAs from both groups. Plasma miRNA profiling studies can offer significant information for breast cancer patient stratification, and can provide minimally invasive tools for early diagnosis and prognosis for this malignancy.

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Epigenetic Regulation of the Cocaine and Amphetamine Regulated Transcript (CART) Gene in Cancer

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Introduction: The cocaine and amphetamine regulated transcript (CART) is well documented in the central nervous system for its role in appetite regulation and reward pathways. In recent studies CART has been demonstrated to be a promising marker of poor prognosis in lymph node-negative, estrogen receptor (ER)-positive breast cancer patients, and has been shown to be associated with worse survival in small bowel carcinoid tumours. The regulation of CART in these different malignancies, however remains largely unknown. The presence of a candidate CpG island in the putative CART promoter region and the lack of expression of CART across several cell lines, suggests a possible epigenetic mechanism of regulation.

Methods: A panel of cancer cell lines were assessed for endogenous CART expression via Western blot and qRT-

PCR analyses. Cells were then treated with two DNA methyltransferase inhibitors (5-Aza-2′-deoxycytidine, RG108) and a histone deacetylase inhibitor (Trichostatin A), followed by examination of CART expression post treatment. Whole methylome sequencing using a novel capture technology, followed by next-generation sequencing, was carried out in order to determine the global effects of treatment with epigenetic modifiers, and treatment with CART conditioned media, which has been previously shown to induce CART expression. Finally, bisulfite sequencing of the CpG island in the putative promoter region of CART was performed in all cell lines to confirm methylation status.

Results & Conclusion: Assessment of a panel of cell lines demonstrates negligible CART expression at a both mRNA and protein level across the panel. Treatment with 5-Aza-2′-deoxycytidine results in increased CART mRNA levels in MCF7 cells, while methyl capture sequencing identified methylated regions within the CART promoter. Moreover, subsequent bisulfite sequencing of the promoter region of CART has confirmed dense methylation at the identified CpG island. Collectively this data suggests the potential involvement of DNA methylation in regulation of CART.

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Application timing critically influences the uptake of fluorescence-labeled Cetuximab (f-CET) when combined with fractionated external radiotherapy

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External beam radiotherapy (EBRT) can be precisely applied to solid tumors but the surrounding normal tissue is limiting the maximal deliverable dose. The usage of radionuclide-labeled-antibodies (Radioimmunotherapy, RIT) mediates internal irradiation with potential to strike also distant metastases but is not able to reach curative doses for solid tumors. The combination of internal and external radiotherapy (CIERT) is a promising treatment strategy as it potentially combines the advantages of both modalities without increasing toxicity. We previously showed that CIERT using Y-90-Cetuximab (Y-90-Cet) massively increased tumor control probability compared to EBRT alone in a head and neck squamous cell carcinoma (HNSCC) xenograft model. In this preclinical setup, single dose EBRT was applied. Further studies are planned to use clinical relevant fractionated (fx) EBRT hence the timing of Y-90-Cet application will be crucial. The aim of the present study was to investigate tumor uptake of fluorescence-labeled Cetuximab (f-Cet) when applied before or during fx-EBRT. NMRI(nu/nu) mice bearing subcutaneous HNSCC xenografts were allocated in three treatment arms: i) f-Cet, ii) fx EBRT -> f-Cet, iii) f-Cet -> fx EBRT. Irradiation was performed with 5x 2.7 Gy over four weeks. To be consistent with previous Y-90-Cet experiments, 13 µg of f-Cet were injected intravenously. Optical imaging was performed using IVIS-Spectrum and f-Cet uptake was longitudinally followed. Tumor:muscle ratio of fluorescence signals in the control group was highest at day 4 post injection. Signal strength and kinetics of uptake were not altered by subsequent EBRT. In contrast, administration of f-Cet after fx-EBRT significantly increased and accelerated tumor uptake. The results indicate that injection of antibody after several fx of EBRT can enhance the bio-distribution. This may improve outcome of CIERT as potentially higher internal doses can be delivered via Y-90-Cet to the tumor if injected after delivery of external dose.

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Functional Genomic Screening Identifies USP11 as a Novel Therapeutic Target in Breast Cancer

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Approximately 70% of breast cancers overexpress the estrogen receptor alpha (ERα) and depend on this key transcriptional regulator for growth and differentiation. The discovery of novel mechanisms controlling ERα function represent major advances in our understanding of breast cancer progression and potentially offer attractive new therapeutic opportunities. Here, we investigated the role of deubiquitinating enzymes (DUBs) in regulating transcriptional activity of the ERα in breast cancer.

To identify DUBs involved in the regulation of ERα transcriptional activity, we performed an RNAi loss-of-function screen using a library of shRNA vectors targeting all human DUB genes. Of particular interest, we found that suppression of the BRCA2-associated DUB, USP11, repressed the activity of an estrogen-response-element (ERE).

Subsequent validation using ZR-75-1 breast cancer cells with stably knocked-down USP11 and multiple independent hairpins revealed a notable reduction in expression of the endogenous ERα target genes, as quantified using qRT-PCR. Growth assays and Western blot analysis also revealed a global decrease in cell survival and decreased ERK and AKT phosphorylation in USP11 knockdown cell lines.

In silico analysis of publically available breast cancer gene expression datasets revealed a highly significant correlation between high expression of USP11 mRNA in ER-positive patients and poor distant metastasis-free survival (HR 1.39, CI 1.1-1.76, p=0.006). This correlation was also significant in ER-positive patients who had received endocrine therapy (HR 1.84, CI 1.22-2.75, p=0.0023). To further investigate the prognostic relevance of USP11, immunohistochemical staining of a breast cancer tissue microarray (n= 144) was

performed. Kaplan-Meier analysis of this cohort revealed a significant association between poor overall survival (p= 0.030) and poor breast cancer specific survival (p=0.041).

These results suggest a role for USP11 in driving cellular growth and identify USP11 as novel therapeutic target in breast cancer.

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Identification of novel molecular targets for TMZ-based therapies against glioblastoma: A comprehensive shRNA-based screen of DNA Damages Response factors

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Current treatment of glioblastoma multiforme (GBM), the deadliest primary tumor of the brain, consists of surgical resection followed by combined radio- and chemotherapy with the DNA alkylating agent temozolomide (TMZ). Unfortunately, the median survival of GBM patients is only 14.6 months, due in large part to the occurrence of resistance to the genotoxic.

Efficient repair and/or tolerance of TMZ-induced DNA lesions plays a crucial role in mediating resistance to chemotherapy. One important factor is encoded by the O-6-methylguanine-DNA methyltransferase (MGMT) gene whose epigenetic silencing is the strongest predictive marker for favorable outcome in GBM patients treated with TMZ. Thus, MGMT promoter methylation, observed in about 40% of GBM patients, is associated with better response to TMZ and increased survival. However, even patients displaying MGMT promoter methylation succumb to tumor relapse, indicating that other DNA repair mechanisms promote resistance to the treatment.

In addition to MGMT, which operates through a single-enzyme, suicidal mechanism, DNA repair factors involved in mismatch repair, base excision repair and the repair of DNA breaks play a role in the removal of TMZ-induced lesions. However, identifying which pathways mediate the resistance of GBM cells to TMZ in the absence or presence of MGMT has remained a crucial challenge.

We have set out to reach this goal by performing large-scale loss-of-function RNAi screens specifically targeting DNA repair genes in isogenic GBM stem-like cells (GSCs) either proficient or deficient in MGMT activity. Our targets of interest include genes required for survival of MGMT-positive GSCs as well as those that are synthetically lethal to the loss of MGMT, either in the absence or presence of TMZ treatment.

Promising candidates will be validated *in vitro* and *in vivo*, using patient derived xenograft animal models. Long-term perspectives include identifying novel druggable targets and personalized treatment strategies tailored to MGMT status.

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PI3K/AKT signaling in breast tumours increases Semaphorin7A expression to enhance tumour cell survival and motility

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Introduction: The study of breast cancer relies heavily upon the identification of tumour-associated proteins involved in tumour growth and metastasis. Our laboratory discovered that mammary tumour cells express high levels of the axonal guidance molecule Semaphorin7A (SEMA7A). SEMA7A expression has been shown to be induced by activation of the PI3K/AKT pathway in murine models of fibrosis.

Material and Method: TGF-beta1 was used to induce PI3/AKT signaling in mammary cells. The SEMA7A gene was silenced in 4T1 mammary tumour cells using shRNA. Cytoskeletal changes and motility were assayed by atomic force microscopy. In vitro proliferation and apoptosis were quantified using flow cytometry. BALB/c mice were inoculated with mammary cells with altered SEMA7A expression. Immunohistochemistry and qPCR were used to correlate SEMA7A and ki67 expression in human breast tumours.

Results and Discussion: Activation of PI3/AKT significantly up-regulates SEMA7A expression. SEMA7A may promote tumour cell survival as an effector of the PI3K/AKT pathway. Gene silencing of SEMA7A in 4T1 cells decreased mesenchymal qualities. SEMA7A silenced 4T1 cells showed decreased ki67 proliferation, decreased activation of the PI3/AKT pathway and increased apoptosis. In vivo, inhibition of SEMA7A resulted in reduced tumour growth, reduced metastasis and enhanced survival. Increased SEMA7A expression positively correlated with high ki67 (>25%) in human breast tumours biopsies.

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Impact of pH and pO_2 on endocytosis of nano-scaled drug carriers in tumor cells

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Nano-scaled polymer drug carriers are discussed for addressing chemotherapy specifically to the tumor tissue. Due to the size of polymers and leakiness of the tumor vascular bed the drug carriers extravasate and accumulate preferentially in the tumor tissue. There the carrier has to be taken up by endocytosis and the cytotoxic drug has to be cleaved. Tumors often show an insufficient oxygen supply leading to severe hypoxia and acidosis due to glycolytic metabolism. Therefore the aim of our study is to analyze whether hypoxia (pO_2 = 1 mmHg) or acidosis (pH6.6) affects the endocytotic uptake of differently structured N-(2-hydroxypropyl) methacrylates (pHPMA) polymers (pHPMA-

homopolymer, random and block pHPMA-LMA-copolymers) in two different tumor cell lines (AT-1 prostate cancer and Walker-256 mammary cancer of the rat).

