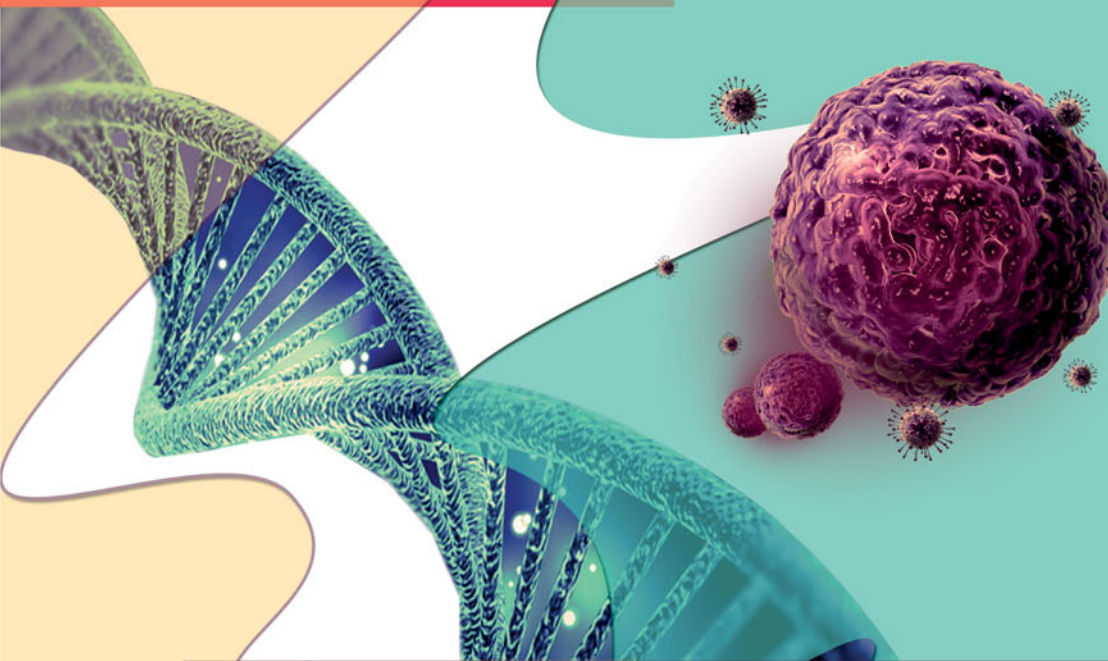


SERBIAN ASSOCIATION FOR CANCER RESEARCH
4TH CONGRESS OF SDIR:
BRINGING SCIENCE TO ONCOLOGY
PRACTICE: WHERE IS SERBIA?

ABSTRACT BOOK



BELGRADE
3–5 OCTOBER

2019

4TH CONGRESS OF THE SERBIAN ASSOCIATION FOR CANCER RESEARCH
WITH INTERNATIONAL PARTICIPATION

ABSTRACT BOOK

“BRINGING SCIENCE TO ONCOLOGY PRACTICE:
WHERE IS SERBIA?”

SDIR-4

Belgrade, 3 - 5 October 2019

THE FOURTH CONGRESS OF THE SERBIAN ASSOCIATION FOR CANCER RESEARCH

with international participation

“BRINGING SCIENCE TO ONCOLOGY PRACTICE: WHERE IS SERBIA?”

SDIR-4

3-5 October 2019, Hotel “M”, Bulevar oslobođenja 56a, Belgrade, Serbia

Serbian Association for Cancer Research (SDIR) is a member of the European Association for Cancer Research (EACR).

President of SDIR-4 Congress

dr sc. med. Mirjana Branković-Magić

THE FOURTH CONGRESS OF THE SERBIAN ASSOCIATION FOR CANCER RESEARCH
with international participation “Bringing Science to Oncology Practice: Where is Serbia?”

Belgrade, 3-5 October 2019

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LETTER OF WELCOME

Dear colleagues,

We are very pleased to welcome you to the 4th Congress of the Serbian Association for Cancer Research (SDIR) with international participation "Bringing Science to Oncology Practice: Where is Serbia?" to be held on 3-5 October 2019 at the Hotel "M", Bulevar oslobođenja 56a, Belgrade, Serbia.

During this three-day congress, lectures will be delivered by a distinguished Serbian and international researchers, that will cover the following topics:

- *Applying translational cancer research in molecular diagnostics: Serbian experience*
- *New technologies and approaches of molecular diagnostics in hereditary cancer*
- *Targeted & Immunotherapy in cancer: Biomarker-based approaches*
- *Current challenges and future perspectives of radiotherapy*
- *The role of oxidative stress in drug resistance*
- *Innovative tools for advancing cancer research*

We are pleased to say that our fourth congress is actively supported by the European Association for Cancer Research.

We are delighted to welcome you in Belgrade!

Kind regards,



*dr sc. med. Mirjana Branković-Magić,
president of SDIR*



ACCREDITATION INFORMATION

The SDIR4 congress is accredited by the Serbian Health Council for Continuing Medical Education (No A-1-1800/19).

Invited speakers will be awarded with 15 CME credits, oral presentations with 13 CME credits, poster presentations with 11 CME credits and passive attendances with 10 CME credits.

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The Serbian Association for Cancer Research is also very grateful to the following sponsors for providing financial support to the SDIR-4 congress:

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- 14.00 – 15.00 **Pre-meeting educational symposium**
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Chairs: Siniša Radulović, Zvonko Magić
- 14.00 – 14.20 Mirjana Branković-Magić. Hereditary breast/ovarian cancer: challenges of next generation sequencing.
Institute of Oncology and Radiology of Serbia, Belgrade, Serbia
- 14.20 – 14.40 Milena Čavić, Radmila Janković. Pharmacogenetics in clinical practice: how far have we come?
Institute of Oncology and Radiology of Serbia, Belgrade, Serbia
- 14.40 – 15.00 Bojana Cikota-Aleksić. Detection of minimal residual disease in leukemia and lymphoma: are we changing definition and concept?
Military Medical Academy, Belgrade, Serbia
- 15.00 – 15.30 Coffee break
- 15.30 – 16.10 **Pre-meeting educational symposium**
Applying translational cancer research in molecular diagnostics: Serbian experience 2
- 15.30 – 15.50 Snežana Šušnjar. How to estimate the indications for administration of adjuvant chemotherapy in patients with luminal HER2 negative breast cancer? The role of uPA and PAI-1 biomarkers.
Institute of Oncology and Radiology of Serbia, Belgrade, Serbia
- 15.50 – 16.10 Marija Đorđić Crnogorac. uPA and PAI-1: prognostic factors for early breast cancer.
Institute of Oncology and Radiology of Serbia, Belgrade, Serbia

Friday, October 4th 2019

- 08.00 **REGISTRATION**
- 08.30 – 09.00 CONGRESS WELCOME
- 09.00 – 10.30 **Session: New technologies and approaches of molecular diagnostics in hereditary cancer**
Chair: Mirjana Branković-Magić
- 09.00 – 09.30 Vita Šetrajčič Dragoš, Srđan Novaković. The relevance of molecular diagnostic in hereditary cancer.
Institute of Oncology, Ljubljana, Slovenia
- 09.30 – 10.00 Ana Krivokuća. The importance of panel testing in high grade serous ovarian cancer.
Institute of Oncology and Radiology of Serbia, Belgrade, Serbia
- 10.00 – 10.30 Short talks
- 10.00 – 10.10 Iva Kirac. Genetic counselling unit-model and experience of University Hospital for Tumours in Croatia.
University Hospital for Tumours, Sestre milosrdnice University Hospital Centre, Zagreb, Croatia
- 10.10 – 10.20 Milica Mihajlović. rs2910164 variant in *mir-146a* gene in high risk hereditary breast and ovarian cancer cases.
Institute of Oncology and Radiology of Serbia, Belgrade, Serbia
- 10.20 – 10.30 Jovana Bročić. *CDK4* codon 24 mutation status in familial melanoma patients - single center study.
Institute for Medical Research, Military Medical Academy, Belgrade, Serbia
- 10.30 – 11.00 Lecture sponsored by Labena - Uršula Prosenc Zmrzljak. Detection of somatic mutations with ddPCR from liquid biopsy of CRC patients.
- 11.00 – 11.30 Coffee break
- 11.30 – 13.00 **Session: Targeted & Immunotherapy in cancer: Biomarker-based approaches**
Chairs: Siniša Radulović, Radmila Janković

PROGRAMME

- 11.30 – 12.00 Davorin Radosavljević. Focus on lung adenocarcinoma.
Institute of Oncology and Radiology of Serbia, Belgrade, Serbia
- 12.00 – 12.30 Lidija Kandolf-Sekulović. Focus on melanoma.
Military Medical Academy, Belgrade, Serbia
- 12.30 – 13.00 Short talks
- 12.30 – 12.40 Tijana Martinov. SOX2-targeted T cell immunotherapy in multiple myeloma.
Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA,
United States of America
- 12.40 – 12.50 Vesna Ćorić. Disturbed redox balance contributes clear renal cell carcinoma
development and progression.
Institute of Medical and Clinical Biochemistry, Faculty of Medicine, University of
Belgrade, Belgrade, Serbia
- 12.50 – 13.00 Vera Jokić. The predictive value of haematological parameters on the progression-
free survival in advanced lung adenocarcinoma patients treated with tyrosine
kinase inhibitors in Serbia.
Institute of Oncology and Radiology of Serbia, Belgrade, Serbia
- 13.00 – 13.30 Lecture sponsored by Roche - Precision medicine in oncology. The importance of
genomic profiling.
- 13.30 – 14.30 Lunch
- 14.30 – 15.30 Poster session and coffee break
- 15.30 – 16.15 Plenary lecture (EACR Sponsored)
Arkaitz Carracedo. Disentangling the molecular drivers of prostate cancer
progression.
Center for Cooperative Research in Biosciences (CIC bioGUNE), Derio, Spain
- 16.15 – 16.30 Lecture sponsored by Superlab. Vladan Kocić. Analytical instruments in cancer
research.
- 16.30 – 18.15 **Session: Current challenges and future perspectives of radiotherapy**
Chairs: Tatjana Stanojković, Marina Nikitović

- 16.30 – 16.45 Milena Krajnovic. DNA methylation as a potential predictor of tumor response to radiotherapy.
Vinca Institute of Nuclear Sciences, Belgrade, Serbia
- 16.45 – 17.00 Emina Mališić. The role of single nucleotide polymorphisms in outcome and toxicity of radiotherapy for prostate cancer.
Institute of Oncology and Radiology of Serbia, Belgrade, Serbia
- 17.00 – 17.15 Barbara Jožef. Comet assay as a tool for evaluation of DNA damage in cancer patient treated with radiotherapy.
Institute for Chemistry, Technology and Metallurgy, University of Belgrade, Belgrade, Serbia
- 17.15 – 17.30 Vesna Stanković, Marina Nikitović. Late genitourinary toxicity after conventionally fractionated conformal radiotherapy for localized prostate cancer.
Institute of Oncology and Radiology of Serbia, Belgrade, Serbia
- 17.30 – 17.45 Jelena Bokun, Marina Nikitović. Pediatric radiotherapy at IORS.
Institute of Oncology and Radiology of Serbia, Belgrade, Serbia
- 17.45 – 18.00 Tatjana Arsenijević, Marina Nikitović. Micro RNA in lung cancer as potential biomarkers.
Institute of Oncology and Radiology of Serbia, Belgrade, Serbia
- 18.00– 18.15 Discussion
- 20.30 Welcome Dinner

Saturday, October 5th 2019

- 09.00 – 11.00 **Session: The role of oxidative stress in drug resistance**
Chair: Milica Pešić
- 09.00 – 09.30 Joanna Kopecka. Targeting mitochondria to overcome multidrug resistance in cancer.
Turin School of Medicine, University of Turin, Turin, Italy
- 09.30 – 10.00 Ana Čipak Gašparović. Lipid peroxidation - double edge sword in fighting cancer (stem) cells.
Institute Ruđer Bošković, Zagreb, Croatia

PROGRAMME

- 10.00 – 10.30 Short talks
- 10.00 – 10.10 Marina Filimonova. Prospects for the use of NOS inhibitors in radiation therapy.
Tsyb Medical Radiological Research Center, National Medical Research Center of Radiology of the Ministry of Health of the Russian Federation, Obninsk, Russia
- 10.10 – 10.20 Marina Stamenković. Repurposing of pantoprazole as anticancer drug through induction of apoptosis, modulation of autophagy and enhancement of the effect of vincristine in cancer cells.
Institute for Microbiology and Immunology, Faculty of Medicine, University of Belgrade, Belgrade, Serbia
- 10.20 – 10.30 Tamara Babić. SMAD4 transcript analysis in human permanent cell lines.
Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Serbia
- 10.30 – 11.00 Discussion
- 11.00 – 11.30 Coffee break
- 11.30 – 13.30 **Session: Innovative tools for advancing cancer research**
Chairs: Engin Ulukaya, Jelena Grahovac
- 11.30 – 12.00 Engin Ulukaya. Spheres: simple and quite reliable models in basic cancer research.
Istinye University, Istanbul, Turkey
- 12.00 – 12.30 Tijana Stanković. Glioblastoma behaviour in 3D biomimetic systems.
Institute for Biological Research “Siniša Stanković”, University of Belgrade, Belgrade, Serbia
- 12.30 – 13.00 Short talks
- 12.30 – 12.40 Marija Nešović, Jelena Dinić. c-Src inhibitors pyrozolo[3,4-d]pyrimidines, Si306 and pro-Si306, evade multidrug resistant phenotype and suppress invasion in glioblastoma.
Department of Neurobiology, Institute for Biological Research “Siniša Stanković”, University of Belgrade, Belgrade, Serbia

- 12.40 – 12.50 Sercan Ergün. Prognostic significance of miR-494 in renal cell carcinoma.
Faculty of Medicine, Ordu University, Altınordu, Ordu, Turkey
- 12.50 – 13.00 Marija Vidosavljević. Nischarin expression in melanoma.
Institute of Oncology and Radiology of Serbia, Belgrade, Serbia
- 13.00 – 13.30 Discussion
- 13.30 – 14.30 Lunch
- 14.30 – 15.00 Closing remarks and best poster award

PRE-MEETING EDUCATIONAL SYMPOSIUM

Applying translational cancer research in molecular diagnostics: Serbian experience

Hereditary breast/ovarian cancer: Challenges of next generation sequencing

Mirjana Branković-Magić

Serbian Association for Cancer Research, Belgrade, Serbia

Introduction of multigene panel testing in the area of hereditary cancer dramatically changed our knowledge about the nature of hereditary predisposition to breast/ovarian cancer. Before new technologies were introduced only mutations in BRCA1 and BRCA2 genes were routinely detected, allowing predictive genetic testing for hereditary breast/ovarian cancer. Simultaneously, the strategies for appropriate clinical surveillance and risk reduction for healthy BRCA1/2 mutation carriers have been developed. In the meantime, we became aware that BRCA1/2 testing do not cover hereditary predisposition for breast/ovarian cancer as a whole – about 30% of hereditary breast cancer predisposition is contributed to BRCA1/2 mutations. Multigene testing can be performed in a large number of individuals and large range of genes can be analyzed simultaneously.

