ORAL PRESENTATIONS

PL1: TARGETING K-RAS DRIVEN LUNG AND PANCREATIC TUMORS

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K-*RAS* oncogenes have been implicated in about one fifth of all human cancers including lung adenocarcinoma and pancreatic ductal adenocarcinoma, two tumor types with some of the worse prognosis. Unfortunately, in spite of intense research efforts, identification of suitable therapies to treat these tumors remains elusive. Indeed, patients suffering from these tumor types are still treated with cytotoxic compounds approved more than two decades ago. In order to identify novel and effective therapeutic strategies, we have developed genetically engineered mouse (GEM) models that closely recapitulate their natural history. We are using these mice to validate targets of potential therapeutic value with the ultimate goal to translate these findings to the clinic. In previous studies, we have crossed these GEM models with mice that carried either germ line or lox-Cre conditional knock out alleles encoding each of the downstream kinases of the Raf/Mek/Erk pathway as well as the cell cycle interphase Cdks to interrogate their role in the development of K-Ras driven lung adenocarcinomas. These studies have led us to validate the c-Raf, Mek1/2, Erk1/2 and Cdk4 kinases as critical targets for these tumors (Puyol *et al.*, Cancer Cell 2010; Blasco *et al.*, Cancer Cell 2011). Following a similar strategy, we have also demonstrated that the EGF Receptor and c-Raf are required for the development of PanIN lesions, a precursor for the formation of K-Ras driven pancreatic ductal adenocarcinomas (Navas *et al.*, Cancer Cell 2012). Moreover, we have established that whereas systemic ablation of the Mek1/2 and Erk1/2 kinases result in the rapid death of the animals, mice tolerate reasonably well the systemic elimination of c-Raf, Cdk4 and EGF Receptors. Hence, making these kinases suitable targets for K-Ras driven lung and pancreatic tumors.

More recently, we have decided to interrogate the role of these targets in advanced tumors. To this end, we have generated new GEM models in which expression of the resident K-Ras oncogene, as well as ablation of the p53 tumor suppressor is mediated by the frt-FLp(o) targeting system, a strategy that allows us the temporal and spatial separation of tumor development from target ablation. In addition, we have generated lox-Cre based conditional knock-in strains for c-Raf, Cdk4 ERF Receptors and PI3K p110α that, upon recombination, direct the expression of kinase dead isoforms instead of inducing protein ablation. We feel that these strains should serve as better models to mimic drug intervention in the clinic. Whereas inactivation of some of these kinases (Cdk4 and EGF Receptor) leads to results comparable to those obtained by ablating protein expression, in the case of c-Raf, the results appear to be significantly different. Finally, we are now combining the ablation and/or inactivation of these targets in K-Ras/p53 mutant lung and pancreatic GEM tumors in order to identify therapeutic combinations that may eventually eradicate these advanced tumors. We hope that these studies will serve to guide the design of future clinical trails to treat patients carrying K-RAS mutant tumors.

PL2: STROMAL CELL ACTIVATION IN PRIMARY AND METASTATIC BREAST CANCER

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The interaction of tumour cells with stroma cells and stromal components not only promotes tumour progression and metastasis, but also influences tumour cell responses to chemotherapy, endocrine therapy and targeted agents. As a consequence, there is an urgent need to identify strategies to efficiently target these interaction pathways for the prevention or suppression of metastatic disease and to overcome treatment-resistant tumour progression in advanced breast cancer.

We are interrogating the tumour-stroma crosstalk pathways underpinning the striking heterogeneity between different breast cancers in their ability to recruit and activate a pro-tumorigenic stroma. These studies are focussed on (a) the mechanisms involved in the activation of cancer-associated fibroblasts (CAFs) and the different functional roles played by activated and non-activated CAFs, (b) the role of activated pericytes in promoting metastatic dissemination, and (c) the mechanisms by which a productive microenvironmental niche is initiated at metastatic sites.

K: EMERGENCE OF PHENOTYPES IN AGING AND DISEASE

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This talk is about concepts rather than mechanisms. Aging and age-related diseases will be considered as emerging phenotypes of increasingly damaged progressively malfunctioning proteomes. Similarity between disease and mortality rates hints to a common cause underlying aging and age-related diseases, called intrinsic aging. From our comparative studies of robust and standard species, dysfunctional oxidatively damaged proteins appear as the likely root cause of radiation-induced and age-related morbidity and mortality with snowballing phenotypic consequences (Radman, DNA Repair 2016; Krisko and Radman, PLoS Genetics 2013). I shall elaborate: (i) how protein polymorphism translates to damage polymorphism that can determine the predisposition to age-related diseases, (ii) how proteome damage produces phenotypes, either directly or indirectly via generation of mutations (Radman and Radman, PLoS Genetics 2013) and acceleration of their phenotypic expression, and (iii) the perspectives for diagnosis, prognosis, prevention and cure of degenerative diseases.