Experiments were performed in bicarbonate buffered HEPES- and MES-Ringer solutions adjusted to the desired pH at 4°C (exclusion of active cellular uptake) and at 37°C (endocytotic uptake) for up to 16 h. Hypoxic conditions were obtained in a hypoxia chamber.

At physiological conditions the mean uptake of pHPMA in the Walker-256 cells was more than 5 times higher compared to the AT-1. Differences were also seen between the different polymer structures with the random HPMA-LMA-copolymer to be taken up better in both cell lines than the homo- and the blockpolymers. Under acidotic conditions the results were variable. The random copolymer was taken up better at pH6.6 whereas the uptake of other polymers was reduced. Under hypoxia a similar distribution pattern was observed.

These results indicate that the polymer uptake depends on the chemical structure of the polymer but also on the specific tumor cell line. In addition it becomes obvious that microenvironmental parameters (pH, pO_2) markedly affect the cellular endocytosis which makes it difficult to select an "optimal" drug carrier.

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EGFR-1 AND HER-2-NEU EXPRESSION IN GALLBLADDER CARCINOMA IN NORTH INDIA

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Background Gallbladder cancer is the commonest biliary malignancy. Reported incidence of gallbladder cancer in north and central India is high. Increased EGFR-1 & Her-2-neu expression has been noted in various cancers and has become a useful target for therapeutic interventions. We have evaluated the expression of EGFR-1 & Her-2-neu in gallbladder adenocarcinoma.

Material & Methods Thirty cases of radical cholecystectomy specimens of diagnosed adenocarcinoma were submitted for EGFR-1 and Her-2-neu evaluation. The standard immunohistochemistry protocol was used with primary EGFR-1 antibody (BioGenex, India) and HER-2/neu antibody (Dakopatts, Denmark) and secondary antibody (Real Envision Detection Kit, Dakopatts, Denmark). More than 10% tumor & 3+ staining were categorized positive, >10% tumor & 2+ staining as equivocal and <10% tumor or >10% tumor with 1+ staining were categorized negative for both EGFR-1 & Her-2-neu.

Results EGFR-1 was found to be positive in 19/30(63.33%), equivocal in 4/30 (13.33) and negative in 7/30(23.33%) patients with tumor positivity ranging 20-90% (mean=69.47). Likewise, Her-2-neu found to be positive in 15/30(50.00%) patients, equivocal in 2/30 (6.66%) and negative in 13/30(43.33%) patients with tumor positivity ranging 30-90% (mean=66.66). Over expression concordance between EGFR-1 & Her-2-neu was seen in 13/30 (43.33%) cases. No significant correlation was evident between EGFR-1 or Her-2-neu expression with grade or stage of the tumor.

Conclusions Majority of the gallbladder carcinoma showed over-expression of EGFR-1 (63.33%) and Her-2-neu (50.00%). Above results in North India further strengthens the rationale in targeting this pathway in gallbladder cancer. Despite the efforts by many investigators, GBC continues to represent a major challenge in oncology. Since this is a pilot study, further larger studies based on molecular understanding are warranted so that new targeted therapies may be developed.

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Identification of SOCS2 and SOCS6 as biomarkers in human colorectal cancer

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Over the last years, some members of the family of suppressor of cytokine signalling proteins (SOCS) have emerged as potential tumour suppressors. This study aimed at investigating the clinical significance of SOCS proteins in colorectal carcinoma (CRC).

We integrated publicly available microarray expression data on CRC in humans, analysed the expression pattern of SOCSs and assessed the predictive power of SOCS2 and SOCS6 for diagnostic purposes by generating receiver operating characteristic (ROC) curves. Using laser micro-dissected patient material we assessed SOCS expression on RNA and protein levels as well as their methylation status in an independent CRC patient cohort. Finally, we investigated the prognostic value of SOCS2 and SOCS6.

The meta-analysis as well as the independent patient cohort analysis reveal a stage-independent down-regulation of SOCS2 and SOCS6 and identify both molecules as diagnostic biomarkers for CRC. We demonstrate a different methylation pattern within the SOCS2 promoter between tumour tissue and normal control tissue in 25% of CRC patients. Furthermore, early CRC stage patients with low expression of SOCS2 display significantly shorter disease-free survival.

Our data offers evidence that SOCS2 and SOCS6 levels are reduced in CRC and may serve as diagnostic biomarkers for CRC patients.

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Survival and its determinants of patients with Kaposi sarcoma in Tanzania, retrospective study

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Background: Kaposi Sarcoma is the most common sarcoma and second most prevalent cancer seen in Tanzania. Its affect all age groups and strongly associated with AIDS. Little is known about disease/patients characteristics and survivorship in our settings, therefore this study expected to determine patient's survival and its associated factors in Tanzania.

Methods: Retrospective descriptive study using structured questionnaires. Patients with Kaposi Sarcoma diagnosed and treated at Ocean Road Cancer Institute, the only cancer care center in Tanzania were followed-up retrospectively using their hospital records and mobile phone communication system, with 199 patients included in the study. Descriptive, Bi-variant Analysis, Ordinal Regression, Life Tables and Kaplan Meier survival analysis as well as SPSS 16.0 and Log rants validity test were used during data analysis

Results: Mean age at KS diagnosis was 40 ± 12.012 yrs. with male (42.60 ± 12.6 yrs.) population older than female (35.1 ± 9.7 yrs.). Patients waited 30 ± 120 days after diagnosis before start of treatment. Male: Female ratio was 1.6:1, with radiotherapy (82%) found to be most preferred modality of treatment. Skin (87.3%) was most primary organ affected followed by oral cavity (12.2%). Median and average survival of KS patients were 8 ± 0.613 months and 15.863 ± 1.407 months respectively. Primary organ affected and patient residence found significantly to influence survival while age, sex, treatment modality, hemoglobin level, time taken waiting for treatments found to be insignificant survival predictors. Serum white cell and platelets counts have not shown to influence survival with HIV found to affect 90% of Kaposi Sarcoma patients.

Conclusions: Social demographic features, overall and median survival of patients with Kaposi Sarcoma in Tanzania, is closely related to other countries with similar settings but the latter two were significantly low compared to the developed countries; This might be caused by differences in disease profile, late presentation, poor diagnostic and treatment resources seen in Tanzania compared with other settings.

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Molecular screening for cancer treatment optimization (MOSCATO-01) in pediatric patients: Feasibility results of a prospective molecular stratification trial

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Background: This feasibility study (NCT01566019) characterizes genomic alterations in recurrent tumors of individual pediatric patients in order to select a targeted therapy approach.

Methods: Pediatric patients with recurrent or refractory solid tumor underwent on-purpose tumor biopsy or surgical resection for molecular characterization. Biopsies were obtained using 18G needles under ultrasound, CT or MRI control from primary tumor or metastatic sites. DNA extracted from fresh samples was analyzed by CGHarray 180K (if ≥ 20% tumor cells in the sample) and by sequencing for 74 target genes (if ≥ 10% tumor cells). A panel of scientists and clinicians reviewed results to determine biological signification of the alteration and match patients to the most relevant targeted therapy available (mainly in early clinical trials).

Results: From December 2012 to November 2014, 38 patients were included. The cohort encompassed 26 patients with solid tumors, 12 patients with brain tumors. In 5 patients the material was insufficient, 2 of them underwent re-biopsy. Tumor cell percentage per sample ranged between 20 and 90% (median 60%). No major complication due to on-purpose intervention occurred. At least 17 patients had gene mutations, 24 had chromosomal abnormalities; at least one theoretically actionable target was identified in 27 patients (74%). Five patients received an adapted targeted therapy; one could not be treated with a matched therapy due to rapid tumor progression, seven due to lack of a pediatric trial. Recently, molecular analysis has been extended to Whole-Exome sequencing and RNA sequencing which is currently under evaluation. This prepares the way for the upcoming MAPPYACTS (MoleculAr Profiling for Pediatric and Young Adult Cancer Treatment Stratification) trial in several European countries.

Conclusions: High throughput molecular analysis of recurrent/refractory malignancies in pediatric patients is feasible. Presence of multiple alterations and limited access to targeted agents within pediatric clinical trials remain the main limiting factors.

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Primary and metastatic liver cancer sensitisation to conventional chemotherapy by the DNA repair inhibitor DT01 in pre-clinical tumour models

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Background & Aims
Primary hepatocellular carcinoma (HCC) and secondary liver tumours lead to significant mortality worldwide. This is mainly attributed to their lack of sensitivity to available chemotherapy and the development of drug resistance due to hyperactivation of DNA repair functions. We investigated the tolerance and efficacy of a novel class of DNA repair inhibitors, Dbait in association with conventional chemotherapy frequently used for HCC and colorectal carcinoma (CRC).

Methods
The cytotoxic efficacy of Dbait in combination with chemotherapy was assessed in vitro, in HCC (HepG2, SNU449) and CRC (HT29, HCT116) cell lines. In vivo, the pharmacokinetics and biodistribution of the cholesterol conjugated clinical form of Dbait, DT01, were assessed.

The chemosensitizing abilities of DT01 were evaluated in association with doxorubicin or oxaliplatin and 5-fluorouracil in intrahepatic HCC and CRC xenografted nude mouse tumour models. DT01 tolerance was investigated in a model of hepatic fibrosis.

Results

In vitro, Dbait treatment increases sensitivity of liver and colon cancer cells to chemotherapy. In vivo, DT01 in combination with conventional chemotherapy, led to a significant decrease in tumour volumes both in HCC (mean: 937.83 vs 2544.44 mm2, p=0.02) and metastatic models (mean: 501.05 vs 872.01 mm2, p=0.02), compared to chemotherapy alone. The high uptake of DT01 indicates that the liver is a specific target. Pre-clinical evaluation of DT01 treatment revealed that it is well tolerated in mice bearing healthy livers or chronic liver disease with no adverse effects.

Conclusions

Combining DT01 with conventional chemotherapy may prove to be a safe and effective therapeutic strategy in the treatment of primary liver cancer and metastases.

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Transcriptomic profiling of KDM4 histone demethylases and therapeutic effect of novel KDM4 inhibitor in breast cancer

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The histone lysine demethylase KDM4 subfamily plays critical roles in controlling transcription, chromatin architecture and cellular differentiation. Although KDM4 family members (A-D) have a high degree of homology, they may play unique roles in various types of breast cancer. The goal of this study is to analyze the genomic and transcriptomic alterations of the KDM4 subfamily across breast cancer subtypes, and explore the therapeutic potential of a novel KDM4 inhibitor in basal breast cancer.