The major advantage of NGS technologies is that we are in fact able to detect genetic variations across the entire genome, large gene panels and exomes. In addition, we need less DNA to detect all variations than required for traditional DNA sequencing approaches, less time and less money to perform comprehensive tests. Even though advantages are undoubtful there are still challenges and limitations that we face using NGS. For many of genetic variations identified through NGS clinical significance is currently unknown. Besides these variants of unknown significance (VUS) we face with pathogenic variations in genes that are not yet associated with the disease (incidental/secondary findings). We find more and more mutations in genes outside recommended panels for the examined hereditary syndromes that often cannot be connected with personal and family history of mutation carrier. There is also insufficient data about significance of mutations in such genes for HBOC. In majority of these genes, spectrum of cancers associated with them is undefined. Even in the cases when pathogenic mutations are detected in the genes that are, or may be associated with HBOC, precise risk for disease development as well as strategies for adequate surveillance and need for risk reduction have not been yet defined.

Besides difficulties in variant annotation and interpretation, NGS also requires sophisticated bioinformatics systems, fast data processing and large data storage capabilities, which can be costly. Specialized, trained bioinformaticists are essential to the analysis of data generated by

NGS, as well as the continued success and growth of precision medicine.

Although gene-panel testing is widely spread in clinical genetics laboratories, full clinical application of the results of genetic testing for HBOC are still limited due to lack of adequate guidelines and recommendations for some of the genes included. It also must be pointed out that risk estimation for HBOC that is including criteria for individual's testing is so far, mainly suitable for BRCA1/2 mutation carriers, but it is not completely suitable for carriers of other HBOC genes. That fact must be taken in consideration in pre-test counselling. The risk for particular gene and particular pathogenic mutation in HBOC genes has to be defined together with factors that can modify cancer risk.

Pharmacogenetics in clinical practice: how far have we come?

Milena Čavić, Radmila Janković

Institute of Oncology and Radiology of Serbia, Belgrade, Serbia

Novel therapies, including the use of directed monoclonal antibodies, tyrosine kinase inhibitors (TKIs), and immunotherapies, have come to full realization as a result of our increasing understanding of the biology of cancer cells. From the identification of the first oncogenes and tumor-suppressor genes to current full gene sequencing for the identification of somatic mutations, knowledge of tumor-cell genetic variant profiles has become critical to the management of the cancer patients.

One projection of the Human Genome Project was to gain a more fundamental understanding of human disease at the genomic level such that novel therapeutics could be designed to provide a more personalized approach to disease management. The rapid development of targeted therapies is also resulting in more advanced clinical trials, aiming to match patients whose tumors harbor specific somatic mutations with those specific therapies targeting the gene or pathway affected. Pharmacogenetics is particularly focused on selection of a therapeutic drug based on the presence or absence of a specific drug target or pathway in the tumor that renders the tumor cells sensitive or resistant to therapy.

Recognizing the importance of this strategy, Institute for Oncology and Radiology of Serbia (IORS), established the centralized pharmacogenetics service in 2008, providing personalized approach to cancer treatment of cancer patients. The same year, RAS mutation testing is introduced in routine clinical practice in Serbia. In the era of personalized medicine, treatment options for patients with metastatic colorectal cancer (mCRC) include blocking epidermal growth factor receptor (EGFR) which is involved in signaling pathways controlling cell proliferation, angiogenesis, apoptosis, and metastasis. Two EGFR-targeted monoclonal antibodies, cetuximab and panitumumab, are approved for treatment of RAS wild type mCRC. From April 2008 to May 2019, 3250 mCRC patients were tested for presence of KRAS and NRAS mutations.

Comprehensive genomic profiling of lung cancers revealed their genetic heterogeneity and

complexity and identified numerous targetable oncogenic driver alterations. These molecular profiling efforts have made it possible to exploit the potential of molecularly targeted therapies. EGFR gene mutations confer sensitivity to targeted therapy with TKIs and their detection has become a companion diagnostic in Serbia in 2011. Until May 2019, 4900 patients have been tested for the presence of EGFR mutations. Patients with sensitizing EGFR mutations were treated with first generation TKIs until progression. EGFR mutation testing from liquid biopsy samples (plasma) of patients who progressed on first generation TKIs was introduced in 2016. So far, 120 patients were tested at progression from plasma samples in order to select a group sensitive to third-generation EGFR-TKIs.

Other services of our pharmacogenetics include BRAF mutation detection in patients with metastatic melanoma in order to select patients for treatment with TKIs and somatic BRCA1/2 mutation testing in ovarian carcinoma to identify patient's eligibility for treatment with PARP inhibitors.

The ultimate promise of precision medicine that treatments will eventually be tailored to the genetic changes in each person's cancer is slowly becoming everyday practice.

Detection of minimal residual disease in leukemia and lymphoma: are we changing definition and concept?

Bojana Cikota-Aleksić

Institute of Medical Research, Military Medical Academy, Belgrade, Serbia

Minimal residual disease (MRD) refers to low-level disease detected by sensitive laboratory techniques, such as flow cytometry and polymerase chain reaction (PCR). The term is used to describe residual disease after induction chemotherapy. The main goal of MRD testing is to distinguish between patients who respond well to therapy and thus should be spared further therapy from those in whom therapy should be continued or intensified to minimize the likelihood of relapse. In Western countries, detection and quantification of MRD has become routine clinical practice in frontline treatment of virtually all childhood and in many adult acute lymphoblastic leukemia (ALL) patients. The importance of MRD testing has been demonstrated not only in ALL, but also in other hematologic malignancies, such as chronic lymphocytic leukemia, lymphoma and multiple myeloma.

The majority of studies on adult ALL measured MRD after end of induction and/or during early consolidation. In addition, all studies confirmed the strong and independent prognostic impact of MRD. A higher specific hazard of relapse was independently associated with postinduction MRD level $\geq 10^{-4}$.

Intensive debate about the sensitivity of MRD techniques imposed the need that MRD technologies should aim for 10^{-4} to 10^{-5} to define the MRD-based risk groups accurately. Thus far, most European clinical trials use PCR-based MRD techniques, such as real-time

quantitative (RQ) PCR of rearranged IG or TCR genes. This approach reaches sensitivity of 10⁻⁴-10⁻⁶ (depending on amounts of DNA analyzed) and can be applied in >95% of all lymphoid malignancies. However, the testing is available in the limited number of laboratories, mainly centralised in the companies. The clinical significance of MRD assessment imposes the need for the improvement of quantitative MRD approaches.

The lecture will include overview of methods for quantitative MRD analysis, instructions for sampling and recommendations for therapeutic decisions in different hematological malignancies (acute and chronic leukemias, lymphoma and multiple myeloma).

How to estimate the indications for use of adjuvant chemotherapy in patients with luminal HER2 negative breast cancer: the role of uPA and PAI-1 biomarkers

Snežana Šušnjar

Institute of Oncology and Radiology of Serbia, Belgrade, Serbia

Breast cancer (BC) is the most frequent malignancy with the highest mortality rate in women of all solid malignant tumors (according to Globocan 2018 there are more than 2 000 000 newly diagnosed BC patients every year). Antineoplastic therapy depends on the disease stage at diagnosis and BC molecular subtype. Radical surgery with or without preoperative systemic therapy is the a therapeutic approach in stages 1 and 2 with curative intent. Adjuvant systemic therapy following surgery is administered with the aim to decrease the risk of disease relapse. This risk depends on tumor size and regional lymph nodes involvement at the diagnosis. The molecular BC subtypes defined according to status of estrogen receptors (ER), progesteron receptors (PgR), receptors for epidermal growth factor type 2 (HER2) and Ki 67 proliferative index, determine the type of adjuvant systemic therapy. Luminal HER2 negative BC is recognised as tumor more or less ER and/or PgR expression, while HER2 is not overexpressed. The therapy of choice for this subtype is endocrine therapy (ET). However it is known that some patients with luminal HER2 negative BC and 0-3 involved axillary lymph nodes had extra benefit from adding chemotherapy (CT) to ET. The clinical factors related to higher benefit from CT in these patients are younger age, larger tumor size, low ER and/or PgR expression and higher Ki67. In everyday practice all four biomarkers are determined by immunohistochemistry (IHC) meaning that their expressions are determined at protein level. Because many patients with luminal HER2 negative BC receiving adjuvant CT had no benefit in terms of reduction of disease relapse but had toxicity, in the past decade several commercially available gene profiling tests has been introduced in clinical practice, such as Oncotype DX, MammaPrint, EndoPredict, Prosigna (PAM 50) and BC index. These tests measure the expression of small number of genes involved in tumor proliferation better discriminating those patients with poor prognosis for whom adjuvant CT is justified. The first biomarkers

used to determine the prognosis in BC patients were urokinase plasminogen activator (uPA) - plasminogen activator inhibitor -1 (PAI-1). These biomarkers play essential roles in tumor invasion and metastasis, being involved in degradation of the tumor stroma and basement membrane. As well as being prognostic, high levels of uPA and PAI-1 were also shown to be associated with benefit from adjuvant chemotherapy

According to Updated guidelines from the European Group on Tumor Markers (EGTM) published in 2017, uPA and PAI-1 should be measured by a validated ELISA (e.g. FEMTELLE, American Diagnostica/ Sekisui) using extracts of fresh or freshly frozen breast tumour tissue, either from biopsy or surgical specimen.

uPA and PAI-1: prognostic factors for early breast cancer

Marija Đorđić Crnogorac

Institute of Oncology and Radiology of Serbia, Belgrade, Serbia

The plasminogen activator (PA) system is an extracellular proteolytic enzyme system associated with different pathological conditions. The expression levels of PA system components, urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitor 1 (PAI-1), are found to be altered in some malignancies. For that reason they are ideal diagnostic and prognostic markers for reducing cancer-associated morbidity. uPA and PAI-1 were identified as independent prognostic factors in breast cancer patients. Since elevated levels of these proteins in tumor tissue are associated with high risk of recurrence, adjuvant chemotherapy would have considerable benefit for these breast cancer patients. The ELISA is the only validated test for quantifying uPA and PAI-1 levels in breast tumor tissue, for now.

In our study we used FEMTELLE® uPA/PAI-1 ELISA which is supported by the American Society of Clinical Oncology (ASCO) Guidelines as a reliable predictor of a woman's likelihood of cancer recurrence. Determination of uPA and PAI-1 tumor tissue levels with ELISA confirmed that these proteins have an influence on disease outcome in early stage, node-negative, HR-positive/HER2-negative breast cancer patients treated with adjuvant endocrine therapy. Our research showed that patients with low intratumor level of uPA and PAI-1 concentrations have better prognosis in comparison to patients whose uPA and PAI-1 tumor tissue levels were higher than standard reference cut-off values. High levels of these markers were associated with tumor aggressiveness. Also, it was shown that high uPA tumor tissue concentration was associated with high concentration of PAI-1 in breast cancer, which points to positive correlation between uPA and PAI-1 levels. Patients with tumor tissue levels of uPA and PAI-1 higher than standard cut-off values 3 ng/mg and 14 ng/mg, respectively, had significantly decreased overall survival (OS), while disease free survival (DFS) was significantly decreased only in patients with higher tumor levels of uPA. Analyzing the best cut-off values for uPA and PAI-1 by Receiver Operating Characteristic (ROC) method, higher values than standard cut-offs were obtained. ROC cut-off values were 5.65 ng/mg of protein for uPA and 27.10 ng/mg for

PAI-1. Using these cut-off values, it was found that patients with tissue levels of uPA and PAI-1 lower than 5.65 ng/mg and 27.10 ng/mg, respectively, had significantly increased OS, DFS and event-free survival (EFS). ROC cut-off values seemed to have more reliable discriminative potential to separate patients into subgroups with better and poorer disease outcome. Our results confirm the benefits of use of FEMTELLE® uPA/PAI-1 ELISA in routine clinical practice and show that this test can help physicians guide their patients care.