L1: TRACING THE EVOLUTIONARY ORIGIN OF TUMORS

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Molecular nature of malignant tumors is well studied in vertebrates, while their evolutionary origin remains to be uncovered. In particular, there is no evidence for cancers in pre-bilaterian animals, although recent computational studies have predicted that all metazoans are prone to develop tumors. We provide the first evidence for naturally occurring tumors in the cnidarian genus Hydra. Histological, cellular and molecular data reveal that these tumors are transplantable, caused by differentiation arrest of female gametes, and their growth is independent on the cellular environment. Tumor bearing polyps have significantly reduced fitness as evidenced by their clonal growth retardation and reduced fecundity. In addition, the Hydra tumors show a greatly disturbed transcriptome that mimics expression shifts in vertebrate cancers. Therefore, this shows that invasive tumors have deep roots in animal phylogeny, and that simple animals may be informative in revealing the fundamental mechanisms of tumorigenesis.

L2: THE METASTASIS SUPPRESSOR GENE FAMILY Nme/Nm23/NDPK: LESSONS FROM MODEL ORGANISMS

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The Nme gene/protein family, initially called nucleoside diphosphate kinase (Nm23/NDPK), consists of evolutionarily conserved genes/proteins present in all three domains of life. This family of proteins was originally named after the first member identified, Nm23-H1/Nme1, which is responsible for metastasis suppression of many tumor types. The Nme1/NDPKA and Nme2/NDPKB are two units of a well-known enzyme nucleoside-diphosphate kinase (NDPK), which transfers the terminal phosphate form (d)NTPs to (d)NDPs and is, therefore, responsible for the maintenance of the cellular nucleotide pool. Besides this housekeeping role, the Nme/NDPK proteins have been assigned several additional biochemical functions. Numerous proteins from evolutionary distinct organisms exhibit extraordinary similarity in their structures with their orthologs in mammals including humans. Therefore, it is presumed that they have similar or identical biochemical and biological functions. The goal of our studies was to determine the structure, function and evolution of several members of the Nme/NDPK family in model systems evolutionary very distinct form humans. Our studies were focused on nme homologs/orthologs in 'non-bilaterian' Metazoa, namely sponges (Porifera). Sponges are simple animals and are considered to be the closest to the common metazoan ancestor. Series of biochemical and biological tests, especially the formation of stable clones expressing the sponge variant of the Group I Nme gene which inhibited the migratory potential of human tumor cells, implied that its function in migration processes was engaged before the origin of true tissues, and genesis of tumors and metastasis. This suggests that the common ancestor of Metazoa possessed a functional NmeGp1 metastatic suppressor gene/protein homolog and that several of its multiple functions existed before the emergence of true multicellularity. Recently we have done a similar study on the Nme homolog from a unicellular filasterean organism *Capsaspora owczarzaki*, which was also able to suppress migration potential of human cells although the human, sponge and filasterean proteins exhibit some structural differences.

L3: ONCOGENIC p63 SIGNALLING IN MELANOMA AND ITS LINK WITH ACQUIRED RESISTANCE TO MAPK INHIBITORS

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Targeted therapy with specific BRAF inhibitors (BRAFi) has improved both overall and progression-free survival in melanoma. Despite impressive initial responses, most patients eventually relapse due to pre-existing or acquired BRAFi resistance. Several mechanisms have been linked to this acquisition of resistance, including NRAS and MEK1 mutations, increased expression of MAP3K, Cot1, cyclin D1 and growth factor receptors. However, the signaling pathways involved in acquired BRAFi resistance in melanoma are not yet fully understood, placing limitations on successful development of second-line treatments which are urgently needed for patients resistant to BRAFi. We have recently provided the first evidence of significant p63 expression in melanoma cell lines and clinical samples and demonstrated its link with poor prognosis. We identified a p63 location/expression pattern in melanoma cells, which differs from that previously reported in cells of keratinocyte lineage or in cutaneous SCC. We have also reported how p63 inhibits p53 apoptotic function in melanoma cells exposed to genotoxic drugs. We have now identified a significant correlation between p63 expression and mutational status of BRAF/NRAS in clinical samples, reinforcing the link between p63 expression and activation of the MAPK signalling pathway in melanoma. In addition, we have established cellular models of melanoma BRAFi resistance that display increased expression of p63 isoforms at both mRNA and protein levels. Further analysis of several p63 interacting proteins in melanoma cells chronically exposed to BRAFi reveals a novel signalling pathway involved in the development of resistance and suggests that strategies aimed at reducing endogenous p63 levels in metastatic melanoma may provide a therapeutic approach to counteract BRAFi resistance-associated MAPK pathway activation.