We conducted a large-scale meta-analysis of KDM4A, B, C, and D in breast cancer and identified associations among recurrent copy number alterations, gene expression and breast cancer subtypes. We examined KDM4 expression in a panel of non-tumorigenic and cancerous breast epithelial cell lines, and assessed global histone 3 methylation levels in a panel of breast cancer cell lines. Next, we tested small molecule NCDM-32B, a novel KDM4 demethylase inhibitor, for its ability to affect basal breast cancer cell growth and impair metastatic potential. Finally, we examined gene expression changes induced by NCDM-32B.

We revealed that KDM4 members show different expression patterns across breast cancer subtypes, and that H3 global methylation levels vary among breast cancer cell lines. We demonstrated that NCDM-32B significantly impaired viability, cellular growth and transforming phenotypes of basal breast cancer *in vitro*. Furthermore, NCDM-32B impaired several critical pathways and classical oncogenes that drive cellular proliferation and transformation in breast cancer.

In summary, our findings add layers of information to the genomic and transcriptomic profiles of the KDM4 subfamily in breast cancer. We provide the first evidence that a novel KDM4 demethylase inhibitor, small molecule NCDM-32B, led to significant inhibition of cellular growth of basal breast cancer *in vitro*. These findings lay the foundation for evaluating KDM4 inhibitors as therapeutic approaches against aggressive breast cancer.

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Physical localization, stage dependent expression and downstream effect of FAM134B (JK1) down regulation in colon cancer

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Background: FAM134B (Family with sequence similarity 134, member B) is a novel gene, encoding a protein of unknown function. Current functional studies on FAM134B suggest that it plays a critical role in cancer pathogenesis. This research aims at examining the cellular distribution and expression pattern of FAM134B in different subset of colorectal cancer tissues and cell lines and also to investigate its impact on cancer cell biology.

Methods: Localization of FAM134B in three colon cancer cell lines (SW-480, SW-48 and HCT 116), normal colon epithelial cell (FHC) and in human tissues of different stage of colorectal cancer was identified with immuno-staining. FAM134B expression levels were quantified at protein and mRNA levels in both tissues and cell lines using western blot and real-time PCR. Impact of FAM134B down-regulation on colon cancer cell proliferation was studied by WST assay. Effect on invasion/migration was investigated by wound healing assay and clonogenic potential was studied by colony formation assay. Apoptosis of cells following FAM134B downregulation examined with fluorescence microscopy.

Results: Microscopic analysis demonstrated cytoplasmic/nuclear localization of FAM134B in colon cancer cells and tissues. FAM134B mRNA and protein expression showed significant changes in different stages of colon cancer. In the advanced stage colon cancer cells (HCT116), the level of FAM134B expression reduced remarkably compared to FHC and early stage cancer cells (SW480). Similarly, FAM34B also altered significantly in different stage of colorectal cancer tissues samples. Advanced stages showed reduced expression compared to early stages of cancer. FAM134B knockdown significantly increased the proliferation of colon cancer cells on different day of transfection compared to control. It was noted that FAM134B down-regulation increased (34-52%; $p<0.05$) the clonogenic capacity and wound healing potential of cells. We did not observed any significances changes in apoptosis potentials among the cells.

Conclusion: Down-regulation of FAM134B in colorectal cancers with advanced pathological stages and its further suppression impacts on the cancer biology has indicated that FAM134B might act as a cancer suppressor gene in colorectal cancers.

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Quantitative proteomics identifies central players in erlotinib resistance of the non-small cell lung cancer cell line HCC827

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Background: Erlotinib (Tarceva®, Roche) has significantly changed the treatment of non-small cell lung cancer (NSCLC) as 70% of patients show significant tumor regression when treated. However, all patients relapse due to development of acquired resistance, which in 43-50% of cases are caused by a secondary mutation (T790M) in EGFR. Importantly, a majority of resistance cases are still unexplained. Our aim is to identify novel resistance mechanisms in erlotinib-resistant subclones of the NSCLC cell line HCC827.

Materials & Methods: We established 3 erlotinib-resistant subclones (resistant to 10, 20, 30 µM erlotinib, respectively), and analyzed these by quantitative proteomics. The resistant subclones were examined both in absence and presence of erlotinib, and in biological triplicates on a Q-Exactive mass spectrometer.

Results: Importantly, the resistant clones did not acquire the T790M or other EGFR or KRAS mutations, potentiating the identification of novel resistance mechanisms. We identified 2875 cytoplasmic proteins present in all 4 cell lines. Of these 87, 56 and 23 are upregulated >1.5 fold; and 117, 72 and 32 are downregulated >1.5 fold, respectively, in the 3 resistant clones compared to the parental cell line. By network analysis, we found cell survival, proliferation and migration to be induced, and apoptosis and adhesion to be repressed across the 3 resistant clones vs the parental cell line. The resistant cells generally lost phosphorylation of EGFR, MET, FGFR and Src, but surprisingly not of AKT and FOXO1/3a, indicating that AKT is the main signaling hub for survival. Also Erk1/2 phsphorylation is pertained although at decreased levels.

Conclusions: In conclusion, cancer-related networks such as proliferation and apoptosis were found to be regulated, supporting the validity of the model. Phosphorylation of EGFR was lost in resistant cells. Activation of AKT was pertained in the resistant cells, indicating a possible mechanism of erlotinib resistance.

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Individuality in FGF signaling influences disease progression and chemosensitivity in ovarian and colorectal cancers

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Background: Ovarian and colorectal cancer are currently treated with platinum-based chemotherapy. Treatment is initially effective in the majority of patients, but longer-term response is frequently hampered by the development of drug resistance. We have shown that specific fibroblast growth factors (FGFs) and FGF receptors (FGFRs) may play a role in the development of platinum resistance in ovarian cancer; FGF1 and FGFR2 expression is significantly increased in cisplatin-resistant A2780DPP ovarian cancer cells and gene knockdown re-sensitises to platinum and additional DNA damaging drugs.

Methods: To investigate whether individuality in the expression of additional FGFs influenced survival or chemosensitivity in ovarian (n=487) or colorectal (n=276) cancer, gene expression datasets for FGF pathway genes were extracted from TCGA (www.cbioportal.org) datasets and gene expression correlated with patient survival and resistance to platinum-based chemotherapy. qRT-PCR analysis was used to confirm differential FGFR expression in colorectal cell lines resistant to oxaliplatin and 5-fluorouracil.

Results: In ovarian cancer (n=487), FGF12 (p=0.012), and FGF22 (p=0.007), expression was significantly increased in drug-resistant patients: FGF12 expression was additionally inversely correlated with progression free survival (p = 0.034). Conversely, reduced FGF18 expression was associated with both decreased progression-free (p=0.043) and overall (p=0.002) survival. In colorectal cancer (n=276) FGFR4 was the most abundantly expressed receptor, allows prioritization of a subset of FGFs which preferentially bind to this receptor for further investigation. Increased expression FGF12 (p = 0.028) correlated with decreased overall survival. In colorectal cell lines made resistant to oxaliplatin and 5-fluorouracil, when compared with their sensitive counterparts FGFR3 (p=0.005) and FGFR4 (p=0.0004) expression was significantly increased.

Conclusions: We have shown that inter-patient differences in the expression of specific FGF ligands and FGF receptors correlates with survival and chemosensitivity in both ovarian and colorectal cancer.

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Intrathecal Chronic Morphine Infusion Pumps For Intractable Cancer Pain

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Objectives: Chronic pain syndrome is currently seen as an independent disease requiring etiopathogenetic treatment. Optimal way for cancer patients is opioid therapy. By reason of permanent presence of symptoms, the method of choice are implantable close sterile systems that do not require regular invasive procedures with minimal side «opioid» effects, that allow to stop strong pain, including “breakthrough pain.” Thus the functional neurosurgery takes the increasing place in oncology.

Methods : At first time in Russia applying programmable morphine infusion pumps for 32 patients in 2013-14. The most significant indications for pump implantation were: the

presence of heavy cancer pain (VAS>60), inefficiency of previous narcotic analgesics at doses equivalent to 30 mg morphine, life expectancy more than 3 months, and positive morphine test. Implantation and pump programming was carried out according to the accepted method.

Results: At 10 day after operation, the intensity of pain in all patients decreased to 0-20 % by VAS (p-value= 0,000301). Morphine dose vary from 180 to 3500 mg/day. All treated patients completely stopped taking opioids. We registered increase of motion activity in 27 cases, absence of sedation effects, improved somatic status. All patients reported more effective pain relief, lack of systemic side effects and increase quality of life after the selection of the Individual mode morphine pump.

Conclusion: In patients with refractory cancer pain, intrathecal drug therapy with programmable morphine pumps is associated with improved pain relive, reduced breakthrough pain and a significant improvement in the quality of life.

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Subtype-specific KRAS mutations and the role of Notch signaling in KRAS-driven lung adenocarcinoma

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Non-small cell lung (NSCLC) cancer is the leading cause of cancer deaths worldwide, with a median 5-year survival of about 10%. Activating mutations of the KRAS gene are detected in more than 30% of lung adenocarcinoma, indicating that oncogenic mutations of KRAS are major driver of lung cancer. Although specific molecular-targeted therapies for the treatment of distinct subtypes of NSCLC have been developed, treatment options for the majority of patients remain unsatisfactory. A better understanding of NSCLC pathogenesis is, therefore, necessary in order to identify new therapeutic targets and develop more effective treatments.

Our group performed a mutation subtype-specific analysis in the so far largest cohort of Caucasian patients with KRAS mutant advanced-stage lung adenocarcinoma treated with platinum-based chemotherapy. 505 Caucasian stage III-IV lung adenocarcinoma patients with known amino acid substitution-specific KRAS mutational status were included. The correlations of subtype-specific KRAS mutations with smoking status, progression-free (PFS) and overall survival (OS) and therapeutic response were analyzed.

We observed no difference in response rate or survival benefit between KRAS mutant or KRAS wild-type patients treated with platinum based chemotherapy. However, patients with G12V KRAS mutant adenocarcinoma tended to respond better to platinum-based chemotherapy and, although non-significantly, had a longer PFS than those with other codon 12 mutations.