SESSION: NEW TECHNOLOGIES AND APPROACHES OF MOLECULAR DIAGNOSTICS IN HEREDITARY CANCER

The relevance of molecular diagnostics in hereditary cancer

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Hereditary cancer cases represent 5 to 10% of all cancers. At present, there are over 50 identifiable forms of hereditary cancer. With regard to inherited germline mutations, hereditary cancers frequently have an unusual clinical appearance, such as early onset, presence of multiple neoplasms and preference toward particular histological pattern. Although the group of hereditary cancers accounts for a small percentage of all malignancies, the carriers of germinal genetic defects are at a significantly higher risk of developing cancer than general population. Owing to the fact that this risk is more or less organ-specific, it is possible to organize meaningful diagnostic and preventive interventions in carriers of specific germline mutations. Oncogenetic testing and counselling has been initiated at the Institute of Oncology Ljubljana as a part of a research project. Since 2008, it has been offered to patients as routine medical care. From 2014 onwards, most of our patients have been tested using the Next Generation Sequencing (NGS) methodology. At present, we are performing genotyping on Illumina Miseq Dx and Illumina NextSeq 550. The main goal of our service is to identify mutation carriers and to enable the appropriate preventive care for healthy individuals and, as recently, to enable the appropriate treatment of cancer patients. Additionally, accumulating large amounts of genomic and phenotypic data allows the implementation of many research projects linked with genetic changes in cancer.

From January 2016 until the end of May 2019, we screened 2650 individuals from Slovene families suspected of having one of hereditary cancer syndromes. By far the most frequently tested individuals – 2112 (79.7%), were individuals tested for hereditary breast and ovarian cancer syndrome. The numbers of individuals tested for other hereditary cancer syndromes were as follows: 271 (10.2%) for Lynch syndrome; 95 (3.6%) for familial adenomatous polyposis (FAP), autosomal recessive adenomatous polyposis and juvenile polyposis syndrome (JPC); 54 (2.0%) for hereditary malignant melanoma; 51 (1.9%) for hereditary diffuse gastric cancer;

LECTURES

20 (0.7%) for neurofibromatosis type I (NF1) and schwannomatosis; 48 (1.8%) for multiple endocrine neoplasia (MEN2A and MEN2B), Cowden syndrome, Peutz Jeghers syndrome, Werner syndrome, paragangliomas, basal cell nevus syndrome and prostate cancer.

The introduction of NGS in routine diagnostic detection of germline mutations allowed for the extension of repertoire of hereditary cancer syndromes, as well as for an increase in the number of diagnosed hereditary cancer cases. However, recent analyses indicate that despite testing an ever-increasing number of individuals for hereditary cancer with ever-broadening gene panels, we are currently identifying less than 10% of carriers of high risk variants. Such findings highlight the need to find novel approaches in the search for individuals at high risk of developing cancer who could benefit from effective preventive measures. Additionally, the NGS technology produces vast amount of sequencing data and increases the likelihood of finding novel genes and novel variants related to hereditary cancer or variants of uncertain clinical significance in already known cancer susceptibility genes. Therefore, significant clinical genetic expertise needs to be involved in interpreting high-throughput DNA tests.

The importance of panel testing in high grade serous ovarian cancer

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Ovarian cancer (OC) is the fifth most common cause of cancer related deaths even though it accounts for only 3 percent of cancers in women. The most frequent type of ovarian cancer is epithelial ovarian cancer (EOC) (85–95%) with four main histological types: serous, endometrioid, mucinous and clear cell. Almost 70% of all epithelial tumors are aggressive high-grade serous carcinomas (HGSOC) and present in advanced stages. One of the major risk factors for OC is family history and associated genetic syndromes that may indicate hereditary predisposition. About 23% of OCs are related to hereditary conditions, and in about 65–85% of those cases the genetic change is pathogenic germline mutation in BRCA1/2 genes. However, more than 15% of hereditary OCs are derived from genetic conditions unrelated to BRCA genes. Family history is still used as the main criterion in the models for calculation of BRCA1/2 carrier probability. In many European countries, referral for genetic counseling and subsequent germline DNA testing is mainly based on the age of EOC diagnosis and family history. However, wider testing unrelated to these criteria in recent years showed that restricting testing to cases with a family history of breast or ovarian cancer results in 8–54% of mutation carriers being undetected. However, criteria for testing other, lower penetrance genes still do not exist since predictive factors have not yet been identified and clinical utility for most of these genes is still being evaluated.

Difficulties for defining adequate criteria for genetic testing of EOC still exist in most of the European countries. There is also an issue with defining specific gene panel for EOC that will

enable balancing clinical utility with cost effectiveness of genetic testing. In that manner, analysis of frequency of deleterious mutations within relevant predisposition genes correlated with specific patients' characteristics may have significant contribution. Thus, we aim to identify predictors for deleterious mutations, to narrow down the criteria for genetic testing and to define gene panel that should be offered for genetic testing of EOC in Serbia. Our three years experience in panel genetic testing of EOC showed that we are still mostly able to identify potential BRCA1/2 mutation carriers in HGSOC if we use current referral criteria for genetic testing such as the age of onset and personal/family history of breast and ovarian cancers. However, if we restrict genetic testing only to those who fulfill these criteria we might still miss a subset of germline BRCA2 mutation carriers as well as carriers of mutations in other OC susceptibility genes (BRIP1, NBN, RAD51C and CHEK2). The lack of predictive factors for mutations in other cancer susceptibility genes presents a challenge in identifying these carriers in OC in Serbia. Until better predictors emerge, our data show that we should be more careful in defining criteria for genetic testing and that it will be necessary to continue performing wider genetic testing of OC, outside these criteria, in order to define population specific gene panel.

SESSION: TARGETED & IMMUNOTHERAPY IN CANCER: BIOMARKER-BASED APPROACHES

Focus on lung adenocarcinoma

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Non-small cell lung cancer (NSCLC) is a heterogenous disease driven by a spectrum of molecular alterations, and this is why it has become probably the most dynamic field of cancer clinical research in past decade. Inspired by numerous, very important fundamental molecular biology and pathology findings, clinicians applied great number of these findings into clinical studies, and consequently into clinical practice. The renaissance has begun with establishing EGFR activating mutations as a target for tyrosine kinase inhibitors, in 2004. Adenocarcinoma of the lung today represents the supstrate for practically all driven mutations that can be effectively inhibited by tyrosine kinase inhibitors (TKI). A very small percentage of activating mutations has been associated with the other non-squamous cell lung carcinomas, while the squamocellular carcinoma still presents as an orphan in terms of activated, drugable mutations.

The mutations of receptor of epidermal growth factor (EGFR) are the most numerous, and the most drugable ones, affecting 10-15% of all advanced NSCLC patients, and having today the three generations of drugs, TKI, with proven efficacy in the first and the second-line

treatment. Osimertinib, as an representative of the third generation of TKI may be very good option for second-line, in patients whose tumor mutated with secondary mutation, T790M, but also revealed supreme median progression-free survival, of almost 19 months, in the first-line treatment. This is very important breakthrough, in the light of fact that molecular and consequent clinical progression is inevitable in almost all patients having genetic alterations in lung adenocarcinoma.

Of particular interest are genetic re-arrangements in anaplastic lymphoma kinase (ALK) gene, the second most frequent (4-5% of all advanced lung adenocarcinoma) driven mutations in non-squamous lung carcinoma: after crizotinib which showed significantly better progression-free survival compared with cytotoxic chemotherapy, in 2010, and very clearly provided the another „proof-of-principle“ in molecular pathology of lung cancer, the next two generations of ALK inhibitors have been formulated, and were put into the practice, firstly in crizotinib-refractory patients, in second and third-line. Alectinib, brigatinib, ceritinib are nowadays pillars of treatment in ALK positive patients, both in first-line, and in later lines, while lorlatinib as the representative of third generation has important activity against secondary mutations and great CNS activity as well. The latest is of high importance, bearing in mind that 30-40% of ALK-positive patients will develop brain metastases.

Less frequent alterations (1-2%) in genes such as ROS1, BRAF, HER2, RET, MET exon 14, NTRK and KRAS should also be assessed, if the next-generation sequencing (NGS) is being used for broader testing. With the increasing number of the next generation kinase inhibitors which are active against these alterations (crizotinib or lorlatinib for ROS1 rearrangement, dabrafenib+trametinib for BRAF mutations, selametinib and AMG-510 for KRAS mutations, capmatinib and tepotinib for exon14 skipping MET mutation, vandetanib and BLU-667 for RET rearrangement, entrectinib for NTRK and ROS1 alterations), more and more efficient targeted therapy is being brought to clinical practice for advanced non-squamous NSCLC.

Focus on melanoma

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Metastatic melanoma is one of the most aggressive human cancers, with an overall survival of 6-8 months in an era of chemotherapy, that was inducing low rate and short-lived responses. A tremendous improvement in overall survival of these patients has been achieved with MAPK pathway inhibitors and checkpoint inhibitors immunotherapy that, for the first time, increased overall survival of these patients. However, even in this era of the new treatment possibilities, 5-year overall survival is not higher than 35%, and only 15% in patients with high tumor load and increased LDH. Therefore, a continuous research is necessary to improve the outcomes in metastatic melanoma patients. Choosing the right treatment for the right patient and optimal treatment sequencing and combination is still a matter of research,

and the results of the new studies are eagerly awaited. Still, the most reliable prognostic markers of the disease outcome are tumor burden (number of organs involved) and LDH level. Several molecular markers were identified, but none of them were until now established as valid enough to be used in everyday clinical practice. One of the emerging ones are tumor mutational burden and IFN-gamma signature, as well as different scores that combine several parameters of metastatic tumor from mutations in signaling pathways, immunological scores of tumor-infiltrating lymphocytes and tumor microenvironment biomarkers. Recent adjuvant and neoadjuvant treatment approaches opened a new chapter in melanoma treatment and necessity to further elucidate which patients do require adjuvant treatment. Until now, sentinel lymph node tumor burden was used to select patient for adjuvant treatment in recent studies with anti-PD1 therapy, but new biomarkers in primary tumor are in development, in order to make selection of patients for adjuvant treatment more reliable. An update on available predictive and prognostic biomarkers in melanoma will be reviewed.

Disentangling the molecular drivers of prostate cancer progression

A career in research: Pitfalls, drawbacks and perspectives

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Researchers in training, just like cancer cells, live and grow in aggressive competition with the environment within the system. Indeed, the Darwinian laws of evolution are conserved in tumors and the early stage researcher population. Whereas we manage to find and learn from tumors that a certain set of rules exist regulating their function, we are hardly trained on the rules and principles that are required to in order to become a mature and successful scientist. In this lecture, I will provide some insights around the key aspects that drove my career, the bottlenecks that could be determinant to define success and failure, and the ingredients to have a motivated life in research combined with a family. I will combine this notion with some flavor of my perception of cancer, and the relevant task that we have of combining high-level research with active lay dissemination to society.

SESSION: CURRENT CHALLENGES AND FUTURE PERSPECTIVES OF RADIOTHERAPY

DNA methylation as a potential predictor of tumor response to radiotherapy

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Radiotherapy is an important modality of cancer treatment; however, radioresistance represents a difficult problem that leads to tumor recurrence and poor prognosis in cancer patients. An understanding of cellular processes that determine the response to ionizing radiation exposure is essential to define potential predictive biomarkers and improve radiotherapy outcome. In the recent years, epigenetic modifications have been extensively explored as potential mechanism of tumor radioresistance. DNA methylation, as a well-established epigenetic mechanism, plays an important role in cancer development, through transcriptional silencing of genes related to cell proliferation, cell cycle process, DNA repair and apoptosis; the key cellular processes that are considered to have an important role in radiosensitive effects, too. It has been shown that different genes DNA methylation profiles can modulate cell radiosensitivity in a way that depends on their various functions in the abovementioned cell processes. Moreover, it has been suggested that radiotherapy itself introduces epigenetic alterations, through modulating gene methylation profiles, which could lead to the acquired radioresistancy. Identification of such epigenetic alterations prior and during the therapy could help in defining potential predictive biomarkers and optimizing personalized treatment. As epigenetic alterations can potentially be reversed by drug treatment, they are interesting candidate targets for anticancer therapy and radio sensitizers. Tumors resistant to ionizing radiation, due to aberrant methylation of particular genes, could be reversed into radiosensitive ones, by the use of epigenetic drugs. The application of demethylating agents to sensitize patients for radiotherapy could provide novel strategies for the treatment of malignant tumors in the future.

The role of single nucleotide polymorphisms in outcome and toxicity of radiotherapy for prostate cancer

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Prostate cancer is the second most frequent malignancy in men worldwide. The most patients with prostate cancer have clinically localized and indolent tumors at diagnosis. Radiotherapy is one of the standard curative treatment options for localized disease. The main aim of radiotherapy is to maximise local control of the tumor. On the other hand, radiotherapy is associated with a spectrum of side effects (toxicity) in the surrounding normal tissues. Acute toxicity is transient and affects high turnover tissues, such as the skin and mucosa, whereas late toxicity can persist for life. It was estimated that either moderate or severe late gastrointestinal toxicity was developed in 15% and 2% prostate cancer patients, respectively. In addition, either moderate or severe genitourinary complications were appeared in 17% and 3%, respectively, of these patients. Approximately half of prostate cancer patients who receive radiotherapy develop erectile dysfunction. Thus, the adverse effects resulting from radiotherapy influence the quality of life for these patients which are particularly important since the 5-year survival rate for early stages of prostate cancer reaches nearly 100%.