L4: TARGETING HEDGEHOG-GLI SIGNALING IN CANCER: NEW CONCEPTS AND STRATEGIES

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Uncontrolled Hedgehog (HH) signaling leads to initiation and progression of numerous cancer entities and therefore constitutes a promising target for cancer therapy. Clinically advanced inhibitors of the HH pathway targeting the essential HH effector Smoothened (SMO) are promising therapeutic drugs. However, development of drug resistance, SMO-independent GLI pathway activation and severe side effects limit the application of these SMO modulators, urgently calling for novel and improved therapeutic strategies.

The oncogenicity of the HH/GLI cascade is also regulated by non-canonical, Smoothened-independent mechanisms by integration of common oncogenic driver signals. Identifying synergistic pathway interactions may therefore guide the way to new therapeutic opportunities based on rational combination treatments. The presentation will summarize efforts to identify cooperative pathway interactions of HH/GLI signaling in cancer as well as discuss new data on kinases and potential drug targets that positively regulate oncogenic GLI transcription factors.

L5: GENETICS AND EPIGENETIC ALTERATIONS OF HEDGEHOG-GLI SIGNALING PATHWAY IN OVARIAN TUMORS

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Hedgehog-Gli (Hh-Gli) signaling pathway is the essential for tissue development and stemness, and its deregulation has been observed in many tumors. Its aberrant activation as a result of genetic and epigenetic alterations has been reported in various types of tumors.

Our research showed Hh-Gli pathway to be active in ovarian tumors, both carcinoma and borderline tumors, and *GLI1* and *SUFU* expression was associated with tumor type and FIGO grade. Point mutations of the main members of Hh-Gli pathway were not detected in any type of investigated ovarian tumors, but some of *PTCH1* polymorphisms were significantly less frequent in both benign and malign ovarian tumors compared to healthy controls. In addition, loss of the entire *PTCH1* gene has been observed in ovarian dermoids, ovarian fibroma and ovarian carcinoma.

Epigenetic alterations of Hh-Gli pathway genes in the development of various ovarian tumors have mostly been reported as a promoter hypermethylation of tumor suppressor gene *PTCH1*. We have shown that in benign ovarian tumors the *PTCH1* promoter was mostly methylated in the CpG island located near the GLI-binding site, thus preventing the *PTCH1* expression and obstructing the pathway negative feedback loop in which GLI1 stimulates the expression of *PTCH1*. On the contrary, it seems that *PTCH1* promoter methylation is not present in ovarian cancer. By whole-genome DNA methylation profiling of ovarian cancer, others found two additional Hh-Gli pathway members, *ZIC1* and *ZIC4*, to be silenced by hypermethylation, what was correlated with increased proliferation, migration and invasion (Huang *et al.*, Epigenetics 2013). An alternative epigenetic modification of Hh-Gli pathway is possible through the inhibition of BET bromodomain proteins: RD4 regulates *GLI1* and *GLI2* transcription occupying their promotors (Tang *et al.*, Nat Med 2014).

We recently started to investigate the role of microRNA in ovarian cancer etiology driven by deregulation of Hh-Gli signaling pathway. We hypothesize that their expression profile can be more accurately correlated with cell differentiation and developmental status compared to mRNA profile. Our first results highlighted more than dozen candidate microRNAs potentially targeting Hh-Gli signaling pathway genes, which have to be additionally verified and investigated.

L6: MULTI-SYSTEM ANALYSIS OF GENOME-WIDE MUTATIONAL SIGNATURES: IMPLICATIONS FOR MOLECULAR CANCER EPIDEMIOLOGY

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Mutational spectra in cancer genomes have the potential to reveal past carcinogenic exposures and can thus point to the causal events underlying the onset and evolution of cancer. Consequently, genome-wide analyses of mutational signatures, carried out in the context of molecular cancer epidemiology studies, can inform hypotheses on particular etiological factors for specific cancer types. Owing to the advances in massively parallel sequencing technologies and associated bioinformatics methods, data on mutation landscapes in human primary tumors accumulate exponentially. However, there remains a need for complementary experimental studies allowing further characterizing of mutagenic insults associated with particular candidate carcinogens. Innovative multi-system approaches using robust cell-based and animal models will be presented, with a focus on their potential for deep investigations of exposure-specific impact on the genome, their capacity to accelerate the identification of cancer-specific etiological factors, and ultimately on their promising role in systematic carcinogen evaluation, classification, and in cancer prevention measures.