Increasing evidence suggests that deregulation of Notch signaling mediate tumorigenesis in lung adenocarcinoma. The interaction between Notch pathway and RTK-signaling has been a subject of several studies. Therefore, we plan to elucidate the role of Notch signaling in KRAS-driven NSCLC.

Understanding the interplay between KRAS and Notch signaling in NSCLC may help to the development of new therapeutic strategies. The clinical relevance of specific mutations in KRAS remains to be elucidated in advanced lung adenocarcinoma and subtype-specific KRAS mutation analysis may help to the development of personalized therapeutic approaches.

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***RAD51C* mutations in high-risk patients from Serbian hereditary breast/ovarian cancer families**

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In 2010 an important finding was published showing that heterozygous mutations in *RAD51C* were highly penetrant and were able to confer an increased risk for breast and ovarian cancers. The role of possible third high penetrance breast cancer susceptibility gene was assigned to *RAD51C*. Because of its rising importance in breast cancer development and the lack of information about this gene in Slavic populations, our goal was to identify potential population specific mutations in *RAD51C* in order to determine more detailed genetic screening strategy and breast cancer risk assessment.

The study included 56 females from Serbian hereditary breast/ovarian cancer families negative for sequence alterations in *BRCA1*/2 genes. Whole coding region and exon-intron boundaries of *RAD51C* were analyzed by dHPLC. All mutations were confirmed by Sanger sequencing. SIFT and Polyphen were used to predict possible impact of non-synonymous variants. We found 5 variants in *RAD51C* including two missense, one intronic, one in the 5'UTR and one variant in the promoter region of the gene. Three detected variants are common- c.1-118G>A (rs16943176, MAF=0,203); c.1-26C>T (rs12946397, MAF=0,207) and c.904+34T>C (rs28363318, MAF=0,186). We detected two missense variants, c.790G>A (p.Gly264Ser) in exon 5 and c.859A>G (p.Thr287Ala) in exon 6. Both of them were previously shown to exhibit reduced protein function but their contribution to cancer risk is still unknown.

Although the initial reports implied that *RAD51C* might be promising candidate for next high penetrance breast cancer susceptibility gene, lack of confirmation suggested that *RAD51C* mutations are not as common as expected. Our study did not reveal truncating mutations in *RAD51C* suggesting that other breast cancer susceptibility genes may account for the increased susceptibility in our cohort of high-risk *BRCA1*/2 negative families.

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Targeting The Immune System To Cancer Using Tumor Homing Poly Inosine/Polycytosine

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The delivery of nucleic acids into cells represents an attractive approach for cancer therapy. Our recent studies have demonstrated that Polyethyleneimine (PEI) can be conjugated to targeting ligands, such as EGF [1,2,3], Her-2 affibody[4] and a PSMA[5] targeting ligand. These vectors deliver synthetic double stranded RNA (dsRNA), Polyinosinic-polycytidylic acid (pIC), to tumors that overexpress receptors for these ligands. Using pIC loaded on liganded Polyethyleneimine-polyethylene glycol (PEI-PEG-Ligand), pIC is internalized in large amounts, into tumors that overexpress EGFR, Her-2 or PSMA. Cellular uptake of pIC triggers pro-apoptotic pathways such as PKR, p38 and JNK. Furthermore, pIC also binds to TLR3 (TLR3), inducing the synthesis of cytokines such as interferon type I, a strong immune activator, TNFg-induced 10kDa protein (IP-10) and Gro-a, which are potent chemo-attractants for T cells. Animal models and tumor spheres demonstrate that these pIC-loaded vectors are highly effective anti-tumor agents. Due to the pIC induced bystander effects, tumor cells, not harboring the target, neighboring the targeted tumor cells are also destroyed, whereas the more robust normal cells remain oblivious to the treatment. The systemic administration of non-targeted pIC was associated with clinical benefit but is associated with severe toxicity, which is avoided when pIC is targeted. The activation of anti-tumor immune system can complement the inhibition of immune checkpoints by antibodies to CTLA-4, PD-1 and PDL-1 and small molecules inhibiting IDO[6] or Stat3[7].

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Inhibition of EphA4 signaling by Dasatinib selectively attenuates amyloid-beta production

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Dasatinib is a FDA-approved drug for chronic myelogenous leukemia (CML), and potently inhibits BCR-ABL kinase, Src family kinases, and Src-related receptor tyrosine kinases (RTKs). It has been shown that Dasatinib exhibits inhibitory potency against members of the Eph RTK family. Accumulated evidence also suggests that members of the Eph RTK family could act as the receptor for oligomeric amyloid-beta (Abeta), the neurotoxic entity eliciting the pathogenesis of Alzheimer's disease (AD). AD is the most common dementia afflicting the elderly, but currently does not have a real cure. We thus sought to determine whether Dasatinib can inhibit Eph RTK activity to reduce Abeta-elicited neurotoxicity in AD. Given that Dasatinib can be co-crystallized with EphA4, we first demonstrated that ligand-activated EphA4 signaling could govern the proteolytic processing of amyloid precursor protein (APP) and control the levels of APP-betaC-terminal fragment (betaCTF), APP intracellular domain (AICD), and Abeta. However, the gamma-secretase cleavage of Notch was not affected by EphA4 signaling, suggesting an EphA4-elicited selective regulation of APP-betaCTF proteostasis without affecting the processing of other gamma-secretase substrates. This EphA4-elicited accumulation of betaCTF and AICD was mediated by a Lyn-dependent pathway. Consistently, inhibition of EphA4 by Dasatinib effectively suppressed the EphA4-induced accumulation of betaCTF and AICD, concomitant with a decrease in Abeta production. Our data also showed that Lyn can physically interact with EphA4, suggesting that an EphA4-Lyn-dependent positive feedback could form to govern the proteostasis of betaCTF and AICD. Our data delineate an EphA4-Lyn pathway that is essential for controlling the metabolism of APP and its proteolytic derivatives, providing the proof-of-principle evidence for repurposing Dasatinib as a novel therapeutic against AD.

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Chitinase-3-like-protein-1 expression increases tumor growth and metastasis

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Metastases account for the majority of cancer deaths. Recently, elevated serum levels of a glycoprotein known as chitinase-3-like-protein-1 (CHI3L1) have been correlated with poor tumor progression in breast cancer patients. We reported elevated levels of CHI3L1 in plasma of mammary tumor-bearing mice. In determining the cause of high plasma concentrations of CHI3L1, we found that tumor cells, splenic macrophages, pulmonary macrophages and myeloid derived suppressor cells (MDSC) secrete CHI3L1. However, the physiological functions of CHI3L1 are still unclear. We demonstrate that while CHI3L1 has an inhibitory role on the expression of (IFN-g) by T-cells, it can also up-regulate the production of MCP-1, IL-8, and MMP-9, all of which contribute towards tumor progression.

To determine if inhibition of CHI3L1 has an effect on tumor progression, tumor-bearing mice were treated with chitin microparticles, a ligand for CHI3L1. In vivo treatment chitin microparticles promoted immune effector functions through increased of IFN-g and decreased MCP-1, IL-8, and MMP-9 expression. Importantly, in vivo administration of chitin microparticles decreased tumor growth and metastasis, while increasing survival. Using CHI3L1 KO mice implanted with mammary tumors, we found decreased levels of MCP-1, IL-8, and MMP-9, decreased tumor growth and metastasis, and increased survival.Inflammation in the lungs, one of the sites of breast cancer metastasis, is characterized by increased CHI3L1. To determine if

increased CHI3L1 expression in the lungs has an effect on accelerating breast cancer metastasis, we developed a mouse model by combining a model of allergic pulmonary inflammation and breast cancer. Allergen induced tumor bearing mice had increased MCP-1, IL-8, MMP-9 production, 5-fold increase in tumor growth, 10-fold increase in metastasis and decreased survival. These studies show that tumor derived CHI3L1 and host derived CHI3L1 plays a role in breast cancer progression. Thus, suggest that CHI3L1 may be useful target for treatment of breast cancer.

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Validation study of ^{1}H-NMR-based metabolomics as a new, complementary tool for the detection of lung cancer via human blood plasma

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Background. Low-dose computed tomography (LDCT) screening has recently been shown to reduce lung cancer mortality. However, >50% of the individuals who undergo LDCT screening are referred for false positive results. This limitation encourages the search for complementary tools which enable to detect lung cancer with an improved specificity. Accumulating evidence has shown that disturbances in biochemical pathways which occur during the development of cancer provoke changes in the metabolic phenotype of the patient.

Objective. This study aims to determine the metabolic phenotype of blood plasma of 233 lung cancer patients and 226 controls by ^{1}H-NMR spectroscopy. Furthermore, the predictive accuracy of the metabolic phenotype was examined by external validation in an independent cohort (98 lung patients and 89 controls). The most discriminating peaks in the ^{1}H-NMR spectrum were used to explain the deregulated biochemical pathways in lung cancer.

Results. The metabolic phenotype allows to classify 78% of the lung cancer patients and 92% of the controls correctly with an AUC of 0.881. Furthermore, it classifies 71% of the lung cancer patients and 81% of the controls from an independent cohort correctly with an AUC of 0.843. The metabolic changes can be mainly linked to a counteraction of the body in response to the well-known Warburg effect in cancer cells. In order to compensate for the lack of glucose as an energy source for normal cells, liver glycogen will be degraded and glucose will be synthesized via the gluconeogenic pathway.

Conclusion. Metabolic phenotyping of blood plasma by ^{1}H-NMR spectroscopy is a promising, complementary tool to detect lung cancer. Furthermore, ongoing research aiming to find out whether the addition of metabolic phenotype data to current risk models used to identify lung cancer patients among high-risk individuals in the general population has added value, looks promising.

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Metabolic phenotyping of human blood plasma: A powerful biomarker to discriminate between different cancer types?

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Background. The application of metabolomics in the search for cancer biomarkers has increased enormously in the past decade. Although many studies have already focused on deregulated cancer cell metabolism, it is not yet clear whether different cancer types share the same metabolic derangements or whether certain derangements are limited to specific cancer types.

Objective. This study aims to investigate whether the metabolic phenotype of blood plasma determined by ^{1}H-NMR spectroscopy allows to discriminate between 54 lung cancer patients and 80 breast cancer patients. Furthermore, the predictive accuracy of the metabolic phenotype was examined by external validation in an independent cohort (81 lung cancer patients and 60 breast cancer patients). The most discriminating peaks in the ^{1}H-NMR spectrum were used to explain the deregulated biochemical pathways.