The cause of normal tissue toxicity is multifactorial and includes genetic factors in addition to radiation dose and volume of exposure, underlying co-morbidities, age, concomitant chemotherapy or hormonal therapy etc. The radiogenomics has focused upon the identification of genetic variants associated with response to radiation (single-nucleotide polymorphisms (SNPs), gene mutation, change in RNA expression, copy number variations etc.).

The human genome includes numerous germline variations in DNA sequence called polymorphisms. In 90% of cases, these variations represent SNPs. The SNPs are defined as polymorphisms in which the minor variant (allele) is present in at least 1% of an investigated population. They are potential biomarkers to predict radiotherapy outcome and normal tissue response after radiotherapy. It is important to mention that many SNPs have only moderately associated with radiotherapy outcome and toxicity. However, in combination, they had a stronger, dose dependent i.e. cumulative effect. Also, many SNPs are connected to each other through "linkage disequilibrium," which is a nonrandom association of alleles at two or more loci. Moreover, it should be investigated whether the clinical variables (such as age, Gleason score, tumor volume, smoking, diabetes, hypertension, prostate-specific antigen, radiation dose and the use of androgen deprivation therapy) could influence predictive SNPs power.

The candidate SNPs biomarkers should look in genes related to endogenous oxidative stress defense, DNA damage signaling and cell cycle control, DNA repair, inflammatory response, cytokine activity related to fibrosis as well as general metabolism and homeostasis. The goal of these investigations is to reveal biomarkers for personalized radiotherapy approach.

Comet assay as a tool for evaluation of DNA damage in cancer patient treated with radiotherapy

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DNA double-strand breaks constitute the most dangerous type of DNA damage induced by ionizing radiation. Standard method for assessing DNA damage in individual cells is the comet assay. In the present study the comet assay was applied to survey the levels of DNA damage in non-target cells collected from cancer patients who were treated with radiotherapy.

Between June 2016 and July 2017, 33 patients with localized prostate cancer were treated with three-dimensional conformal radiotherapy (3DCRT) in the Institute of Oncology and Radiology of Serbia (Belgrade, Serbia). The patients were recruited as follows: group 1, with 3DCRT (72 Gy in 36 fractions, n=24), and group 2, 3DCRT postprostatectomy (66 Gy in 33 fractions, n=9). The effect of Radiotherapy on DNA damage was preliminarily analyzed on ten patients using the Comet assay. The study used each cancer patient as his own control. Blood samples were collected at baseline, during and after 3DCRT according to the following dynamics: before the radiotherapy, after 5, 15, 25 fraction, at the end of therapy, as well as on control, a month after radiotherapy. Peripheral blood lymphocytes were isolated using LymphoPrep[®]. DNA damage was measured by comet assay and expressed as % tail DNA2.

The results obtained indicate two groups of patients: one with elevated DNA damage (46 to 80%) and second with no DNA damage ($\leq 10\%$) before the treatment. Patients with lower sensitivity indicate milder trend in DNA damage, while the other patients show more pronounced DNA damage response during the treatment. After the end of a treatment DNA damage was from 2 to 60%, indicating great individuality in DNA damage response to therapy. Basal and residual levels of DNA damage in PBMCs can be influenced by many external factors resulting in a wide intra-individual and inter-individual variability. Thus, a larger number of patients and further studies are necessary to confirm the overall outcome of a treatment.

Late genitourinary toxicity after conventionally fractionated conformal radiotherapy for localized prostate cancer

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Three-dimensional conformal radiotherapy (3DCRT) makes it possible to spare normal tissue. Reductions in radiation toxicity as a result of using 3DCRT compared with previous techniques have already been reported, but randomized trials have shown no significant difference in the occurrence of acute or late genitourinary (GU) toxicity between conventional radiotherapy and 3DCRT. These facts lead to the assumption that in addition to the irradiation technique, other factors are probably involved in the occurrence of symptoms of GU toxicity. In addition to the dose-volume effect reported in the literature, other factors that may affect the incidence of acute and late GU toxicities such as age, smoking, hormonal therapy, diabetes mellitus, use of certain drugs, and genetic markers have been mentioned in the literature. The association between the acute and late effects of RT in PC has been studied by only a few investigators. Therefore, we conducted a longitudinal study among patients with localized PC in order to assess the incidence of acute and late GU toxicity after 3DCRT at a single institution. Furthermore, we wanted to explore which of the clinical and patient-related factors are involved in the occurrence of acute and late GU toxicity and determine possible correlations between them.

Between September 2009 and September 2013, 225 patients with localized PC were treated with 3DCRT at the Institute for Oncology and Radiology of Serbia. Ninety-four of these patients fulfilled the following inclusion criteria for this study: localized disease stage (T1-2), prostate specific antigen (PSA) level ≤ 20 , Gleason score (GS) < 8 , Karnofsky index (KI) ≥ 80 , and an estimated risk of lymph node involvement $\leq 15\%$ according to the Roach formula. We performed a retrospective analysis of the individual, clinical, and toxicity records, and dose volume histograms (DVHs) for these patients.

Late GU morbidity was graded according to the European Organization for the Research and Treatment of Cancer (EORTC) scoring scale, slightly modified by Peters and coworkers. Patients were seen in routine follow-up visits every 3-4 months for the first 2 years, and every 6 months during years 2-5. At each follow-up visit, the patient underwent a physical examination, additional examinations (e.g. imaging, endoscopy), PSA determination and assessment of specific genitourinary and gastrointestinal morbidity.

Median follow up was 66 months (range 6-101 months).

Prior abdominal or pelvic surgery have a significant impact on the occurrence of grade 2 or higher late GU toxicity.

In our study, the occurrence of any grade of acute GU toxicity was significantly influenced by the development of any grade of late GU toxicity.

Pediatric radiotherapy at IORS

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Constant progress of techniques and computer technology enabled further development of radiotherapy. New generation of linear accelerators and contemporary software systems for radiotherapy planning enabled the development of new radiotherapy techniques. Except for technical possibilities and research in the field of medical physics and radiobiology, improvement in immobilization of the patients and education of all members of radiotherapy team enabled the development of highly conformal radiation techniques. They provide high uniformity and conformity of the dose distribution in the target volume with the decrease of irradiated volume of normal tissue and organ at risk. It is of particular importance in pediatric radiotherapy. Highly conformal radiation techniques, intensity modulated radiotherapy (IMRT) and volumetric modulated arc therapy (VMAT), can be helpful in treating children of a young age and in the treatment of special sites such as: head and neck, skull base and brain, spinal or paraspinal region, retroperitoneal space close to liver and kidneys and pelvis. When applying IMRT and VMAT techniques in the treatment of children caution is advised. Leading radiotherapy centers still summarize their clinical findings and published data.

In the Institute for Oncology and Radiology of Serbia from 2007 to 2018 806 children aged from 1 to 18 years were treated with radiotherapy. More than 90% are treated with a three-dimensional radiotherapy which is a standard radiotherapy technique. During the past 12 years we have gained our own experience that helped us to start with new radiotherapy techniques. During 2017 and 2018 installation of new radiotherapy machines with additional equipment the conditions for new techniques have been created. With a highly educated radiotherapy team with a great experience in pediatric radiotherapy in a short period we started with IMRT and arc therapy. According to recommendation we use IMRT and arc techniques for head and neck tumors, retroperitoneal tumors, lymph nodes of abdomen and pelvis, with a reduction in radiotherapy volumes. Given the limitations and caution in using arc therapy we will use it and wait for further recommendations.

MicroRNA in lung cancer as potential biomarkers

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Despite advances in early diagnosis and novel targeted therapies, lung cancer still remains leading cause of cancer related deaths. The need for a precise biomarker of diagnosis, prognosis and therapy put molecular complexity of lung cancer in investigation focus. Since the discovery of microRNAs in humans in 2000, up to now, numerous studies have shown that they can be used not only as a diagnostic tool, but as a marker of prediction and prognosis, as well as therapeutic target.

The stability of MicroRNAs (miR) in biological fluids (such as sputum, blood, plasma, and urine), and the possibility to reveal the tumor origin, could lead to becoming a noninvasive biomarker in lung cancer.

In histological differentiation, MicroRNA expression patterns permit precise determination between squamous NSCLC and non-squamous NSCLC as well as SCLC even in poorly differentiated tumors and small biopsies. They can also distinguish primary tumor from lung metastases from other sites (i.e. colon cancer metastases in the lung-miR-592 and miR-522). MiRs are associated with lung cancer driver mutations (EGFR, ALK, Ras) and tumor suppressors (PTEN, p53) and therefore affect numerous biological pathways and even function as oncogenes. Several studies revealed that the level of miRs expression is associated with tumor stage and metastases, response and resistance to radiation, chemotherapy and TKI, promotes proliferation, angiogenesis and recurrence, and even impacts survival.

It seems that MicroRNAs can also serve as directed therapies. In animal model lung cancer there are two ways for using miRs as therapeutics: restoring tumor-suppressor miR function or blocking oncogenic miR function. Restoring tumor-suppressor miR function can be accomplished in several ways: by transcription factor (c-myc and p53) and through epigenetic modification (methylation). The other way uses miR mimics which reduce off-target effects and can be personalized by tumor's miR signature. Vector based delivery system, well known in gene therapy, can also be utilized using adenoviral and lentiviral delivery, lipid emulsion, liposomes and nanoparticles. Strategies for blocking oncogenic miRs are primary based on anti-sense oligonucleotides. This approach includes antagomirs, locked nucleic acid (LNA) miRs and miR sponges.

However, all this knowledge is accumulated through a variety of in vivo and in vitro preclinical studies. Up to now, only one human clinical trial with miR targeting therapy is performed and it is for hepatitis C viral infection...Still, promising results of these studies clearly show that microRNAs are the near future of oncology.

SESSION: THE ROLE OF OXIDATIVE STRESS IN DRUG RESISTANCE

Targeting mitochondria to overcome multidrug resistance in cancer

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Multidrug resistance (MDR) of cancers is major obstacle in successful chemotherapy. One of the most frequent mechanism of MDR is the overexpression of membrane efflux pumps such as P-glycoprotein (Pgp) that extrude drugs from tumor cells limiting their therapeutic effects. MDR tumor cells have high energy requirements and increased mitochondrial metabolism. Targeting tumor cells mitochondria increases chemotherapy efficacy. To this aim, we used different approaches.

First, we found that we can impair mitochondrial metabolism in MDR tumor cells simply by changing administration timing and doses of the chemotherapeutic agent. We used this metronomic chemotherapy comparing the efficacy of two repeated low doses of doxorubicin versus one single higher dose. The former treatment was significantly more cytotoxic *in vitro* and *in vivo* against doxorubicin resistant tumors, where the conventional treatment failed. The greater efficacy of the repeated low doses treatment was due to the increased level of intracellular reactive oxygen species, produced by the higher electron flux from complex I to complex III of the mitochondrial respiratory chain. This process induced mitochondrial oxidative damage, decrease in mitochondrial ATP, loss of mitochondrial potential and activation of cytochrome c/caspase 9/caspase 3 pro-apoptotic axis in drug resistant cells (Riganti C et al, Cancer Lett 2015).

Second, we test new synthetic doxorubicins vectorised to be delivered into mitochondria (Chamberlain GR et al, ACS Chem Biol 2013; Riganti C et al, Mol Pharm 2013). Here, the mitochondrial targeted-doxorubicin increased the generation of reactive nitrogen species and reactive oxygen species, decreased the TCA cycle, the electron transport chain, the synthesis of ATP and induced peroxidation of mitochondrial lipids, triggering mitochondrial damage and tumor cells apoptosis (Buondonno I et al, Mol Cancer Ther 2016). The liposomal formulations of this mitochondrial targeted-doxorubicin (Pedrini I et al, Mol Pharm 2014) rescued doxorubicin efficacy in preclinical model of drug-resistant tumors (Gazzano E et al, J Control Release 2018). In conclusion, mitochondria might be Achilles' heel of MDR tumor cells and are worth of future investigation as potential targets to design new therapeutic approaches against aggressive and unresponsive cancers.

Lipid peroxidation - double edge sword in fighting cancer (stem) cells

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Oxidative stress is both, cause and consequence of tumor transformation. This disturbed balance between oxidants and antioxidants has disturbed cellular redox signaling as a consequence. In the light of cancer, oxidants cause mutations in normal cell which could lead to malignant transformation. Paradoxically, conventional chemotherapy is based on drugs that increase intracellular ROS and thereby achieving their toxic effects. Changes of ROS level alter redox signaling pathways and can contribute to differential therapy response. Numerous metabolic factors can influence redox homeostasis both, in normal cells as well as in cancer cells. Therefore, our weapon in fighting cancer can turn against our efforts and through adaptation to stress conditions. Here, new aspects of cancer therapy and metabolic changes will be presented in order to provide understanding of events which could create difference between the outcome of the disease.

SESSION: INNOVATIVE TOOLS FOR ADVANCING CANCER RESEARCH

Spheres: simple and quite reliable models in basic cancer research

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Spheres are trendy approaches to allow the researchers to do more realistic experiments due to their 3D structure. Especially, for the evaluation of anticancer activity of newly synthesized compounds, they produce more reliable results, compared to their 2D counterparts. For quite a long time, the systemic treatment of malignancies has been based on physicians' empirical judgement, relying on data obtained from clinical trials (population-based approach). However, even histopathologically identical tumors behave so differently that the response rate of tumors to the chemotherapeutics is varied. In other words, each patient responds differently. Therefore, the effectiveness of current therapeutic approaches is limited mainly by tumor heterogeneity, which often causes the failure of the successful treatment of cancer patients. Detecting the chemosensitivity and/or chemoresistance of tumor tissue freshly removed from the patient during routine surgical operation to anti-cancer agents *in vitro* (*ex vivo*) could overcome this difficulty.