L7: RECENT ADVANCES IN CANCER IMMUNOTHERAPY

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By being successful in advanced stages of several solid tumors, novel treatments using modifications of patients' immune system have changed the current models of oncologic therapy. Collectively named oncologic immunotherapy, they show promise to change the therapeutic regimens in many malignancies in the future. The recent increase in clinical trials with immunotherapy has been encouraged with the success of the use of immune checkpoint inhibitors. These follow previous trials using chimeric antigen receptor modification, various tumor-antigen "specific" vaccines and viral vectors to combat cancer. The immunotherapeutic "arsenal" can boost, empower or tailor patient's own immune system to seek and kill cancerous cells throughout the body. Therapies like immune checkpoint inhibition (anti-PD1, anti-CTLA4) in melanoma, non-small-cell lung cancer and renal cancer, peptide vaccination in prostate cancer and glioblastoma, and oncolytic immunotherapy in melanoma have been approved by regulatory authorities and are already in clinical use. Ongoing clinical trials are currently pursuing novel therapeutic settings and treatment combination for appropriate patient populations trying to maximize benefits of immunotherapeutic regimens. There is a search to find biomarkers that could be used for continuous monitoring of treatments' successes and identification of therapeutic targets. These biomarkers will be important for the ideal implementation of immunotherapy into cancer therapies of the future. Some biomarkers might belong to a group of hereditary risk factors for cancer, which could be essential for early diagnostics and prognosis. Our recent search for genetic risk factors in various types of solid tumors will be discussed.

L8: EXTRACELLULAR VESICLES AND THEIR THERAPEUTIC POTENTIAL

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The story of extracellular vesicles (EVs), these cell-secreted phospholipid bilayer-bound structures started about 70 years ago. The release of membrane vesicles is a process conserved in both prokaryotes and eukaryotes and compelling piles of evidence support the significance of exosomes, microparticles/microvesicles (MV/MP) and apoptotic bodies in a broad range of physiological-pathological conditions.

EVs derived from cancer cells have been shown to contribute to horizontal cellular transformation, cellular reprogramming, functional alterations, even metastasis. Besides that identification of the tumour-derived material carried by EVs isolated from different body fluids can help the early detection of different cancers, the more accurate diagnosis and prognosis today, and these small vesicles might become efficient therapeutic tool in the future.

The main goal of the talk is to convince you about the existence, importance and therapeutic potential of extracellular vesicles, as well as to give an insight into the difficulties of their isolation and identification.

L9: PRECISION MEDICINE IN MYELOID LEUKEMIA: PREDICTIVE PHARMACOGENOMICS BIOMARKERS OF DRUG SENSITIVITY AND RESISTANCE

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Myeloid leukemia represents a highly variable and heterogeneous disease on the basis of clinical course and molecular indicators. Precision medicine in myeloid leukemia is required in frontline induction and post-remission consolidation therapy, as well as in salvage post-relapse therapy. Personalized approach enables the identification and use of pharmacogenomics biomarkers selected from comprehensive landscape of genetic alterations. Due to technical advancements, molecular diagnostics and response monitoring are applied in effective and molecularly targeted therapy. Detailed insight into the genetic and epigenetic biomarkers of the disease is implemented in leukemia classification and pretreatment risk-categorization. In addition to clinical and molecular response parameters, biomarker-guided targeted molecular therapy response represents the most sensitive evaluation approach and prognostic system. It is applied to distinguish the complete, suboptimal and lack of response, indicating the need for change of treatment or bone-marrow transplantation. Biomarkers of targeted response are successfully applied in elucidation and detection of molecular mechanisms of primary/secondary resistance development in order to initiate adequate treatment for drug-resistant cases in a timely manner. With medical progress, application of pharmacogenomics biomarkers in myeloid malignancies is crucial to effectively predict drug sensitivity/resistance and to effectively guide treatment decisions. The goal of this talk is to summarize the latest advancements in precision medicine in myeloid leukemia with particular emphasis on evaluation and application of predictive pharmacogenomics biomarkers of drug sensitivity and resistance.