Results. The metabolic phenotype allows to classify 93% of the lung cancer patients and 99% of the breast cancer patients correctly with an AUC of 0.961. Furthermore, it classifies 89% of the lung cancer patients and 82% of the breast cancer patients from an independent cohort correctly with an AUC of 0.935. The metabolic changes can be linked to a stronger counteraction of the body in response to the Warburg effect for lung cancer as compared to breast cancer. More specifically, increases in hepatic glycogen degradation and *de novo* synthesis of glucose to compensate for the lack of glucose are more pronounced in lung cancer patients.

Conclusion. Metabolic phenotyping of blood plasma by ^{1}H-NMR spectroscopy seems to discriminate significantly between breast and lung cancer. Although our results already give an indication of which metabolites may serve as general cancer biomarkers and which are more specific for either lung or breast cancer, future research is needed (and ongoing) to investigate whether the metabolic phenotype allows to discriminate between other frequently occurring cancer types.

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Association between Dukes stage and CDH1 Methylation Status in Colorectal Cancer

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Aim: The colorectal adenoma-carcinoma sequence is associated with epigenetic and genetic modifications, which arise during oncogenesis. These modifications include the methylation status of different gene promoters, and mutations in the K-ras and B-raf genes among others. Included are methylation of the promoters of H-cadherin, of MGMT deregulating removal of toxic methyl adducts on guanine bases and inducing the activation of K-ras through G to A point mutations, activation of B-raf gene by a V600E mutation, methylation of E-cadherin and PTEN inactivation by promoter hypermethylation in more than 200 patients.

Methods: DNA was extracted by standard methods from resected tumour samples from 203 colorectal cancer patients. PTEN methylation was determined by methylation-specific PCR, gel electrophoresis after Sybr green staining and UV-photography. From each patient we examined germline DNA from white blood cells as described above. In these patients the Dukes stage was determined and classified as early (Dukes A & B) or late (Dukes C & D). The association between methylation status, mutation status and Dukes stage (early vs. late) was evaluated by logistic regression.

Results: The logistic regression of Dukes stage (early vs. late) on CDH1 status was statistically significant, P=0.033. No such relationship between stage and each of the other variables (methylation status for CDH13, MGMT and PTEN; mutation status for K-RAS, BRAF and PIK3CA). P values ranged from 0.92 for PIK3CA to 0.30 for K-RAS. All results were tumor specific as all the sequenced blood controls were unmethylated or wild type.

Conclusions: A significant association was found between Dukes stage and CDH1 methylation status, however, no such association was detected between stage and the methylation or mutational status at the other loci. Loss of function of the CDH1 gene is thought to contribute to progression in cancer by increasing proliferation, invasion, and/or metastasis.

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Increase in survival, accompanied with quality of life in patients suffering from metastatic prostate cancer

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34 patients over 65 years old, all of them suffering from prostate cancer, with radical prostatectomia without surgical castration and undergoing treatment with *zoladex* (goserelin) were studied.

All the patients presented moderate to severe anemia, moderate leucocitocis, severe thrombocytopenia, with macrocitic elements, very high levels of plasmatic homocystein, hepatic profile, CPK, LDH, prostatic specific antigen (PSA) above normal.

During six months the patient´s were treated with ferrous fumarat and folic acid so as to raise the heamoglobin level and with a minimun dose of acenocoumarol and an appropiate dose of prednison to avoid hepatic disorders.

At the next hematology control of the following semester it was clear-sighted that slowly and gradually all the hematologycal values, including the hepatic profile and prostatic specific antigen were encouragingly changing, that´s why it was decided to continue with acenocoumarol (adjusted to INR), a mimimun dose of prednison and an intradermic injection of *zoladex* every exact 40 days instead of every 21 days as it is suggested in the therapeutic antitumoral, also to suspend the folic acid to allow the new diet to do the corresponding supply.

The corresponding hematologycal control was done, the patients reached the analytical values expected, the plasmatic homocystein had decreased to normal level, the hemoglobin between 12 and 13 g/dl accompanied with normocitic elements, with values of count ot thrombocytes placed between 300 to 400 x 10^9/l, together with the hepatic profile stabilization, CPK and LDH and the decline of indetectable of prostatic specific antigen and getting social, therapeutic empathy resources.

The patients in this study, subjected to the treatment given, have been able to improve their life-forecast, considering that their survival time has increased, which added to their rights to enjoy a better quality of life as longevous patients allows gaining psycho-social stability.

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Inhibition of Akt kinase increases cytotoxicity of meta-iodobenzylguanidine to neuroblastoma cells

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Neuroblastoma is a malignant neuroendocrine tumor, arising from the sympathetic nervous system. It is the most common extracranial solid cancer in childhood. Meta-iodobenzylguanidine (MIBG) and its radioactive form ^{131}I-MIBG are functional analogues of the neurotransmitter norepinephrine that are taken up by 90 to 95% of all neuroblastomas. Whereas most tissues accumulate MIBG by a nonspecific process, cells of the neuroadrenergic tissues and their malignant derivatives exhibit active uptake of the tracer that is mediated by the norepinephrine transporter. Although MIBG is selectively taken up and stored by tumours derived from the neural crest and might stress them in several ways (lipidperoxidation, inhibition of mitochondrial respiration), its toxicity is not very high. Thus, the goal of this study is to increase cytotoxicity of MIBG to neuroblastoma cells.

We found that Akt 1/2 kinase inhibitor synergistically increases cytotoxicity of MIBG to neuroblastoma SK-N-Be(2) and SH-SY5Y cells as assessed by combination index analysis. Similar results were obtained for combination of MIBG with L- Buthionine-sulfoximine (BSO), an inhibitor of glutathione synthesis. Cytotoxicity of the MIBG/BSO combination depends on ROS production and diminishes in hypoxia (1% O2). Nevertheless, cytotoxicity of MIBG in combination with Akt1/2 kinase inhibitor is not ROS dependent and is preserved in hypoxic culture conditions. Thus, inhibition of the Akt kinase might be a promising

strategy to increase cytotoxicity of MIBG to neuroblastoma.

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Role of clathrin- and caveolae-mediated pathways in galactodendritic conjugated phthalocyanine uptake by bladder cancer cells

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Bladder cancer (BC) is the most common malignancy of the urinary tract being characterized by high risk of progression and recurrence rates. Photodynamic therapy (PDT) is a promising anticancer therapy, which combines a photosensitizer (PS), light at a specific wavelength and molecular oxygen to generate in situ cytotoxic reactions. The urinary bladder is a suitable organ for PDT, because of its accessibility for both intravesical installation and illumination. As a hollow organ, it is possible to irradiate the whole bladder allowing the treatment of multifocal tumors [1]. Since there is no difference in light penetration between normal and tumor bladder tissues, PDT effect will depend on the specific accumulation of the PS within the tumor [2].

We have hypothesized that PSs coupled with galactodendrimers [3,4] should be effective in targeting BC because galactose carbohydrates with a dendritic structure have increased affinity for lectins (namely galectin-1) overexpressed in cancer cells. Amongst our new PSs, a phthalocyanine coupled with eight dendrimers of galactose (PcGal16) demonstrated that its internalization in UM-UC-3 cancer cells occurs in , time- and concentration-dependent fashion and it can be blocked by knockdown of galectin 1. Recently, endocytosis inhibition results proved that PcGal16 entered cells mainly through the clathrin-mediated endocytosis pathway, and caveolae-mediated endocytosis was involved to a small extent.

These results suggest that dendrimers of galactose can serve as a new targeting strategy to promote specific accumulation of PSs into bladder cancer cells through mediated interaction with galectin 1. In addition, the endocytic pathways involved in the entry of PcGal16 have been investigated to shed some light on the possible mechanism of enhanced cellular uptake of PSs conjugated with dendrimers of galactose.

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PI3K and MEK pathway inhibitors differentially regulate autophagic markers: exploitation in novel therapeutic protocols

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Autophagy is the basic catabolic mechanism that involves cell degradation of unnecessary or dysfunctional cellular components through the actions of lysosomes. Autophagy plays an important role in cancer – both in protecting against tumour progression by isolating damaged organelles, also, depending on the tumour, by potentially contributing to cancer growth in promoting survival of tumour cells that have been starved.

The differential impact of autophagy in RAS induced transformation and apoptosis has been shown, and still remains to be further analysed, depending on the tumour cell context. Notably, resistance of colorectal neoplasms has been observed for anti-cancer agents against components of RAF/MEK and PI3K pathways. Resistance pathways are currently under investigation, among which autophagy has attracted a major interest.

In the present study, the role of KRAS/BRAF and PIK3CA oncogenic pathways on the autophagic cell properties and expression of main components of the autophagic machinery in colon tumour cells has been analysed . Moreover, the effect PI3K and MEK pathway inhibitors on autophagy and cell death have been analysed and search of novel combinatorial antitumour therapeutic protocols has been performed for further exploitation.

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KRAS, BRAF, and PIK3CA Pyrosequencing Reveals Remarkable Genetic Colorectal Tumour Heterogeneity

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Colorectal cancer (CRC) diagnostics and therapeutics have to develop novel methodologies yo tackle the disease complexity, tumor heterogeneity, and resistance to targeted therapeutics. In the present study, we examined 171 CRC adenocarcinomas from Greek patients undergoing surgery for CRC to determine the frequency of KRAS, BRAF, and PIK3CA point mutations from different areas of tumors in heterogeneous specimens. Ninety two out of 171 (53.8%) patients were found to bear a KRAS mutation in codons 12/13. Of the 126 mutations found, 57.9% (73/126) were c.38G>A mutations (p.G13D) and 22.2% (28/126) were c.35G>T (p.G12V). Remarkably, RAS mutations in both codons 12 and 13 were recorded in the same tumor by pyrosequencing. Moreover, differences in KRAS mutations between tumor center and periphery revealed tumor heterogeneity in 50.7% of the specimens. Most PIK3CA mutations were revealed by pyrosequencing 6/171 (3.5%). In summary, double mutations of KRAS in the same tumor

and different KRAS mutation status between tumor core and margin are detected with high frequency. These findings as well as other recent findings on tumour heterogeneity are expected to have a major impact in cancer diagnosis and personalized therapies.