Working on malignant cells isolated from individual fresh cancer tissues or biopsy specimens removed from cancer patients during surgical operation needs such models (spheres or 3D structures). They more or less mimics a 3D cell culture and includes some steps: 1) isolation

of cells from the patient tumor tissue; 2) seeding the cells in a ultralow-attachment U-shape plate (mimicking a 3D culture); 3) incubation of cells with chemotherapeutics/compounds for 7 days; 4) assessment of cell viability by ATP assay and interpretation of the results.

If a medium that specifically allows the cancer stem cells to grow, then a sphere formation can occur. If the ATP assay is performed on these cells, then the response to treatment can be regarded as the response of cancer stem cell-enriched population. This is so valuable tool to evaluate the efficiency of drugs for a particular patient..

Another benefit of these models is that they allow to work on cancer stem cells (CSCs). CSCs are also highly trendy currently because of their resistant nature to anticancer drugs. By using some special mediums and growth factors etc, it is easy to obtain CSC-enriched population that permits to perform diverse assays on them.

Very recently, organoids (although not the scope of this talk) are expected to be highly promising for the development of personalized medicine approaches although there seem to be some concerns such as the lack of immune system and patient's psychiatric status.

Glioblastoma behaviour in 3D biomimetic systems

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Glioblastoma, although not the most common, is one of the deadliest human cancers. Since implementation of Stupp protocol in clinical practice (surgery + radiation + temozolomide chemotherapy), which prolonged patients survival for only 2.5 months, there were no major advances in glioblastoma treatment. Therefore, it is an imperative to better understand mechanisms behind glioblastoma behavior in order to efficiently treat it in the future. Conventional, two-dimensional, cell cultures are affordable and easy to perform in vitro systems for cancer research but they do not recapitulate tumor structure complexity and lack actual microenvironmental conditions. On the other hand, animal studies are quite expensive and time consuming and do not adequately reproduce the disease status present in humans, due to species-specific differences. Therefore, recent studies are focused on bridging this gap by developing tri-dimensional (3D) in vitro cultures to study cancer progression and its response to therapy under biomimetic conditions. We used two types of 3D perfusion culture systems, microfluidic chips and bioreactors, to study glioblastoma behavior and response to different therapeutic approaches in more realistic environmental setup. Specifically, microfluidic chips with collagen hydrogels were used to test combined effects of temozolomide and Coenzyme Q10 on invasion, cell death and reactive oxygen species production in drug-sensitive and resistant rat glioblastoma cell lines. On the other hand, bioreactors loaded with human glioblastoma cells, immobilized in alginate microtubes, were utilized to establish and characterize long term 3D perfusion cultures for testing novel temozolomide protocol in glioblastoma treatment. Overall, results showed that these 3D systems successfully mimic

glioblastoma behavior in situ and emphasized their value as platforms for more reliable treatment evaluation.

P1

The implementation of somatic *BRCA1/2* testing in advanced ovarian cancer patients: Serbian experience

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Background: Detection of both germline and somatic *BRCA1/2* mutations in ovarian cancer (OC) is used to select candidates who may benefit from treatment with poly (ADP-ribose) polymerase inhibitors (PARPi). However, the high complexity of this analysis and lack of competent staff hampered its clinical application in Serbia. In 2016, as part of pharmacogenomic service, we were the first who started implementing *BRCA1/2* somatic genetic testing in OC patients. **Patients and Methods:** Between 2016 and 2019, tumor samples from 36 patients with platinum-sensitive relapsed high-grade serous OC (HGSOC) were referred to *BRCA1/2* mutation testing using next-generation sequencing (NGS). A total of 22 samples (61.1%) passed the quality control and were sequenced on Illumina's MiSeq sequencing system using multiplex PCR-based NGS protocol according to the manufacturer's instructions. **Results:** We have accomplished our goal in terms of efficient optimization of sequencing protocol and data analysis workflow. In addition, the *BRCA1/2* somatic analysis in HGSOC identified two frameshift mutations (9.09%) that confer sensitivity to PARPi: one in *BRCA1* and one in *BRCA2*. **Conclusion:** Our results showed that we have successfully established very complex and challenging somatic *BRCA1/2* mutation analysis. Thus, we became the only Institution in Serbia that not only offers this particular type of genetic testing, but also it is able to analyze demanding results by experienced staff and to refer patients to clinical oncologists for further treatment. *BRCA1/2* tumor testing at IORS will identify additional HGSOC patients who, along with germline carriers, could benefit from PARPi therapy.

Keywords: *BRCA1/2*, next-generation sequencing, ovarian cancer, PARP inhibitors, somatic mutations

P2 - O

Disturbed redox balance contributes clear renal cell carcinoma development and progression

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Background: Renal cell carcinoma (RCC) belongs to tumours in which significant changes occur in cellular redox balance, probably as the consequence of deregulated Nuclear factor erythroid 2-related factor 2 (Nrf2) pathway. Nuclear accumulation of Nrf2 modulates the expression of a large number of genes, including antioxidant enzymes, as well as detoxification phase II enzymes, such as glutathione transferases (GST). Therefore, changes in cellular redox balance in RCC might also be attributed to alterations in GST phenotype, affecting both the development and the progression of clear cell renal cell carcinoma (ccRCC). **Patients and Methods:** In order to elucidate the possible role of GSTP1 in ccRCC, *GSTP1* genotypes (rs1695 and rs1138272) were determined in 204 RCC patients and 280 matched-controls, in whom overall survival was also evaluated. The expression of GSTP1, regulatory (JNK1/2) and executor (Caspase-3) apoptotic molecules in ccRCC tissue samples were analyzed by immunoblot. The presence of GSTP1:JNK1/2 protein:protein interaction was determined by immunoprecipitation. **Results:** *GSTP1*-variant genotype (rs1695) was significantly associated with the risk of ccRCC development and almost 2-fold increased hazard ratio in prediction of overall mortality. Haplotype C (*Val105+*Val114) was associated with 4-fold increased ccRCC risk. The presence of GSTP1:JNK1/2 protein:protein interaction was found in all ccRCC tissue samples studied. **Conclusion:** It might be concluded that *GSTP1* polymorphisms might be associated with ccRCC risk, while the presence of GSTP1:JNK1 protein:protein interaction might be the possible molecular mechanism underlying the role of *GSTP1* in ccRCC progression. **Keywords:** GST, progression, RCC, redox balance, risk

P3

Lower glutathione level in papillary thyroid cancer patients: method optimization and case-control study

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Spectrophotometric method based on the reaction of sulfhydryl group with 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) is one of the most commonly used method for detection of reduced glutathione (GSH). However, there is a need for method optimization in order to improve its specificity and to evaluate the storing capacity when performing human biomonitoring studies. To address these knowledge gaps, we performed several sample preparation protocols, traced the stability of GSH in frozen blood samples, and finally determined the GSH concentration in 36 papillary thyroid cancer patients. The optimal approach for GSH determination included treatment of blood samples with 5 % TCA followed by centrifugation (4000 rpm, 15 min). To 500 μ L of blood supernatant 350 μ L phosphate buffer (1.0 mol/L) and 50 μ L DTNB (1.0 mmol/L) were added and the absorbance was read at 412 nm on UV-Vis spectrophotometer. GSH was quantified based on the absorption coefficient factor (ϵ) 14150 M⁻¹ cm⁻¹. Testing the stability of GSH in blood samples demonstrated that GSH is stable in blood samples for up to a month. Then we applied this protocol on patients' blood samples (aged 51.6 \pm 14.0 years, 28:8 female:male ratio, 33% of active smokers, and BMI of 28.01 kg/m²). The levels of patients' blood GSH (76.4 \pm 38.2 μ mol/L) were significantly lower ($p < 0.05$) compared to matched control population (107.5 \pm 34.9 μ mol/L). Taken together, we have provided the optimized spectrophotometric method for determination of blood GSH. Moreover, we detected lower non-enzymatic oxidative stress defence mechanism in papillary thyroid cancer patient. Keywords: glutathione, papillary thyroid cancer, oxidative stress, method optimization

P4 - O

Prognostic significance of miR-494 in renal cell carcinoma

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Background: Truncated KIT (tr-KIT) is an alternative variant of c-KIT protein. tr-KIT has only one tyrosine kinase domain of c-KIT, not cytoplasmic domain and preserve its oncogenic function. tr-KIT transcript as a different 5'UTR than c-KIT and this provides docking of miR-494 on it. In our previous studies, we proved increased expression of tr-KIT in renal cell carcinoma (RCC). In this study, we hypothesized that increased tr-KIT transcripts would sponge miR-494 and decreased miR-494 expression would have a prognostic value for RCC cases. **Material and Methods:** Kidney Cancer cDNA Array containing a total of 48 cDNA samples from the normal kidney tissues of 9 healthy subjects and kidney tumor tissues of 10 stage-1, 5 stage-2, 13 stage-3 and 11 stage-4 RCC patients was used for gene expression analysis. miR-494 expressions were measured in these samples and associated with clinopathological characteristics of RCC patients. **Results:** miR-494 expression is significantly lower in RCC samples ($p=0.001$). Also, miR-494 expression is significantly differed between normal-stage I, normal-stage IV and stage I and stage IV (TNM stage) (respectively, 0.027, 0.036, 0.049). Moreover, miR-494 expression is significantly discriminative between normal-grade 1, grade 2-3 and grade 3-4 (Fuhrman grade) (respectively, 0.001, 0.001, 0.06). Finally, miR-494 expression is significantly decreased in clear cell and papillary subtypes than normal (respectively, 0.011, 0.020). **Conclusion:** Our results suggest that miR-494 may have a tumor suppressive role in RCC and decreased miR-494 expression level might be useful as a prognostic marker for RCC patients.

Keywords: miR-494, tr-KIT, c-KIT, renal cell carcinoma

P5

Impact of Factor V Leiden Mutation on Thrombosis Risk in Patients with Diffuse Large B-Cell LymphomaVesna Ilić¹, Tarabar Olja², Bojana Cikota Aleksić¹, Zvonko Magić¹¹*Institute for medical research, MMA, Belgrade, Serbia*²*Clinic for Haematology, MMA, Belgrade, Serbia*

Background: Clinical course of patients (pts) with diffuse B cell lymphoma (DLBCL) may be complicated with venous thromboembolic events (VTE). The aim of this study is to examine the impact of FV Leiden mutation on the risk of VTE in pts with DLBCL. **Patients and methods:** The

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present study included 122 patients with DLBCL. Patients were treated with rituximab-CHOP. Monitoring included a period from the diagnosis of lymphoma to finished treatment. Factor V polymorphism was detected by PCR-RFLP. **Results:** Of 122 pts with DLBCL, in 16 pts (13.11%) was diagnosed deep vein thrombosis (DVT)/pulmonary thromboembolism (PTE), 10 pts had DVT in lower extremities, 1 pt with symptomatic PTE, 2 pts with combined DVT + PTE and 3 pts with DVT development at other sites. In 12 out of 122 pts (9.8%), heterozygous mutation for Factor V Leiden (FVL) was present in 7 pts (6.6%) without DVT and in 5 pts (31.25%) with DVT, $p = 0.001$. Also, patients with DVT compared to non-DVT patients had a significantly more frequent performance status (PS) gr2 (45.5% versus 17.2%, $p = 0.003$), the international prognostic score (IPI) gr 3-5 (68% vs 27% $p = 0.001$) and bulky disease (68.2% vs 26.9%, $p = 0.001$). In multivariate analysis, IPI gr 3-5 (HR 4.037, $p = 0.04$) and FVL (HR 10.598, $p = 0.004$) were associated with the development of DVT. **Conclusion:** FVL could be a promising disease independent parameter associated with DVT in patients with DLBCL
Keywords: DLBCL, Factor V Leiden

P6

Phenotypic and clinicopathologic characteristics and *CDKN2A* mutation status of familial melanoma in population of Serbia

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Background: Melanoma is a malignant disease with rapidly growing incidence, especially among Caucasoid population. According to the literature, 5-12% of patients have hereditary predispositions for melanoma, and around 40% of these cases are associated with mutations in high-penetrability gene *CDKN2A*. **Patients and Methods:** We collected data about behavioral and phenotypic melanoma risk factors, clinicopathological parameters and occurrence of melanoma and/or other malignant diseases in families, for 564 patients with earlier diagnosed melanoma. These data were analyzed and compared between familial melanoma group (at least one first- or second-degree relative that has or has had melanoma) and sporadic melanoma group. The Sanger sequencing method was used to determine mutation in promoter, exons 1 α , 2, 3 and intron 2 of *CDKN2A* gene. **Results:** Familial melanoma was determined for 37 patients (6,6%) while 527 patients (93,4%) had sporadic melanoma, with almost equal sexual distribution. Analysis showed a statistical significance of eye color ($p=0,009$) and number of lifetime sunburns ($p=0,01$) for two analyzed groups. Sequencing analysis for 6 samples and comparison to NM_000077 referent sequence show SNPs: c.116G>A in promoter of all 6 samples, c.442G>A in

exon 2 of 1 sample and c.806G>C in exon 3 of 4 samples. **Conclusion:** Our results on frequency of familial melanoma correspond to literature finding. Lifetime history of sunburns and fair eye color were found more frequently in familial melanoma group. According to our preliminary research, found SNPs c.116G>A and c.806G>C are common variants with no reported clinical significance and one rare variant c.442G>A is reported as benign.