L10: CELL COMPOSITION IN p53 ISOFORMS DEFINES CELL FATE DECISION

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All genetic models, experimental and clinical data indicate that *TP53* gene play key roles in cancer formation, progression and treatment. For 30 years, it was thought that *TP53* is a dedicated tumor-suppressor encoding a single transcription factor, p53, which protects cells from genotoxic stress. However, it is poorly understood how a single protein, p53, can be responsive to so many stress signals and orchestrates very diverse cell responses to maintain/restore cell/tissue functions.

The uncovering that *TP53* gene physiologically expresses, in a tissue-dependent manner, not one p53 protein but twelves different p53 proteins with distinct biochemical activities (p53 isoforms) may prove fundamental to decipher the p53 pathway and improve cancer treatment. Over the last decade, all genetic animal models of p53 isoforms (zebrafish, drosophila and mouse), have consistently indicated that altering expression of a few p53 isoform, without affecting canonical p53 protein expression (i.e. p53alpha), promote different pathologies: premature ageing, (neuro)degenerative diseases, inflammation, cancer, embryo malformation and altered responses to ionising irradiation or infectious diseases. The p53 isoforms are dynamically reforming in depth the p53 field.

I will summarise a decade of research on p53 isoforms and present our latest experimental and clinical studies in breast cancer that could be extended to predict response to treatment in other type of cancer.

ST1: THE EXPRESSION OF p53/p73 ISOFORMS, NME AND GLI IN METASTATIC MELANOMA AND THEIR INDUCTION BY γ-IRRADIATION

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Although mutations of p53 occur infrequently in melanoma, it fails to function as a tumor suppressor. This may result from alterations in p53 family members, including the diverse isoforms of p53 and its homologue p73. Moreover, we assume that the p53 function in malignant melanoma might be altered through interactions with p53 small molecular weight and p73 isoforms, NME and GLI families of proteins. Therefore, we are studying the expression profile of p53 and its potential interaction partners (p73/NME/GLI) in metastatic melanoma and in adjacent healthy skin tissue. In the study on 30 patients with metastatic melanoma, the expression of p53, p73, NME and GLI protein families was determined by western blot analysis and quantitative RT-PCR.

Protein expression analysis has shown that only Δ133p53α expression is significantly different in the two tissue types: it is poorly expressed in healthy tissue (only 12% of samples), but strongly expressed in tumor tissue (72% of samples). There is no difference of expression of other p53 isoforms between healthy and tumor tissue samples. Protein p73 is expressed in almost all tumor samples and healthy tissue samples. TAp73α and ΔNp73α, are more frequent in tumor samples and TAp73 β and ΔNp73 β, are less frequent in tumor samples than in healthy tissue samples. Proteins NME1 and NME3 are expressed in 96% of tumor samples and 70% of healthy tissue samples. Surprisingly, protein expression of NME1 and NME2 was several fold higher in tumor samples than in healthy tissue samples. GLI1 and both GLI3A and GLI3R have higher expression in tumor than in healthy tissue samples. GLI2 is expressed in only 8% of tumors and not in healthy tissue samples.

Comparison of gene expression in the melanoma tissue samples and corresponding normal tissue revealed that *NME2* is significantly stronger expressed in normal tissue while *NME1* is expressed equally in healthy and tumor tissue. All other examined genes are significantly less expressed in the tumor tissue.

To obtain higher expression of proteins of interest, we irradiated two melanoma cell lines with different mutational status of p53 (Mel505/p53mut and A375M/p53wt) with γ rays with the dose of 5 and 10 Gy. The results have shown that γ irradiation increases the level of proteins of interest to certain extent and their protein expression profiles obtained after γ irradiation provide the molecular environment where the proteins possibly interact and inhibit activity of p53 in melanoma.

ST2: INTERACTION OF THE HUMAN PAPILLOMAVIRUS E6 ONCOPROTEIN WITH SORTING NEXIN 27 MODULATES ENDOCYTIC CARGO TRANSPORT PATHWAYS