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A Transcriptomic Signature Mediated by HOXA9 Promotes Human Glioblastoma Initiation, Aggressiveness and Resistance to Temozolomide

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Objective: Glioblastoma is the most common and malignant subtype of glioma, exhibiting remarkable resistance to treatment. Here we investigated the oncogenic potential of HOXA9 in gliomagenesis, the molecular and cellular mechanisms by which HOXA9 may render glioblastoma more aggressive, and how HOXA9 affects response to chemotherapy and prognosis.

Methods: Expression microarrays were used to identify HOXA9 target genes. Stable glioblastoma cell lines with ectopic HOXA9 overexpression or shRNA-mediated knockdown of HOXA9 were established to evaluate the roles of HOXA9 in cell viability, death, invasion, and response to temozolomide. Subcutaneous and orthotopic intracranial xenograft models of glioblastoma were established to evaluate the oncogenic potential of HOXA9 *in vivo*, and its role in response to temozolomide and overall survival.

Results: Transcriptomic analyses identified novel HOXA9-target genes that have key roles in critical cancer processes, including cell proliferation, adhesion, DNA metabolism and repair, and stem cell maintenance. Functional assays with a variety of glioblastoma cells revealed that *HOXA9* promotes cell viability, stemness, and invasion; conversely, *HOXA9* displayed anti-apoptotic functions. Additionally, ectopic expression of *HOXA9* promoted the malignant transformation of human immortalized astrocytes in an intracranial orthotopic mouse model of glioblastoma, and caused tumor-associated death. *HOXA9* also mediated resistance to temozolomide treatment both *in vitro* and *in vivo*. Mechanistically, BCL2 was identified as a novel HOXA9 target that may be therapeutically targeted. Indeed, the pharmacological inhibition of BCL2 with ABT-737 specifically reverted temozolomide resistance in HOXA9-positive cells.

Interpretation: These data establish HOXA9 as a critical driver of glioma initiation, aggressiveness and resistance to therapy.

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Triple Negative Breast Cancer Mutation Evaluation using Next Generation Sequencing

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Breast cancer is the first cause of death in Europe and Romania, with 8189 new cases each year in our country. Among the different subtypes of breast cancer, triple negative breast cancer (TNBC) presents the most interest because is more aggressive and does not responds to common therapy approaches. Thus, recent studies have been aimed to identify the mutational profile of this type of tumors, in order to explain these limitations of TNBC treatment. The purpose of our present study was to use Next Generation Sequencing in the identification of mutations in 46 genes involved in cancer in 31 patients with TNBC operated at the Oncology Institute "Prof. Dr. I. Chiricuta", Cluj-Napoca between 2006-2007. We used FFPE tissue samples which were sequenced using the Ion Torrent Personal Genome Machine and analyzed with the Ion Reporter 1.6 software. After data analysis, we obtained 103 mutations in 34 of the 46 studied genes. The clinical assessment of the identified mutations showed that 42 were likely pathogenic, 28 were pathogenic, while 29 had no assessment. This study also identified KDR, TP53, PIK3CA, FGFR3 and FGFR2 genes as being the most frequently mutated. In the PIK3CA gene we were able to identify the c.2119G>A mutation, which is a mutation associated to a significantly worse overall survival. For the TP53 gene we identified the c.743C>T mutation, which is characteristic for invasive ductal carcinoma, as well as other mutations specific for breast cancer tumors – c.637G>A, or for metastatic breast cancer tumors – c.818C>T, c.817C>T and c.853G>A. Also, we were able to identify several other unknown mutations that could be associated to the poor prognostic of the TNBC tumors. Our results show that TNBC has specific mutations leading to resistance to therapy and to the poor outcome of these patients.

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Inhibition of dopamine receptor signaling sensitizes glioblastoma multiforme for temozolomide

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Glioblastomas are highly resistant to therapy and carries a dismal prognosis. Current standard of care includes surgery followed by radiotherapy (RT) with concurrent and adjuvant chemotherapy with temozolomide (TMZ). The addition of TMZ to RT increases the overall survival by approximately 3 months compared to RT alone. In this work we sought to identify drugs that would sensitize GBM for TMZ. By integrating results from a genome-wide synthetic lethal RNAi screen with the Connectivity Map database, we have identified signaling networks critical for cancer cell survival during chemotherapy. We transfected human GBM cell lines with a pooled lentiviral shRNA library targeting approximately 5000 genes, and incubated the cells at three sub-lethal TMZ concentrations or in control medium. Individual silencing of 292 genes led to significantly reduced cell growth in the treated population. The Connectivity Map database was then interrogated using these results to identify small-molecule inhibitors that would induce analogous changes in gene expression. From this approach we identified Thioridazine, a dopamine receptor antagonist, as a potent sensitizer for TMZ. We show, both pharmacologically and genetically, that the specific anti-cancer effect of Thioridazine is mediated by blocking the dopamine receptor 2 (DRD2). Furthermore, we show mechanistically that the therapeutic action of Thioridazine was mediated by inhibiting a DRD2/β-arrestin-2/Akt signaling complex leading to impaired autophagy and non-apoptotic cell death. These results could be translated in-vivo by delayed tumor growth and increased survival in tumor-bearing mice. We believe that DRD2 represent a nodal point connecting autocrine survival cues in a subset of glioblastomas. Our findings suggest that, like androgen and estrogen dependence in prostate- and breast cancer, dopamine addiction may represent a fundamental tumor survival mechanism with important therapeutic implications.

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Revealing Regulatory RNAs in the response to Temozolomide in Glioblastoma

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The poor prognosis of glioblastoma (GBM) is mainly attributed to tumor recurrence and resistance to therapy. Understanding how gliomas become resistant to chemotherapy (i.e. Temozolomide, TMZ) is therefore a clinical question of priority. Although some TMZ response markers such as the O6-methylguanine methyltransferase (MGMT) have been identified, there is currently little knowledge on the molecular pathways that may help to overcome therapeutic resistance. So far, transcriptional analysis upon TMZ treatment has mainly focused on mRNA profiling using classical adherent GBM cell lines that do not display typical genetic features and *in vivo* phenotypes of GBM. In this project, we provide an in-depth molecular characterization of the transcriptional response to TMZ, in GBM patient-derived cells grown as 3D spheres under serum-free conditions. We and others have shown, that such cells represent relevant pre-clinical models that reflect typical GBM characteristics in vivo, including infiltrative growth and angiogenic processes. To monitor TMZ response at the cellular level, we applied a multi-color flow cytometric approach combining markers of cell cycle, proliferation, cell death, DNA damage and repair. Using RNA-Sequencing, we have uncovered a complex transcriptional response to TMZ involving different classes of RNAs, both coding and non-coding. We identified miRNAs, mRNAs and lncRNAs regulated by TMZ that are associated with glioma patient prognosis. This integrative analysis of small RNA-seq and RNA-seq data could provide new RNA-based predictors of GBM chemoresistance, as well as potential targets to counteract such a resistance.

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Towards a blood test for esophageal adenocarcinoma

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While the incidence of most cancers are now steadying or declining, esophageal adenocarcinoma (EAC) continues an upward trend. The rapid increase in EAC is likely attributed to the increased prevalence of risk factors of obesity and gastro-esophageal reflux. Despite aggressive treatment, the survival rate for EAC is low at 9-24% five years post diagnosis. The precursor condition, Barrett's esophagus (BE), affects 0.2-2% of the adult population and increases EAC risk 25-100 fold. However, due to the low conversion rate of BE to EAC, studies indicate that current endoscopic screening programs may not be beneficial. Furthermore, a significant proportion of EAC patients do not have prior BE diagnosis, hence there is an urgent need for better detection of EAC. Our goal is to develop blood biomarker panels that can be used to screen at-risk patients, with positive results triggering follow-on endoscopic screening.

We focused on alterations in circulatory protein glycosylation, using a panel of 20 lectins to enrich serum glycoproteins based on glycan structures. Serum samples from healthy, BE and EAC patients (n=29) were analyzed by lectin magnetic bead array (LeMBA) (Choi et al., 2011)-coupled discovery proteomics for biomarker discovery, followed by targeted proteomics using multiple reaction monitoring-mass spectrometry for biomarker verification (n=61). Data analysis was performed using customized database and analysis packages "GlycoSelector" for biomarker discovery and "Shiny mixOmics" for biomarker verification (Lê Cao et al., 2011).

We have identified a ranked list of glycoprotein biomarkers that distinguish a) EAC from BE and b) EAC from healthy phenotypes. Selected biomarkers were further

validated using an orthogonal technique, immunoblotting. A multivariate panel achieved area under the receiver operating curve (AUROC) over 0.90, indicative of high diagnostic value. Continuing work will evaluate clinical performance of the EAC biomarker panel in a large independent patient cohort.

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Stem cell markers and early precursor phenotype in T lymphoblastic lymphoma.

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Precursor T lymphoblastic lymphoma (T-LBL)/T lymphoblastic leukemia (T-ALL) is a neoplasm of lymphoblasts committed to the T-cell lineage. LBL is comparable to ALL and often difficult to distinguish from one another morphologically. The major distinction between LBL and ALL is that the degree of BM involvement is often greater in ALL. Recent studies have identified a subtype of T-ALL termed ‘‘early T-cell precursor’’ (ETP) ALL comprising up to 15% of T-ALL, and associated with high risk of treatment failure and poor prognosis. However, little is known about such ETP phenotype or expression of other precursor molecules in T-LBL. It is plausible and hypothesized that subsets of cells that retain stem cell-like features, also exist in T-LBL and would respond poorly to lymphoid-cell directed therapy and therefore poor outcome or high risk of relapse in these patients. Based on this hypothesis we reviewed 30 cases of precursor LBL/ALLs reported from our department. These were analysed for clinicopathologic parameters, and expression of early precursor phenotype, stem cell markers and aberrant myeloid markers. A correlation of clinicopathologic parameters, multidrug combination therapy regimes, cell marker profiles and survival outcome was also done. The details would be presented and discussed.

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Can metabolomics predict early disease recurrence in patients with early-stage non-small cell lung carcinoma?

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Background. Complete surgical resection is the treatment of choice for patients with early-stage I-IIIA non-small cell lung carcinoma (NSCLC). However, 10 to 25% of these patients will have a disease relapse or die within 12 months after surgery.