Keywords: *CDKN2A*, Characteristics, Familial Melanoma.

P7 - O

***CDK4* codon 24 mutation status in Familial Melanoma patients - single center study**

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Background: Familial melanoma constitutes 5-12% of all melanoma. Association with *CDKN2A* and *CDK4* genes is found in 45% of familial melanoma patients and mutation in this genes increase familial melanoma risk by 40% and 20% respectively. *CDK4* gene, located on 12q14.1, codes for cyclin-dependent kinase, a protein which is a member of Ser/Thr kinases family. Protein kinases participate in cell cycle regulation. Changes in their function cause abnormalities in cell cycle regulation, resulting in neoplastic transformation. **Patients and Methods:** Our study included 60 patients - 29 male (48,3%) and 31 (51.7%) female patients, average age 55.2 years, mediana 58, from Melanoma outpatient clinic, Military Medical Academy, Belgrade, Serbia who had been surveyed in order to gather information about the existence of melanoma and/or pancreatic cancer within their first or second degree relatives. Hotspot mutation in *CDK4* gene (R24H and R24C) were analyzed on 7500 Real Time PCR System (Applied Biosystems, USA) using TaqMan assays (rs104894340 and rs11547328). **Results:** The results for all of 60 familial melanoma patients did not indicate presence of mutation on the abovementioned position and all patients had wild type genotype for rs11547328 (GG) and rs104894340 (TT). **Conclusion:** Those findings proves the absence of *CDK4* mutations in our patients with melanoma in family. Keywords: *CDK4*, familial melanoma, hotspot mutation

P8 - O

The predictive value of haematological parameters on the progression-free survival in advanced lung adenocarcinoma patients treated with tyrosine kinase inhibitors in Serbia

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Background: Systemic markers of host inflammation have been proposed as new factors that influence cancer development and response to standard and targeted therapies. The aim of this study was to analyze the predictive value of pre-treatment haematological parameters in EGFR-mutated NSCLC patients treated with tyrosine-kinase inhibitors (TKIs). **Material (Patients) and Methods:** This retrospective study included 101 advanced lung adenocarcinoma patients (stage IIIB/IV, ECOG performance status 0, 1 or 2). *EGFR* mutation testing was performed by real-time qPCR. Patients with sensitizing *EGFR* mutations were treated with EGFR TKIs in the first line until progression. Haematological parameters were derived from the absolute differential counts of a complete blood count (CBC) before TKI treatment, and analyzed parameters included: neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), platelet-to-monocyte ratio (PMR), lymphocyte-to-monocyte ratio (LMR) and neutrophil-to-monocyte ratio (NMR). Cut-off values were determined using ROC curves, and correlation with progression-free survival (PFS) was examined by Kaplan-Meier method and Cox regression. **Results:** Although 21 % of patients progressed in the first 3 months, and 43 % in the first 6 months, the median PFS in the whole group was 10.9 months (8.1-13.7, CI 95%). Predictors of a shorter PFS were LMR ≥ 6.0 (8.6 vs 10.9 months, $p=0.0003$), NLR ≥ 3.8 (8.7 vs 10.9 months, $p=0.004$), PLR ≥ 278.0 (8.7 vs 11.1 months, $p=0.001$) and PMR ≥ 387.5 (8.7 vs 11.1 months, $p=0.0001$). **Conclusion:** Pre-treatment haematological parameters LMR, NLR, PLR and PMR might be proposed as low-cost and minimally invasive predictive markers of EGFR TKI response in EGFR-mutated NSCLC patients.

Keywords: EGFR tyrosine kinase inhibitors, lung adenocarcinoma

P9 - O

SOX2-targeted T cell immunotherapy in multiple myeloma

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Background: Multiple myeloma (MM) results from the uncontrolled growth of clonal antibody-producing plasma cells in the bone marrow. Adoptive cell therapy (ACT) is emerging as a promising treatment for MM, but it requires further improvement, as many patients have relapsed due to the outgrowth of antigen-negative MM cells. These observations suggest that targeting a protein involved in the induction or maintenance of the malignant phenotype may be critical for ACT success. Recent studies suggest that the transcription factor SOX2 may regulate cell proliferation and self-renewal in MM and other cancers. Spontaneous T cell immunity to SOX2 in MM patients has been associated with slower progression and with a prolonged response to therapy. We have therefore hypothesized that adoptively transferred T cells targeting SOX2 will have a clinical benefit in MM. **Materials and Methods:** We have begun isolating high affinity SOX2-specific T cell receptors (TCRs) from healthy HLA-A*02:01 donors. To date, we have generated 40 T cell lines that recognize six distinct SOX2 epitopes in the context of HLA-A*02:01 with varying affinities. **Results and Conclusion:** Expanded SOX2-specific T cells can respond to endogenously processed and presented SOX2 peptides. We will next sort the highest affinity SOX2-specific CD8+ T cells from these lines for sequencing and cloning of the TCRs. Assembled TCR pairs will be tested in vitro and in vivo for specific tumor cell killing. Collectively, these experiments should reveal if SOX2 is a safe and effective T cell target for treating MM.

Keywords: multiple myeloma, adoptive cell therapy, T cell, epitope

P10 - O

rs2910164 variant in *mir-146a* gene in high risk hereditary breast and ovarian cancer cases

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Background: *miR-146a* downregulates the expression of *BRCA1*, influencing protein activity and, presumably, cancer risk. rs2910164 variant occurs in the conserved region, possibly affecting miR-146a function. The goal of this study was to examine the role of rs2910164 variant in cancers that occur in high-risk families with accumulated cases of breast and ovarian cancer.

Material and methods: Study group consisted of 154 examinees: (I) healthy, (II) breast cancer, (III) bilateral breast cancer/ breast and ovarian cancer/ multiple primary cancers and (IV) ovarian cancer/ bilateral ovarian cancer. DNA was isolated from peripheral blood with salting-out method and genotypes were determined with Real Time PCR and TaqMan genotyping assay.

Results: Subgroups I, II, III and IV showed following genotypes distribution (respectively): GG (62.3%,52.8%,45.8%,50%), GC (35.8%,43.4%,54.2%,41.7%) and CC (1.9%,3.8%,0%,8.3%).

No significant association was found between rs2910164 and types of cancer found in the subgroups ($p=0.492$). Investigating the frequency of recessive allele, no association was found between rs2910164 variant and groups of healthy and affected examinees ($p=0.163$), the same result was also obtained between the same groups with *BRCA1/2* wt and *BRCA1/2* mutated examinees ($p=0.430$, $p=0.824$, respectively). rs2910164 was not associated with the risk for breast cancer in individuals from high-risk families ($OR=1.618$, $p=0.165$). After introducing the years of cancer occurrence as confounding variable, the risk for breast cancer grows ($p=0.077$).

Conclusion: This study shows that rs2910164 in *mir-146a* gene could be studied as an independent risk factor in younger affected individuals from high-risk families. Increasing the study group might lead to statistically significant results.

Keywords: *BRCA1/2*, hereditary breast and ovarian cancer, *miR-146a*, rs2910164

P11

Difference in amplification of *CCND1* oncogene in triple negative and hormone receptor positive breast cancer

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Background: The crucial biological markers in breast cancer are estrogen receptor – ER, progesterone receptor – PR and human epidermal growth factor receptor 2 – HER2. Based on their expression, breast cancer can be divided into hormone dependent (ER+, PR+, HER2+/HR+) and triple negative (ER-, PR-, HER2-/ TNBC). TNBC and HR+ subtypes differ markedly in clinico-pathological characteristics and response to therapy which implies that different genetic alterations drive these tumors. Our aim was to identify possible difference in amplification status of *CCND1* oncogene in both types of breast cancer and to investigate whether the amplification of *CCND1* oncogene was associated with the clinical course and outcome. **Material and methods:** *CCND1* amplification was analyzed in 78 TNBC specimens by TaqMan based quantitative real time PCR assays. Gene copy number status was evaluated according to the Livak ($2^{-\Delta\Delta CT}$) method. *CCND1* amplification status in 46 HR+ cancers was obtained using the revised patient cohort from our previously published data. **Results and Conclusion:** *CCND1* was amplified in only 5% (4/78) of TNBC samples. *CCND1* was amplified in 20% (9/46) of HR+ cancers. *CCND1* was significantly more frequently amplified in HR+ tumors compared to TNBC ($p=0.017$). No associations between *CCND1* amplification status and clinicopathological parameters, in both TNBC and HR+ cancers, were observed. Our results imply that *CCND1* oncogene status contributes to the differences observed between HR+ and TNBC tumors. Identification of genetic alterations that induce the disparate behavior of HR+ and TNBC tumors could contribute to the development of new therapeutic strategies.

Keywords: *CCND1*, gene amplification, hormone receptor positive breast cancer, triple negative breast cancer

P12

GSTO1 (rs4925) polymorphism might be determinant of postoperative prognosis among male clear cell renal cell carcinoma patients

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Background: Single nucleotide polymorphisms in genes encoding omega class glutathione transferases, GSTO1 and GSTO2, contribute to development of several neurodegenerative diseases and solid cancers. We aimed to determine the potential role of GSTO polymorphisms (*GSTO1**C419A (rs4925), *GSTO2**A424G (rs156697) and *GSTO2**A183G (rs2297235)) as determinants of postoperative prognosis in patients with clear cell renal cell carcinoma (ccRCC). Furthermore, we assessed phosphorylation status of PI3K/PTEN/Akt/mTOR and Raf/MEK/ERK signaling pathways in non-tumor and tumor ccRCC tissue, as well as possible association of GSTO1 with signaling molecules known to be regulated by glutathionylation. **Material and methods:** *GSTO1* (rs4925) and *GSTO2* (rs156697, rs2297235) genotyping was performed in 239 ccRCC patients, while ccRCC tissue and non-tumor specimens were used for immunoprecipitation and immunoblot. Immunoprecipitation with GSTO1 antibody was followed by immunoblot using anti-GSTO1, Akt, phospho-Akt (pT308), β -actin antibodies and Akt/MAPK signaling pathway antibody cocktail. **Results:** We showed that *GSTO1**CC (rs4925) genotype, exhibiting higher deglutathionylase activity, predicts shorter survival of male patients ($p=0.049$). Additionally, male carriers of *GSTO1**CC (rs4925) genotype had significantly increased mortality risk compared to the carriers of *GSTO1**A allele ($p=0.037$). Expression of phosphorylated proteins of Akt/MAPK signaling pathway (p90RSK1 (pS380), Akt (pS473) and ERK1/2 (pY204/187)) was increased in ccRCC tumor compared to corresponding non-tumor specimens. What is more, immunoprecipitation has shown an association of GSTO1 with Akt, phospho-Akt, p90RSK1 (pS380) and RPS6 (pS235/236). **Conclusion:** *GSTO1**CC genotype might be determinant of postoperative prognosis among male ccRCC patients.

Keywords: Akt, clear cell renal cell carcinoma, GSTO1, GSTO2, MAPK

P13

Individual epithelial cells dispersed in the stroma of breast tumours prognosticate the risk of distant metastases

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Background: Survival and life quality of breast cancer patients could be improved by more aggressive chemotherapy for those at high metastasis risk and milder treatments for those at low risk. Such personalised treatment cannot be currently achieved due to the insufficient reliability of metastasis risk prognosis. The purpose of this study was thus to improve the metastasis risk prognosis through extensive optimisation of the computational analysis of epithelial cell growth structures in breast carcinoma histopathology specimens. **Patients and Methods:** The group of 102 patients had a follow up median of 12 years, without lymph node spread and systemic treatments. Epithelial cells were stained by the AE1/AE3 pan-cytokeratin antibody cocktail. Optimization of the computational histopathology image analysis included 80 different combinations of binarization and particle selection filters. **Results:** The optimal prognostic performance was obtained by histopathology image binarization by the 250-threshold and circularity filter at 0.6-1.0 and object size at 20-infinity pixels. The count (AUC=0.82), total area (AUC=0.78), average size (AUC=0.68) and circularity (AUC=0.71) of the extracted epithelial patches associated with the increased metastasis risk. The majority of such prognostically relevant particles were identified as individual epithelial cells. **Conclusion:** This study thus points to small epithelial stromal spots sized from one to two cells as invasion markers with strong metastasis risk association in breast carcinoma.

Keywords: Breast cancer; prognosis; metastasis; image analysis; pan-cytokeratin; histopathology.

P14

Do mutations in *FANCM* gene predispose to colon cancer?