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A subset of high-risk Human Papillomaviruses (HPVs) are the causative agents of a large number of human cancers, of which cervical is the most common. Two viral oncoproteins, E6 and E7, contribute directly towards the development and maintenance of malignancy. A characteristic feature of the E6 oncoproteins from cancer-causing HPV types is the presence of a PDZ binding motif (PBM) at its C-terminus, which confers interaction with cellular proteins harbouring PDZ domains. Here we show that this motif allows E6 interaction with Sorting Nexin 27 (SNX27), an essential component of endosomal recycling pathways. This interaction is highly conserved across E6 proteins from multiple high-risk HPV types and is mediated by a classical PBM-PDZ interaction but unlike many E6 targets, SNX27 is not targeted for degradation by E6. Rather, in HPV-18 positive cell lines the association of SNX27 with components of the retromer complex and the endocytic transport machinery is altered in an E6 PBM-dependent manner. Analysis of a SNX27 cargo, the glucose transporter GLUT1, reveals an E6-dependent maintenance of GLUT1 expression and alteration in its association with components of the endocytic transport machinery. Furthermore, knockdown of E6 in HPV-18 positive cervical cancer cells phenocopies the loss of SNX27, both in terms of GLUT1 expression levels and its vesicular localization, with a concomitant marked reduction in glucose uptake, whilst loss of SNX27 results in slower cell proliferation in low nutrient conditions. These results demonstrate that E6 interaction with SNX27 can alter the recycling of cargo molecules, one consequence of which is modulation of nutrient availability in HPV transformed tumour cells.

ST3: IDENTIFICATION OF NOVEL GENES RESPONSIBLE FOR CHEMORESISTANCE IN BREAST CANCER

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Tumor microenvironment (TME) plays a critical role in cancer progression and response to therapies. Cancer therapeutic intervention strategies can induce inflammation, resulting in changes in the TME. To investigate the response of cancer cells to chemotherapy in the context of an inflammatory microenvironment, we studied the effects driven by the chemotherapeutic drug doxorubicin (D), and the inflammatory cytokine TNFα (T).

We previously demonstrated using expression microarrays (validated also through qPCR) that the D+T combined treatment determined a strong up-regulation of migration-related genes as well as increased motility in breast cancer cells (MCF7).

We confirmed the synergistic activation of a group of 6 different genes upon D+T combined treatment in different cancer cell line models (A549-lung, U2OS-bone). Moreover, we demonstrated that the effect was only partially p53-depenedent but strongly doxorubicin-dependent using p53 mutated MDA-MB-231 breast cancer cells or knocking-out wild-type p53 in MCF7 or U2OS cells through the cutting edge technology CRISPR/Cas9.

We also demonstrated that the combined D+T treatment was not only able to disrupt the 3D architecture of mammary acini observed with MCF10A primary cells, but also to stimulate the tube-forming potential of Human Umbilical Vein Endothelial Cells (HUVEC).

Furthermore, a signature of D+T highly synergistic genes (DT-29) was shown to exhibit prognostic value for luminal breast cancer patients, with adverse outcome correlating with higher relative expression (based on Kaplan-Meier plotter tool).

Since STAT3 pathway is often activated in response to cancer-related inflammation, we demonstrated a STAT3-dependent regulation for some of the selected genes and STAT3 inhibition resulted also in a reduced migration potential of cancer cells.

Among the group of up-regulated genes upon D+T, we identified *ETV7* and *LAMP3* as putative mediator of the increased migratory potential of cancer cells. ETV7 is a member of the large family of ETS transcription factors that usually acts as transcriptional repressor; LAMP3 is a lysosome-associated membrane protein reported to be important for autophagy-dependent cell migration. Interestingly, we have recently proved that over-expression of ETV7 and LAMP3 was able to stimulate the migration potential of breast cancer cells.

In summary, we have identified pathways and genes whose activation can be related to breast cancer chemoresistance and aggressiveness.

ST4: A CASE-CONTROL STUDY OF CYTOGENETIC AND OXIDATIVE STRESS PARAMETERS IN PATIENTS WITH THYROID DISEASES

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Although thyroid cancer comprises only about 2% of new cancer cases, it is one of the fastest-growing cancer types, according to GLOBOCAN2012. Incidence of thyroid cancer in Croatian population is among the highest in Europe. At the same time it is more frequent in female population (4-fold compared to male population) and is most usually detected at ages 50-59.

We performed a case-control study of 100 untreated patients with thyroid diseases (papillary thyroid cancer, follicular thyroid adenoma, etc.) and 100 controls. The case population reflected the characteristics of a global population with thyroid cancer in mean group age 52.23±13.21 years and 4:1 female:male ratio. The control population was matched in age, gender, and smoking status to minimize the influence of confounding factors.

Among the patients, the average plasma-protein carbonyl concentrations were higher 1.26-fold, but the catalase activity was lower 35% compared to control population. With regard to cytogenetic parameters, higher total number of chromosome aberrations (1.47-fold), comet assay tail moment (2.25-fold) and total numbers of micronuclei (2.32-fold), nuclear buds (2.34-fold), and nucleoplasmic bridges (3.98-fold) were detected in the case group. About 98% of patients were positive for either B-Raf or Ret protein expression in thyroid tissue. Papillary thyroid cancer patients expressed B-Raf protein more frequently (97.06%) than did the patients with follicular thyroid adenoma (78.38%) or other thyroid diseases (79.17%).