Objective. Therefore a biomarker that could stratify patients between outcomes within one year at time of diagnosis would be very useful in clinician’s decision making. Metabolomics has already proven to be capable for distinguishing lung cancer patients from healthy subjects. This study evaluated if the metabolic phenotype of blood plasma as determined by ^{1}H-NMR spectroscopy could predict which patients will have a disease relapse within a year.

Results: Between 2011 and 2013 the blood plasma of seventy-two patients with early-stage NSCLC were evaluated by ^{1}H-NMR spectroscopy. In total 44 men and 28 women were included, with a mean age of 65 year. The study population consisted of 37 patients with an adenocarcinoma, 30 patients with a squamous cell carcinoma, 3 patients with a NSCLC not otherwise specified and 2 patients with an adenosquamous carcinoma. In total 10 patients (14%) had a disease recurrence within a year. Unfortunately multivariate statistics with principal component analysis and orthogonal partial least squares-discriminant analysis could not find a significant difference in the metabolic profile between patients with en early disease relapse and patients without early disease recurrence. Even when patients where subdivided according to histological type or pathological state metabolic profiling could not distinguish between aforementioned patients.

Conclusion: Metabolic profiling by ^{1}H-NMR spectroscopy could not predict which patients with early-stage NSCLC had an early disease relapse. Possible explanations are the heterogeneous study population and the small number of observations. Nonetheless it is also possible that ^{1}H-NMR spectroscopy isn’t sensitive enough to detect the differences in the metabolism between an aggressive and non-aggressive lung tumour.

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NoSarC.org, – Norwegian Sarcoma Consortium.

Towards individualized therapy for orphan cancer

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Abstract

New therapies targeting aberrant signalling pathways offer great promise for cancer treatment. However, even with increasing insight into molecular mechanisms involved in rare cancers like sarcomas, the investments required to develop new drugs are prohibitive for such limited patient populations. However, there is increasing understanding that the same oncogenic mechanisms may contribute to subsets of cancers of various tissue origins. Thus, one may by molecular diagnostics identify tumours which are sensitive to therapies already developed and approved for more common cancers. A prime example is the sarcoma subtype GIST, which was found to be driven by mutations in c-KIT and thus targetable by the leukaemia drug Imatinib with very good results. We are now initiating mutation screens of 2-3 annual cohorts of sarcoma to identify targets for existing therapies. This unique approach is based on the collaboration with the clinical sarcoma groups in all health regions, and is expected to feed new candidate approaches to trial designs by the World Sarcoma Network. Furthermore, we will investigate the potential of new

treatments in preclinical studies on sarcoma models and, based on ethical approval, also small-scale or "single patient" studies. This project (NoSarC) builds on our national cancer genomics network, NCGC, (see cancergenomics.no) and will serve as a clinical scale model for the introduction of personalized strategies for orphan cancers.

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Prognostic significance of microenvironment expression of PD-1 ligands and cytokines in classical Hodgkin's lymphoma

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The clinical and pathologic features of classical Hodgkin's lymphoma (cHL) reflect an abnormal immune response resulting from the expression of a variety of cytokines by HRS cells, altering the composition and function of HRS cells in the surrounding microenvironment. The aim of our study was to investigate the prognostic significance of microenvironment expression of PD-1 ligands and cytokines in cHL.

The case group comprised 49 patients with cHL, who received chemotherapy (ABVD or BEACOPP-14/esc) and radiotherapy by indications. PD-L1/2, MIP-1α, RANTES, IFN-γ, IL-10 mRNA expression levels were analyzed in pre-treatment fresh biopsies of lymph nodes using real-time RT-PCR.

For 49 patients the overall response rate after the first line therapy was 95.9% with a complete response (CR) of 73.5% and a partial response – 22.4%. Progression during the therapy was observed in 2 patients. Among patients who achieved a CR during the follow up (24-36 months) – 9 had relapses. We noticed that 26.5% of cHL cases were PD-L1 negative, 16.3% -RANTES negative, 14.3% – both PD-L1 and RANTES negative. MIP-1α and RANTES levels were higher in mixed cellularity cHL, IFN-γ – in lymphocyte rich, PD-L1 – in nodular sclerosis and advanced cHL stages. PD-L2 and IL-10 levels were independent of histological variant or disease stage. All cases with the absence of both PD-L1 and RANTES had a CR to the therapy and long-term remission. A trend for a higher risk of relapse was observed for patients with increasing RANTES/MIP-1α and decreasing IFN/IL-10 rates (P=0.09, P=0.12, respectively).

High PD-L1 expression was associated with the reduced progression-free survival (PFS) in cHL patients. A 2-year PFS rate for cHL patients with high PD-L1 expression was 47% compared to 95% for low/absent of PD-L1 expression (P=0.04).

PD-L1 expression level can be used as a marker of prognosis in cHL patients and represents an attractive target for a cHL immunotherapy in patients with poor outcome.

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A cytoskeletal LIM domain protein promotes invadopodial activity and cell invasion in aggressive breast cancer

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Metastasis is the primary cause of cancer mortality. The metastatic cascade involves a number of steps which largely rely on the ability of tumor cells to digest the extracellular matrix (ECM) and invade tissues. We identified a nucleo-cytoplasmic LIM domain-containing protein, namely cysteine rich protein 2 (CRP2), whose up-regulation closely correlates with basal-like breast carcinoma, a breast cancer subtype associated with poor prognosis. Accordingly, we found that CRP2 is weakly or not expressed in non-/weakly metastatic, epithelial-like, breast cancer cell lines, whereas it is highly expressed in highly metastatic, mesenchymal-like, cell lines. Using the MDA-MB-231 aggressive breast cancer cell model, we provide evidence that CRP2 substantially promotes cell invasion in various *in vitro* assays. In contrast, CRP2 only modestly contributes to 2D cell and 3D spheroid proliferation. By investigating the molecular mechanism underlying the role of CRP2 in cell invasion, we found that CRP2 is required for efficient ECM digestion. For instance, CRP2-deficient MDA-MD-231 cells exhibited a 50% reduced capacity to digest a gelatin matrix as compared to control cells. Noticeably, this functional defect was rescued in a complemented cell line. In addition, zymography analyses revealed that CRP2-deficient cells secrete reduced amounts of metalloproteinases (MMPs). Collectively our data point to a role for CRP2 in the formation and/or activity of invadopodia which are actin-rich protrusions whose primary function is to secrete MMPs and degrade the ECM for invasion. Supporting this scenario, CRP2 (non exclusively) localizes in active invadopodia formed by MDA-MB-231 cells. In addition, our biochemical analyses revealed that CRP2 autonomously crosslinks actin filaments into parallel bundles, a type of cytoskeletal component that constitutes the backbone of invadopodia. In vivo mouse experiments aimed at validating the role of CRP2 in metastasis are currently conducted.

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Next generation sequencing reveals clinically relevant genetic signatures for the identification of novel therapeutics for oral cancer

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Oral squamous cell carcinoma (OSCC) remains one of the most difficult to treat head and neck cancer with a survival rate of approximately 50% over 5 years. Clearly, the existing treatments for head and neck cancer have failed to make a significant difference in the prognosis of oral cancer patients underscoring the urgent need for development of more effective therapies for treatment of these cancers. Recent extensive molecular characterization of cancer cell line panels has led to the identification of new therapeutics targets or repurposing existing drugs. A key component of such studies is cancer cell lines that are representative of the cancer. We have profiled a panel of 16 patients-derived OSCC cell lines (ORLs) by RNAseq and identified gene expression profiles consistent to those observed in HNSCC

tissues, including a closely clustered subset of ORLs enriched in cell cycle pathways (CC cluster). To demonstrate the clinical relevance of the molecular subtypes, cell lines from different clusters were treated with clinically relevant therapeutics including cisplatin, mitomycin C, Irinotecan and topotecan and analyzed with MTT assays. Further, based on genes that are enriched in the CC cluster, cell lines were treated with CDK inhibitors and analyzed with CLICK-it assays to determine whether there was differential response from the different clusters. We demonstrated that ORLs from different gene expression clusters respond differentially to targeted drugs but not to general DNA crosslinking agents. For example, ORLs from CC cluster were significantly more sensitive to Topoisomerase 1 inhibitors (Irinotecan and Topotecan) and to CDK1 inhibitor (RO-3306) compared to other cell lines. Taken together, the data demonstrate that cancer cell lines that are representative of oral cancer can identify clinically relevant subtypes of the disease and affords an opportunity to identify new targets for therapeutic interventions.

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The role of Fbxw7 expression in hepatocellular carcinoma and adjacent non-tumor liver tissue

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Background and aim: Fbxw7 is a tumor suppressor gene through ubiquitination and degradation of multiple oncoproteins. Loss of Fbxw7 expression is frequently observed in various human cancers. In the present study, we examined the role of Fbxw7 expression in both non tumor liver tissues and tumor tissues on clinicopathological significance.

Methods: Sixty six patients with hepatocellular carcinoma (HCC), who underwent hepatectomy, were divided into two groups: high and low gene expression group, based on the Fbxw7 expression level. We compared the clinicopathological factors between the high expression and low expression groups in both tumor and non tumor tissues.

Results: Fbxw7 mRNA expression level in the non tumor tissues was significantly higher than that in the tumor tissues. In the analysis of Fbxw7 expression in tumor and non tumor tissues, disease free survival rate in the Fbxw7 high expression group was significantly higher than that in the low expression group. In multivariable analysis, Fbxw7 low expression in both tumor and non tumor tissue was detected as the strongest independent risk factor for HCC recurrence.