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Background: Current scientific publications indicate possible role of *FANCM* germline mutations in increased breast and ovarian cancer risk. There are no data if such mutations increase colorectal cancer (CRC) risk. **Material and Methods:** Our female proband, age 47, developed CRC in 45. Her family history is indicative of Lynch syndrome: her mother's brother developed larynx cancer in 74, CRC in 77, and prostate cancer in 84, while her mother's sister developed endometrial cancer in 65 and CRC in 69. DNA isolated from peripheral blood samples of all three family members was sequenced using TruSight Cancer Panel on Illumina Miseq system. **Results:** New *FANCM* class 4 gene mutation, c.5048_5052delAAAGA, p.Lys1683ArgfsTer3, was detected in our proband and her aunt. Her mother's mutation carrier status was confirmed by targeted mutation detection. In addition, MSH2 VUS, c.1597C>G (rs786202987), was detected in our proband only. No additional class 4 or 5 mutations were detected in remaining 94 genes. **Conclusion:** Detected *FANCM* mutation is not described in scientific literature. MSH2 VUS might be inherited from her father, suggesting its benign character. Two detected mutations cumulatively might increase cancer risk resulting in early onset of CRC in our proband. Incomplete penetrability of *FANCM* mutations explains its incomplete segregation with disease: the proband's mother is a healthy carrier, while proband's uncle is not a carrier but has developed three cancers. Late onset of these three cancers suggests that detected *FANCM* mutation may have no clinical importance. Further investigations of the clinical importance of detected *FANCM* mutation are needed.
Keywords: *FANCM* mutations, hereditary predisposition, colorectal cancer, Lynch syndrome

P15 - O

Genetic counselling unit- model and experience of University Hospital for Tumours in Croatia

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Genetic counselling unit exists as a separate functional unit in University hospital for tumours from the year 2015. It is based on cooperation and teamwork of experts of different profiles: medical geneticist, surgeons, oncologists, molecular geneticists, specialized nurses and psychologists. The aim is to recognize and counsel patients with family and personal history indicative of the hereditary cancer syndrome. Most of the patients are those suspected to have hereditary breast and ovarian cancer syndrome, although there are also patients with familial adenomatous polyposis, Lynch syndrome (hereditary non-polyposis colorectal cancer syndrome), neurofibromatosis and few other very rare hereditary cancer syndromes (e.g. von Hippel-Lindau disease, Li Fraumeni).

Clinical signs pointing to the possibility of pathogenic mutation in some of the predisposing genes (early age of onset, typical histology, multiple tumours...), along with confirmed family history are the base of selection. Patients with such disease characteristics are referred to a genetic counselling centre with the assessment of indication for further genetic testing.

Genetic testing of *hereditary breast and ovarian cancer syndrome* (*BRCA1* and *BRCA2* genes), as the most commonly identified hereditary cancer, is now available and covered by Croatian health Insurance Fund through the programme of genetic counselling in an authorized institution. The analysis is performed by next generation sequencing technology coupled with quantitative polymerase chain reaction (qPCR) for detection of large deletions and duplications. The positive result is confirmed by Sanger sequencing. Genetic testing is performed in a Laboratory for hereditary cancer and Laboratory for advanced genomics at Rudjer Boskovic Institute, Zagreb. Patient receives genetic counselling before and after genetic testing.

Primary and secondary prevention measures that could be undertaken after the results of hereditary breast cancer genetic testing in a high risk population contribute to savings in the health care system and the better quality of life in a group of high risk individuals.

Keywords: genetic counselling, genetic testing, hereditary cancer syndromes, Croatia

P16

Small nucleotide polymorphism Arg72Pro of the tumor suppressor *TP53* as a low-cost molecular predictor of chronic myeloid leukemia risk and resistance to imatinib

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Background: No specific molecular predictors of risk for chronic myeloid leukemia (CML) and patients' response to standard treatment with the tyrosine kinase inhibitor imatinib have been validated in the clinic so far. The aim of this study was to determine whether the small nucleotide polymorphism (SNP) Arg72Pro of the tumor suppressor *TP53* which reduces its pro-apoptotic potential might be used as a genetic predictor of CML risk and response to imatinib. **Material (Patients) and Methods:** An age and gender matched case-control study of 57 CML patients and 63 healthy volunteers was performed. Patients with clinically confirmed CML were treated with imatinib and complete molecular response was evaluated after three months. Genomic DNA was isolated from blood leukocytes and *TP53* genotyping performed by PCR-RFLP. Descriptive analyses included genotype and allelic frequencies; the odds ratio (OR) and 95% confidence interval (CI) were calculated as an estimate of relative risk ($p < 0.05$). **Results:** The *TP53* 72Pro allele was associated with CML in the dominant model (Arg/Pro+Pro/Pro vs. Arg/Arg) [$p = 0.04$; OR (95% CI) = 2.62 (1.11–6.22)], and with poor response to imatinib in the recessive model (Pro/Pro vs. Arg/Arg+Arg/Pro) [$p = 0.04$; OR (95% CI) = 4.92 (1.28–16.24)]. **Conclusions:** CML patients were statistically more often carriers of the *TP53* 72Pro allele which leads to the production of a protein with a reduced pro-apoptotic potential. The ProPro genotype is also associated with poor response to imatinib, thus analysis of this SNP might be proposed as a low-cost minimally invasive molecular screening and predictive tool in CML.

Keywords: chronic myeloid leukemia, small nucleotide polymorphism, *TP53*

P17 - O

Nischarin expression in melanoma

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Background: Melanoma is one of the most aggressive malignancies. If left untreated, patients with distant metastasis have a median survival of 6-9 months. Despite promising improvement in treatment with immunotherapy and targeted BRAF and MEK inhibitors, complete and durable responses are still not achievable. Therefore, novel targets and agents that target melanoma are of great interest. Nischarin (NISCH, IR1, IRAS) is a non-adrenergic imidazoline-1 receptor protein that has been described as a tumor suppressor in breast cancer and ovarian cancer. Its expression and biological role in melanoma have not been investigated to date. **Material and Methods:** We examined NISCH mRNA levels in normal skin, benign nevi, primary and metastatic melanoma samples in publicly available TCGA and GEO databases, and an independent cohort by qRT-PCR. To get better insight into the possible role of NISCH in melanoma, we performed Gene Set Enrichment Analysis (GSEA) using Broad Institute software. **Results:** NISCH expression decreased with melanoma progression. GSEA showed that NISCH expression positively correlated with genes involved in the regulation of migration, extracellular matrix remodeling and invasion. **Conclusion:** Contrary to the reports in breast cancer, nischarin may not have tumor suppressive role in melanoma. Our future efforts will be in the function of elucidation of NISCH role in melanoma progression.

Keywords: melanoma, nischarin

P18

miR-31-3p as a potential discriminatory biomarker between oral cancer and non-cancerous tissue: preliminary results

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Background: Approximately 20% of oral cancer patients develop recurrence after surgical removal of cancerous tissue. That indicates a need for novel biomarkers suitable to sensitively discriminate between oral cancer and non-cancerous tissue. Micro RNAs (miRNAs) have been

nominated as promising molecular biomarkers due to its stability in tissue. Results of a previously conducted meta-analysis shown that *miR-31-3p* is one of the most commonly up-regulated miRNAs in oral cancer. We aimed to validate findings from meta-analysis in an independent set of oral cancer clinical specimens. **Material and Methods:** Total RNA was isolated by miRVana kit from paired oral cancer and adjacent non-cancerous tissue from 12 patients. RNA was converted into cDNA by TaqMan microRNA Reverse Transcription kit. Relative gene expression, normalized to RNU6B, was quantified by TaqMan gene expression assay. Relative expression was reported as $2^{-\Delta Ct}$. **Results:** Relative expression of *miR-31-3p* was increased in oral cancer compared to adjacent non-cancerous tissue. However, the difference was not significant between analyzed tissues ($p=0.272$, Wilcoxon sign ranked test). Possibility of using *miR-31-3p* expression as a potential diagnostic test for discrimination of oral cancer and non-cancerous tissue is illustrated by area under the receiver operating characteristics curve (AUC=0.681, 95% confidence interval: 0.456-0.905, $p=0.133$). **Conclusion:** Preliminary results show that *miR-31-3p* is up-regulated in oral cancer, and has ability to discriminate between cancerous and non-cancerous tissues, but with no significance. Enlarging the study group will provide more reliable data on potential using of *miR-31-3p* as a discriminatory biomarker in oral cancer.

Keywords: oral cancer, miR-31-3p, expression

P19

Can cytokine profiling predict the onset of radiation toxicity?

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Background: Adjuvant radiotherapy (RT) reduces recurrences in operable stages of cervical cancer (CC) patients, but can be associated with adverse effects. State-of-the art RT techniques such as intensity-modulated radiotherapy (IMRT) have improved target dose coverage and reduced treatment toxicities. We investigated the relationship between cytokines profile and onset of radiation toxicities in patients with CC. **Material and Methods:** Forty-five CC patients UICC stage I-III underwent adjuvant external beam IMRT (40-45 Gy) combined with brachytherapy (18-24 Gy). Concomitant cisplatin chemotherapy (40 mg/m²) was administered to some patients according to pathohistological risk factors. Due to onset of severe treatment toxicities found in 2 patients that are supposed to be less common after IMRT, we examined gene expression levels of *IL-6*, *INF-γ* and *TGF-β1* in lymphocytes and concentration of IL-1β, IL-2, IL-6, INF-γ, TGF-β1 and also at 2 patients without toxicity and at 2 healthy volunteers. **Results:** The highest expression

level of *TGF-β1* gene in lymphocytes was found in patient who developed severe acute toxicity, while the lowest expression level was observed in patient without acute toxicity. The highest expression levels of *INF-γ* gene and *IL-6* gene were found in healthy volunteers. Expression level of *IL-6* gene was the lowest in patient without acute toxicity. In patient with severe acute toxicity the lowest expression level of *INF-γ* gene and the highest serum concentration of IL-6 were measured. The highest serum concentration of INF-γ and TGF-β1 were detected in patients who didn't develop toxicity. **Conclusion:** In present study overexpression of *TGF-β1* gene and high serum concentration of IL-6 might be associated with onset of acute radiation toxicity. Our preliminary findings suggest the need for further study of the impact and significance of cytokines profile on larger number of patients.

Keywords: Cervical Cancer, Cytokines, Radiotherapy, Toxicity

P20

Analysis of prothrombin expression at the RNA and protein level in tumor cell lines

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Background: Although a close relationship between thrombotic disorders and cancer is recognized for more than a century, its underlying mechanism has not yet been resolved. Current literature data show that thrombin plays a role in cancer, stimulating proliferation, migration and invasion of malignant cells, angiogenesis and metastasis. However, data regarding expression of thrombin precursor-prothrombin in cancer are lacking. The aim of this study was to determine whether several different cancer cell lines express prothrombin at RNA and protein level.

Material and Methods: The experiments have been performed on the following permanent cancer cell lines, representing some of the most common malignancies: Caco-2 (colorectal adenocarcinoma); MCF-7 and SK-BR-3 (breast adenocarcinoma); U-87 and U-251 (glioblastoma). Since prothrombin is expressed in hepatocytes, HepG2 cell line (hepatocellular carcinoma) was used as a control. Qualitative (by PCR on cDNA as a template) and subsequent quantitative analyzes (by qPCR) of prothrombin gene expression were performed. For detection of prothrombin at protein level, Western blot and ELISA methods were used. **Results:** Prothrombin cDNA was detected only in Caco-2 cell line with 5 times lower prothrombin expression in comparison to HepG2 cell line. At the protein level, prothrombin was detected also only in Caco-2 cell line, in 8.5 times lower amount in the comparison to HepG2 cells. **Conclusion:** Results of this study provide a background for further research of (pro)thrombin role in colorectal adenocarcinoma.

Keywords: cancer, expression, prothrombin

P21

Asymmetrically substituted bis-(thiocarbohydrazones) bearing *N*-heterocyclic moiety are strong apoptotic inducers in LoVo cell line

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Background: As a continuation of our efforts to develop novel anticancer agents based on thiocarbohydrazone (TCH) moiety we report the activity of 14 novel, asymmetrically substituted bis-TCHs. **Material and methods:** Anticancer activity has been evaluated on human cell lines of colorectal (LoVo), ovary (SkOV-3), non-small cell lung (A549), and mammary (MCF-7) adenocarcinoma, while toxicity was assessed on human keratinocytes (HaCaT) by means of Calcein AM/propidium iodide (PI) staining. The type of induced cell death (Annexin V/PI staining), cell cycle changes, activation of caspase-8/-9, generation of mitochondrial superoxide radicals, and anti-migratory activity (wound healing assay) were assessed for the most potent compounds. **Results:** Calcein AM/PI staining revealed that 6 of 14 compounds at 100 μ M stimulated death in more than 50 % of LoVo cells after 24 h, while 4 of them (compounds 1-4) were equally effective at lower concentrations. Apoptosis was the dominant type of cancer cell death after treatment with 2-4, while 1 was excluded because of notable percentage of necrosis. While all three compounds challenged accumulation of LoVo cells at the G1-to-S transition point of mitotic division, 2 and 4 exclusively activated caspase-8 whereas 3 triggered independent activation of both caspase-8 and 9. None of compounds displayed as powerful generator of mitochondrial superoxide radicals. Only compound 3 significantly inhibited the cellular mobility, while all four compounds caused lower incidence of cell death in HaCaT than LoVo cells. **Conclusion:** Current results highlight the need for further investigation and development of asymmetrically substituted TCHs as promising hit molecules for the treatment of colorectal carcinoma. **Keywords:** Anticancer Agents, Apoptosis, Bis-thiocarbohydrazones, Colorectal Carcinoma

P22

Antiproliferative activity of Sloe's extracts from Bosnia and Herzegovina against prostate cancer cells (DU145 and PC3)

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Background: The sloe (*Prunus spinosa L.*) traditional medicinal plant of Central and Eastern Europe indicated for the treatment of urinary tract disorders, inflammation, and in the treatment of various disorders. Also, it is known that for prostate cancer, there is no effective treatment for advanced stages of the disease. There is increasing evidence that nutritional agents such as *Prunus spinosa L.* may play a role in chemoprevention and treatment of prostate cancer. We in vitro investigated the anti-proliferative potential of sloe's extracts towards prostate cancer cells.