We have observed altered oxidative and cytogenetic status of patients with thyroid diseases, supported by changes in protein expression and localisation. We hope that such data could be used for identification of subgroups at increased risk.

ST5: NOTCH AND AIOLOS IN SURVIVAL OF NEOPLASTIC B-CELLS

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B-cell chronic lymphocytic leukaemia (B-CLL) is the most common blood cancer in adults. The disease is characterized by progressive accumulation of mature monoclonal but functionally incompetent lymphocytes in blood, bone marrow, lymph nodes and spleen/liver that co-express the CD5 antigen and B-cell antigens CD19, CD20 and CD23. The presence of the aberrant B-cell clone (CD5+CD19+) greatly facilitates the disease diagnostics as well as the characterization of individual cell groups within the sample. This clone is essentially an uncontrollably multiplied precursor of mature B-cells which makes CLL a model for studying the development and differentiation of B-cells. The effects of the Notch signalling pathway in the immune system have been shown to affect hematopoietic stem cells, as well as committed progenitors. By guiding the specification, maturation and survival of progenitors it effectively moulds the immune compartments and shapes the faith of hematopoietic cells. The role in promoting survival and apoptosis resistance of Notch signalling has been thoroughly explored in T-cell acute lymphoblastic leukaemia but the conclusions of the work done with this specific pathway in B-CLL have been contradictory. B-CLL cells are characterized by in vivo resistance to apoptosis which remains a major clinical challenge for successful treatment of the disease. It has been shown that Notch 1 is being mutated in 12% cases of B-CLL and it has been also implied that Notch 1 mutations are predictors of poor prognosis. However, the relationship between the genetic aberrations and drug resistance or disease progression remains unclear. In addition to Notch, we have turned our attention towards other modulators of lymphocyte differentiation, such as Aiolos and other members of the Ikaros family zinc -finger proteins. These transcription factors modify the expression of genes by binding and remodelling chromatin thus regulating the activity in the nucleus and guiding the lineage specification of B cells. We and others have shown that Ikaros family member, Aiolos is highly expressed in B-CLL cells and there is evidence supporting their role in survival of mature neoplastic B-cells. The expression of the downstream genes was assessed by gene expression assays and multiplex PCR. In this work we have made some promising steps towards elucidating the intricate mechanism of B cell lineage specification and the genes involved in guiding it.

ST6: NOVEL THERAPIES FOR BREAST CANCER

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Breast cancer is the most commonly diagnosed cancer in female population worldwide and it is estimated that approximately one out of every eight Croatian women will develop invasive breast cancer over the course of her lifetime. Advances in understanding tumor biology, particularly signaling pathways, have led to the development and approval of many novel agents and have changed the landscape of therapy for patients with metastatic breast cancer. However, metastatic breast cancer still remains an incurable disease for most patients. The most important future strategies will be those for overcoming endocrine and human epidermal growth factor 2-neu (HER2) resistance, targeting triple-negative breast cancers through novel receptors, harnessing the immune system, and new ways of targeting angiogenesis.

Estrogen receptor (ER) is expressed in approximately 70% of newly diagnosed breast cancers and has an important role in tumor growth and progression. Endocrine therapy forms the backbone of treatment for hormone-dependent breast cancer. Despite the benefits of endocrine therapy, resistance eventually occurs in a large number of patients and represents important clinical challenge in the management of breast cancer. Understanding the mechanisms of resistance to endocrine therapies which have been identified helps us develop novel potential drugs to overcome resistance. A new strategy in treating patients with ER-positive breast cancer is to target cyclin-dependent kinases 4 and 6 (CDK4/6), a key pathway involved in regulation of the G1/S transition of the cell cycle.

Cancers over-expressing a protein receptor HER2 (HER2 IHC 3+, or FISH-positive) may be treated with targeted therapies such as trastuzumab, pertuzumab, lapatinib, and ado-trastuzumab emtansine. Despite the important treatment advances with these potent anti-HER2 therapies, these drugs are generally not curative in the metastatic setting, likely due to several mechanisms of anti-HER2 therapy resistance.