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CD133^{+}CD15^{+} Subpopulation of Chordoma Cells are Candidate Cancer Stem Cells

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Chordoma is a rare type of malignant bone tumor and is known to arise from the remnants of the notochord. It is known to be resistant to chemotherapy and radiotherapy. It is similar characteristic with cancer stem cells (CSC). As a result, there may be a CSC subpopulation in chordoma. CD133 also known as prominin and CD15 also known as SSEA1 have been identified as a marker for various kinds of stem cells in cancer. In several studies suggested that CD133+ and CD15+ cell populations showed high tumorigenicity, self-renewal capacity and resistance to chemotherapy. These features make cancer cells more aggressive. Aim of our study is to identify CSC subpopulation in chordoma. METHODS: In this study chordoma cells were sorted by cell surface marker including CD133 and CD15. 3 chordoma cell line U-CH1, U-CH2 and MUG-Chor were used in this study. CD133+CD15+ and CD133-CD15- subpopulations were sorted from all of chordoma cell lines using FACS AriaIII. Genes that are up-regulated in stem cells were compared including Oct4, Klf4, c-myc, Nanog and Smad2 and genes that are marker for chordoma were compared including Brachyury, EMA, Vimentin and Gal-3. We then analyze miRNA profile bot CD133+CD15+ and CD133-CD15- subpopulation using Affymetrix Gene Atlas. RESULTS: Our result showed that CD133+CD15+ subpopulation of chordoma is very limited in chordoma cell lines(Approximately %1). According to the results expression level of genes that are up-regulated in stem cells are higher in CD133+CD15+ subpopulation when compared with CD133-CD15- subpopulation. CONCLUSION: The results suggest that CD133+CD15 subpopulation of chordoma cell lines share similar characteristic with stem cells. This finding may lead to the development of new approaches toward treatments of chordomas.

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Targeting acute myeloid leukemia with a small molecule inhibitor of the Myb/p300 interaction.

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The transcription factor c-Myb is highly expressed in hematopoietic progenitor cells and controls the transcription of genes important for lineage determination, cell proliferation, and differentiation. Deregulation of c-Myb has been implicated in the development of leukemia and certain other types of human cancer. c-Myb activity is highly dependent on the interaction of the c-Myb with the KIX domain of the coactivator p300, making the disruption of this interaction a reasonable strategy for the development of Myb inhibitors. Inhibition of Myb is therefore emerging as a potential therapeutic strategy for these diseases. However, due to lack of suitable inhibitors the feasibility of therapeutic approaches based on Myb inhibition has not been explored. We have identified the triterpenoid Celastrol as a potent low molecular weight Myb inhibitor that disrupts the interaction of Myb with its cooperation partner p300. We demonstrate show that Celastrol suppresses the proliferative potential of acute myeloid leukemia (AML) cells while not affecting normal hematopoietic progenitor cells. Furthermore, Celastrol prolongs the survival of mice in a model of an aggressive AML. Overall, our work demonstrates for the first time the therapeutic potential of a small-molecule inhibitor of Myb for the treatment of AML. In addition, the

identification of Celastrol as a potent Myb inhibitor provides a starting point for the further development of Myb-inhibitory compounds for the treatment of leukemia and, possibly, other tumors driven by deregulated Myb.

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Preclinical efficacy and safety studies of PEK fusion protein in GPI-0100 adjuvant for HPV-infectious diseases immunotherapy

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Human papillomavirus 16 (HPV16) protein E7 is considered a potential target for cancer therapy based on the evidence of its interaction with the products of the Rb tumor suppressor protein. Several lines of evidence suggest that cell-mediated immunity is important for control of both HPV infection and HPV-associated neoplasia. A vaccine comprising the cell binding and cytoplasmic translocation domains of *Pseudomonas aeruginosa* exotoxin at the N-terminus, the full length HPV 16 protein E7 in the middle, and the triple KDEL ER retention signal in the C-terminus (termed PEK), has the potential advantage of therapy by stimulating T cell responses targeting HPV16 E7 protein. Here we test PEK formulated along with the adjuvant GPI-0100, a semi-synthetic quillaja saponin analog that was developed to promote both humoral and cellular immune responses. Subcutaneous administration to mice of PEK with GPI-0100, three times at weekly intervals, elicits strong HPV16 E7 specific T cells and protected mice from HPV16-transformed TC-1 tumor cell challenge. Notably, Vaccination with PEK in GPI-0100 adjuvant also enhanced the amount of tumor infiltrating lymphocytes in mice. Vaccination with PEK in GPI-0100 also completely prevented tumor growth after challenge with 5×10^4 TC-1 tumor cells. Furthermore, 3+1 weekly vaccinations with 1.2 mg of PEK in 1.2 mg GPI-0100 were well tolerated by New Zealand White Rabbits and Cynomolgus Monkeys. These results revealed that PEK formulated with GPI-0100 adjuvant is a potential active and safe therapeutic vaccine for HPV-infectious disease immunotherapy.

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A novel role for Wnt signaling pathway, regulating the response to cisplatin in lung and ovarian cancer cells through the action of the Transcription Factor TCF4.

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The standard treatment for non-small cell lung cancer (NSCLC) and ovarian cancer is Platinum-based chemotherapy, although the main clinical problem associated is the progression of the disease to a platinum-resistant state. This fact has limited its efficacy in these tumor types, which is one of the first causes of cancer deaths in developed countries. Thus, it is of great interest to identify predictive molecular biomarkers that could help in the patient treatment selection. In this study we used array-CGH to analyze the cytogenetic alterations that arise in NSCLC and ovarian cancer cells after cisplatin treatment, by using four paired sensitive(S) and resistant(R) cell lines: H23S/R, H460S/R, A2780S/R and OVCAR3S/R. Our experimental approach revealed the presence of a common deletion of the gene TCF4 in a mosaic manner in at least 50% of the resistant cells in both tumor types, while a decrease in TCF4 expression was confirmed through qRT-PCR in the same cells. As TCF4 is a downstream transcription factor of Wnt signaling, we analyzed its potential role regulating the CDDP response in resistant cells through its action in the Wnt pathway. Combination of Top-Fop vectors and TCF4-cDNA overexpression plasmids showed firstly, that resistant cells responded easily to the activation of Wnt pathway, an effect in part mediated by the decrease in TCF4 expression; secondly the overexpression of TCF4 induced an increase in the Cisplatin sensitivity. These results indicate that TCF4 could be acting as a Wnt transcriptional repressor, maintaining the sensitivity to Cisplatin in A2780-S cells. Our translational approach in a total of 40 ovarian and lung primary tumors and in 14 normal tissues confirmed that TCF4 expression is frequently downregulated in these tumor types. Altogether we present a novel role for Wnt signaling pathway, regulating the response to CDDP, which could be a potential target for cancer treatment.

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Development of Gene Therapy Products for Cancer Treatment in Russia

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Unique preparations for treatment of cancer patients with direct antitumor effect and detoxifying and immunomodulating properties have been developed in Russia. Three preparations for gene suicide antitumor therapy were created using the PEI-PEG-TAT non-viral vector and CMV promoter: AntioncoRAS-M on the basis of herpes simplex virus thymidine kinase and GM-CSF genes, AntioncoRAS-F – with yeast cytosine desaminase and GM-CSF genes, and GeneTherPlus – with CD gene and modified Cre-LoxP system enhancing the target gene expression. It has been shown in vitro and in vivo that transfection of murine and human tumor cells with these constructs caused the production of target proteins, while addition of prodrugs to the transfected cells led to their death. Cytotoxic activity of all three systems depends on the amount of the transfecting construct and the prodrug, on the time intervals between injections of system components, and on the duration of cell incubation with reagents or duration of animal treatment. All studied systems inhibit growth of the transplanted murine and human tumors by 30-90%, increase animal lifespan by 20-75%, and enhance chemo- and radiation treatment efficacy. An original gene

therapeutic construct (RPAN-Lf) producing human lactoferrin was obtained on the basis of recombinant serotype 5 adenovirus with inserted human lactoferrin gene (hLf). Pharmacodynamics, pharmacokinetics, and safety of RPAN-Lf were studied. It is efficient for inserting hLf gene into 293 cells of permissive culture. Recombinant hLf produced in those cells is similar to native hLf by MW (78±2kDa), antigenic, antioxidative, and antimicrobial properties. RPAN-Lf provides the expression of hLf gene in animal cells for 30 days after the transfection. Recombinant hLf, as well as native hLf, is efficient as a detoxifying agent in cases of acute toxic reactions caused by CCl4, or CDDP, or transplanted tumors. The range of safe and efficient doses was evaluated in experimental models using animals.

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The Roles of Single Nucleotide Polymorphisms in the p53 Pathway in Human Cancer and Evolution

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There is great heterogeneity between individuals in their risk of developing cancer and response to treatment. This heterogeneity is a major obstacle in designing uniformly effective prevention and treatment strategies. Common inherited genetics have great potential to help us understand cancer and serve as biomarkers. GWAS studies have identified hundreds of SNPs associated with cancer susceptibility. However, discerning the causal-SNP from linked nonfunctional-SNPs and the molecular mechanism behind the association have proven challenging. These uncertainties have limited our ability to integrate SNPs into the clinic. To address this we focus on the identification and analysis of functional, cancer-associated SNPs residing in well-defined cancer pathways. We focus in the p53 tumor-suppressor-network. Knowledge of how SNPs impact p53 signaling and cancer will most certainly deepen our understanding of tumor suppression and aid in selecting better screening and treatment strategies. For instance, the ability of p53 to regulate transcription is crucial for tumor suppression and implies that SNPs in functional p53 binding sites could influence cancer. Indeed, we identified a polymorphic p53 responsive element, and demonstrated its influence on cancer risk using genome-wide datasets of cancer susceptibility loci, p53 occupancy and subsequent experimental validation and modeling. The SNP resides in *KITLG* and associates with one of the largest risks identified among cancer GWASs. We established that the SNP has undergone positive selection, signifying a selective benefit: possible UV protection in lighter-skin individuals. We go on to show that similar SNPs are rare in the genome due to negative selection, indicating that polymorphisms in p53 binding sites are primarily detrimental to humans. Together, this work provides an example for why an inherited mutation that increases cancer risk could be positively selected for in a population and found at such high frequencies and provides further evidence for the importance of a tightly regulated p53 response.

EACR, OECI and FNR Participation Grant Winners

Congratulations to the winners of EACR, OECI and FNR Participation Grants. Each winner received an award to the value of up to 1000 Euros to assist with the cost of accommodation, travel and registration to support full attendance at the conference.

EACR European Association for Cancer Research

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Highly multiplexed amplification-free Cancer Pathway analysis on challenging samples (FFPE) using the NanoString nCounter technology.

DATE: Monday 2 March 2015
TIME: 13:30 - 14:00
ROOM: José Ensch

Rudy van Eijsden, Ph.D.
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Monday 02 March 2015 13.30 - 14.00

Rudy van Eijsden (Netherlands)

"Highly multiplexed amplification-free Cancer Pathway analysis on challenging samples (FFPE) using the NanoString nCounter technology."

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Tuesday 03 March 2015 13.00 – 13.30

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"Unravelling breast cancer progression through geographical and temporal sequencing"

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