Triple-negative breast cancer tends to have an aggressive phenotype with higher recurrence rates and lower survival rates. To date, there are no approved targeted therapies specifically for this subtype; however, many are in development. Ongoing research is investigating targetable novel cell surface receptors, the use checkpoint inhibitors, and identifying subgroups likely to benefit from platinum-based therapies and poly(adenosine diphosphate-ribose) polymerase inhibitors. The androgen receptor (AR) has been identified as a possible predictive biomarker for antiandrogen therapy in ER- breast cancer.

While numerous studies investigating anti–vascular endothelial growth factor (VEGF) therapy in the neoadjuvant setting suggest improved pathologic complete response rates, especially in TNBC, studies to date have not demonstrated a survival benefit in the adjuvant setting or metastatic setting.

A promising field of clinical research in breast cancer is the use of immune checkpoint inhibitors. By blocking inhibitory molecules or, alternatively, activating stimulatory molecules, these treatments are designed to enhance pre-existing anti-cancer immune responses. Several studies investigating checkpoint inhibitors are currently enrolling breast cancer patients. Approximately 20% of TNBCs express PD-L1, and expression of PD-L1 is associated with poor prognosis, thus making this aggressive phenotype attractive subtype in which to investigate PD-L1 blockade.

ST7: DESIGN OF NOVEL HYBRID COMPOUNDS OF VITAMIN C AND 1,2,3-TRIAZOLE WITH ANTICANCER PROPERTIES: IMPACT OF PHYSICOCHEMICAL PROFILE ON BIOLOGICAL ACTIVITY

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Vitamin C has been known as a reducing agent with a number of biological functions (Du *et al.*, Biochim Biophys Acta 2012). On another side, compounds with a 1,2,3-triazole fragment have shown antiproliferative activities against a variety of cancer cell lines (Kraljević *et al.*, Eur J Med Chem 2016). Design of a library of novel hybrid compounds of vitamin C and 1,2,3-triazole with various physicochemical characteristics (such as lipophilicity and solubility) and antioxidant capacity, through interdisciplinary approach will be outlined. The molecular properties have been modulated through introduction of various substituents at the C-4 atom of 1,2,3-triazole and their impact on biological activities has been estimated by using in silico modelling (Cruciani *et al.*, J Mol Struct Theochem 2000), which will be presented.

ST8: CANCER STEM CELLS INFLUENCE RENAL CELL CANCER TYROSINE KINASE RESISTANCE

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This study was designed to verify the effect of multi-targeted tyrosine kinase inhibitors on renal cell cancer cells along with subpopulation of cancer stem cells. Cells analyzed covered: Human Kidney Cancer Stem Cells (HKCSC) isolated from nephrectomy specimen of papillary and clear cell renal cell carcinoma (Celprogen Inc), cell line Caki-1 – derived form human metastasis of clear-cell renal cell carcinoma to the skin with wt *VHL* (von Hippel Lindau) gene (ATCC® HTB-46™), 769-P - primary clear- cell renal cell carcinoma with *VHL* mutation (ATCC® CRL-1933™), 786-0 (TCC® CRL-1932™) - primary clear cell adenocarcinoma with *VHL* mutation, ACHN – papillary renal cancer pleural metastasis with wt *VHL*, and SK-RT-42 – clear cell renal cancer bone metastasis. Cells were primarily cultured in monolayer standard 2D conditions and in order to mimic in vivo conditions cells were cultured also in 3D conditions and in ECM coated plates with laminin, collagen I or poly-D-lysine. Cells were also cultured in hypoxia (1-2% oxygen tension) to recapitulate intratumoral conditions. Cells were treated with sunitinib, sorafenib or axitinib in 2D and 3D conditions. Cell proliferation and TKI toxicity were evaluated with Alamar Blue and MTT assay. Renal cell cancer - cancer stem cells proliferation is inhibited by tyrosine kinase inhibitors. Hypoxia reduced proliferation of renal cancer cells - increase of percentage of quiescent cells (Ki67-) and cells arrested in S phase is found. Efficacy of TKIs is limited by hypoxic conditions and cancer cell cell-cell interactions. Hypoxia induces downregulation of expression of eukaryotic translation initiation factor B (encoded by *EIF4B* gene) and dual specificity mitogen-activated protein kinase 1 (encoded by *MAP2K1* gene). Specific response to tyrosine kinase inhibitors (TKIs), including primary resistance was also observed. HKCSCs cells cultured in hypoxia develop primary resistance to sorafenib, while HKCSCs cultured in normoxia were resistant to axitinib. Understanding the complex molecular feedback loops between cancer cells, cancer stem cells and the tumor microenvironment in 3D culture may promote the identification of novel treatment targets in renal cancer.

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