Material and methods: Anti-proliferative activity of eighteen *Prunus spinosa L.* extracts from three different areas from Bosnia and Herzegovina (Trnovo, Borije, Vareš) was investigated in vitro against DU145 and PC3 cells. Flowers, leaves and fruits were fractionated using ethanol by two extraction methods: microwave-assisted extraction (MAE) and ultrasound assisted maceration (UAM). The anti-proliferative activity was measured by MTT assay. **Results:** The obtained results indicate that tested extracts show dose dependent cytotoxic activity against tested cancer cells in vitro. Ethanol extracts of sloe's leaves were the most active fractions against analyzed cell lines. The IC₅₀ values ranged from 220,00±0,63 µg/mL to 409,46±78,76 µg/mL for PC3 cells, and from 216,91±28,16 µg/mL to 444,58±9,36 µg/mL depending on area and type of extraction methods. The best result of anti-proliferative activity have been obtained for leaves extracts from area Borije. **Conclusion:** Comparing values of IC₅₀ for all tested ethanol extracts samples obtained by MAE show a slightly better anti-proliferative effect than samples obtained by UAM. **Keywords:** Medicinal plant, Ethanol extracts, Phytosterols Anti-proliferative, Prostate.

P23 - O

SMAD4 transcript analysis in human permanent cell lines

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SMAD4 is a key mediator of TGFβ signal transduction pathway and acts as a tumor suppressor in most human tissues. Two human *SMAD4* transcripts coding for full-length protein have been identified: ENST00000342988.7- SMAD4-201 and ENST00000398417.6- SMAD4-202. They

differ in their 5'UTR regions and originate from different promoters. Aberrant usage of genes' alternative promoters by a malignant cell is a known phenomenon, which can be exploited for biomarker development. The aim of this study was to investigate the activity pattern and genetic structure of *SMAD4* transcripts in human permanent cell lines and evaluate their potential usability as biomarkers for malignant diseases. Eight cancer cell lines originating from colon, lung, liver, bones, brain, breast and cervix were used in this study, as well as human healthy lung tissue samples from biopsies as controls. Analysis of genetic variants in genomic promoter regions was performed by polymerase chain reaction (PCR) and sequencing. In order to analyze the amount of *SMAD4* transcripts, total RNA was isolated and used in quantitative polymerase chain reaction (qPCR). Sequencing analysis revealed no new genetic alterations: in *SMAD4*-201 all nucleotides were wild-type, while in *SMAD4*-202 already known variant rs67325153 was detected. In healthy lung tissue, *SMAD*-201 was present with up to 20% and *SMAD*-202 with up to 80% of total *SMAD4* transcripts. In cancer cell lines, higher amount (up to 70%) of *SMAD*-201 was detected. Transcript *SMAD*-201 was overrepresented in cancer cell lines in comparison with healthy tissue, which makes it a biomarker candidate. Future studies should include clinical samples to confirm this finding.

Keywords: cancer biomarker, *SMAD4*, transcription

P24

Cytotoxic and cytoselective profile of novel ruthenium(II)-arene complexes with (fluoro substituted) picolinic acid

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Background: Ruthenium containing compounds represent the most promising alternative to platinum-based chemotherapeutics whose therapeutic value has been limited by significant side effects. Advantageous features such as good aqueous solubility and relatively inert arene ligand make them very attractive for structural optimizations aimed for improved in vivo potency. **Material and Methods:** Reported complexes were obtained in a reaction of $[\text{Ru}(\eta^6\text{-benzene})\text{Cl}(\mu\text{-Cl})_2]$ or $[\text{Ru}(\eta^6\text{-toluene})\text{Cl}(\mu\text{-Cl})_2]$ with picolinic acid or 6-fluoropicolinic acid in a 1:2 molar ratio in ethanol and characterized by IR and NMR spectroscopy and MS spectrometry. **Results:** The cytotoxic profile was investigated by the colorimetric MTT assay, in a panel of human non-malignant cell line (MRC-5), and cancer cell lines (A549, HTB177, PC3, A375, HeLa, HCT116, MDA-MB-453). The complexes carrying picolinic acid, displayed moderate antiproliferative effect particularly toward colorectal carcinoma (HCT116) and cervix adenocarcinoma cells (HeLa).

The highest activity and cytoselectivity was observed for complex with $[\text{Ru}(\eta^6\text{-benzene})\text{Cl}(\mu\text{-Cl})_2]$ toward HCT116 cells: it was capable of reducing viability of HCT116 cells 1.5 times more efficiently ($\text{IC}_{50} = 27.5 \mu\text{M}$), than of the MRC-5 cells ($\text{IC}_{50} = 41.3 \mu\text{M}$). **Conclusion:** The complex with improved activity and selectivity is candidate for further investigations regarding its binding modes, sites, and affinities.

Keywords: Cytoselectivity, Antiproliferative effect, MTT test, Organoruthenium complexes

P25 - O

Prospects for the use of NOS inhibitors in radiation therapy

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Background: The need and feasibility of radiotherapy in oncology does not cause doubts. However, the improvement of radiological technology does not significantly affect the number and severity of complications - which are very torpid and difficult to treat. A new approach to prevent or mitigate these side effects of radiotherapy is to use low-toxic and well-tolerated radioprotectors capable to selectively protect normal tissues surrounding the tumor. We have synthesized and studied a range of compounds – selective inhibitors of inducible and endothelial nitric oxide synthase (NOS), N,S-substituted isothioureas, with hypoxic radioprotector properties in non-toxic doses. This paper presents the prospectivity of two of them (compounds named T1004 and T1023) for the prevention of radiotherapy complications. **Methods:** Studies were carried out on the model of radiation therapy of transplantable sarcoma M-1 in Wistar rats (KYO). INOS1 and INOS2 were administered in the optimum radioprotective dose (1/4 LD16) once before single (32 or 36 Gy) or fractionated (two doses of 20 Gy) local γ -irradiation of the tumor. The dynamics of tumor growth were evaluated with an interval of 2 days. The degree of radiation damages of the skin was evaluated by RTOG/EORTC-95 classification. **Results:** Although T1004 and T1023 are quite effective radioprotectors (DRF - 1.4-1.8), these compounds did not alter the growth and radiosensitivity of sarcoma, and didn't modified antitumor effects of γ -radiation. At the same time, both compounds statistically significantly limited the severity of skin damages in all modes of irradiation. These compounds did not affect the development of inflammatory and regenerative processes, but significantly reduced the post-radiational alterations of the deep layers of the skin and underlying tissues - thereby, manifested itself as preventive radioprotectors, causing short-term reduction in radiosensitivity of non-malignant tissues. **Conclusions:** The results present that NOS-inhibitors can become the basis for new effective means of prevention of radiotherapy complications in oncology.

Keywords: Radiation therapy, complications, prevention, NOS inhibitor

P26

Expression of PPAR γ in pancreatic ductal adenocarcinoma may have opposing roles in early and late stages of the disease

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Background: Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal forms of cancer with a 5-year survival rate of 6 percent. PDAC is highly resistant to conventional chemotherapy and development of new treatments is urgently needed. Being at the crossroads of obesity and diabetes – known risk factors for PDAC – PPAR γ is considered to be an important target for drug repurposing. Regrettably, there are opposite reports on the function of PPAR γ in preclinical models and on its expression and prediction of prognosis in patients. The aim of this study was to settle the contradictory findings regarding the PPAR γ role in the progression of PDAC. **Materials and Methods:** *In silico* and GSE analyses were performed to examine mRNA expression level and PPAR γ activation status in patient tumor samples. Expression was confirmed by immunohistochemistry in tumor samples and PDAC cell lines. ShRNA technology, Seahorse XF analysis, qRT-PCR array and western blotting were used for validation of the role of PPAR γ in cell phenotype. **Results and conclusions:** We found that in the early stages of the PDAC progression PPAR γ is over-expressed and active thus enabling higher energy consumption and proliferation. Later in the disease PPAR γ expression decreases conferring mesenchymal phenotype and supporting metastatic spread. *In vitro*, we confirmed that down-regulation of PPAR γ in PDAC cells induces epithelial-to-mesenchymal transition (EMT) and promotes autophagy, two processes necessary for metastatic cell spread and survival. Our findings suggest that PPAR γ agonists are not good candidates for repurposing in PDAC without detailed stratification of patients. **Keywords:** autophagy, EMT, PDAC, PPAR γ

P27

***In Vitro* Anti-Migration and Anti-Angiogenic Effects of *Fucus spiralis* Seaweed**

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Background: Cancer cell migration and tumor angiogenesis enhance tumor aggressiveness. It is important to find novel agents that can inhibit cancer migration and angiogenesis. Seaweeds from the *Fucus* genus are known for their anticancer potential. The aim of this study was to examine anti-migration and anti-angiogenic activities *in vitro* of extract and fractions of *F. spiralis*. **Material and Methods:** *Fucus spiralis* Linnaeus was collected from the Atlantic coast of Morocco. Dichloromethane-methanol extract, petrol-ether, ethyl-acetate and n-butanol fractions were made. They were tested for their anti-migration and anti-angiogenic properties (subtoxic IC₂₀ concentrations) against human endothelium-derived permanent EA.hy926 cell line using scratch and tube formation assays. **Results:** The ethyl-acetate fraction showed the best anti-migratory activity (percentage of gap reduction was 2.34±2.11% after 24h and 6.35±1.49% after 48h), followed by the dichloromethane-methanol extract (6.85±0.64% after 24h; 25.14±6.92% after 48h), and petroleum-ether fraction (8.00±5.07% after 24h; 44.18±5.03 after 48h), while n-butanol fraction exerted the poorest effect (46.20±3.56% after 24h; 65.39±1.28 after 48h); the percentage of gap reduction in control cells was 57.60±1.83 after 24h and 100.00±0.00 after 48h. *In vitro* anti-angiogenic assay showed that the *F. spiralis* whole extract, petroleum-ether, and ethyl-acetate fractions inhibited elongation and connection of endothelial EA.hy926 cells and prevented their organization into tubular structures. Control cells and cells treated with n-butanol fraction showed formation of large vessel structures and complex meshes. **Conclusion:** Our research showed anti-migration and anti-angiogenic activities of extract and fractions. Ethyl-acetate fraction compared to the whole extract exerted better anti-migration activity. However further analysis is needed.

Keywords: angiogenesis, anti-migration, cancer cells, *in vitro*, *Fucus spiralis*, seaweed

P28

Heteromerization of adenosine A2A and dopamine D2 G protein-coupled receptors in human lung cancer cells *in vitro*

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Background: Dopamine and adenosine G-protein coupled receptors (GPCRs) have been recognized as new potential pharmacological targets in lung cancer (LC). GPCR heteromerization leads to the formation of new pharmacological entities and has not been studied in detail in LC. The aim of this project was to determine the existence and the functional properties of the A2aR-D2R heteromer in human LC cell line NCI-H460, and its multidrug resistant counterpart NCI-H460/R. **Material and Methods:** The resistant NCI-H460/R cell line was derived from NCI-H460 cells by doxorubicin treatment with gradually increasing concentrations. The existence of A2aR-D2R heteromers was investigated using Proximity Ligation Assay (PLA), and the functional cross-talk studying the MAPK pathway, Dynamic Mass Redistribution (DMR) and the production of cAMP. **Results:** The existence of the A2aR-D2R heteromers was confirmed in both cell lines, with 80% and 85% of cells containing heteromers, and a ratio of 13 and 16 heteromers per heteromer-containing cell, respectively. A functional negative cross-talk and cross-antagonism was detected in both cell lines (MAPK and DMR results), but the study of the production of cAMP showed the existence of a functional negative cross-talk in both cell lines and cross-antagonism only in the NCI-H460 cell line (cAMP results). **Conclusions:** There are functional differences between the A2aR-D2R heteromer in sensitive and multidrug resistant LC cells. Ongoing characterization of this heteromer in patient samples might contribute to the elucidation of resistance mechanisms that occur during chemotherapy and the development of new GPCR-targeted therapies.
Keywords: adenosine, dopamine, drug resistance, GPCR, lung cancer.

P29 - O

c-Src inhibitors pyrozolo[3,4-d]pyrimidines, Si306 and pro-Si306, evade multidrug resistant phenotype and suppress invasion in glioblastoma

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Background: Glioblastoma multiforme (GBM) are the most frequent and aggressive (WHO grade IV) brain tumors in adults. GBM have high expression of c-Src tyrosine kinase involved in survival, migration and invasiveness of tumor cells. Thus, c-Src emerged as a potential target for GBM therapy. **Materials and methods:** Antiproliferative effect of c-Src inhibitors pyrozolo[3,4-d]pyrimidines, Si306 and its prodrug pro-Si306, was assessed in human GBM cell line U87, multidrug resistant (MDR) U87-TxR, and primary GBM cells by MTT assay. Anti-migratory and anti-invasive effects of c-Src inhibitors were evaluated by gelatin degradation and transwell invasion assays. Their effect on c-Src, extracellular signal-related kinase (ERK), and focal adhesion kinase (FAK) expression was analyzed by western-blot and flow-cytometry. Zebrafish model was used to evaluate anti-invasive potential of pro-Si306 in U87 xenografts *in vivo*. **Results and conclusions:** c-Src inhibitors were more efficient in cell growth inhibition compared to dasatinib, a well-known tyrosine kinase inhibitor. The potency of Si306 and pro-Si306 was not affected by the MDR phenotype. Migratory potential of U87, U87-TxR, and primary GBM cells was significantly decreased by both inhibitors. Si306 and pro-Si306 also compromised cells' ability to degrade the matrix and invade through basement membrane. Both compounds reduced phosphorylation of c-Src, and its downstream signaling components, ERK and FAK, in GBM cell lines. *In vivo*, pro-Si306 showed anti-invasive effect against U87 xenografts in zebrafish model. Considering their ability to suppress migration and invasion and overcome MDR, Si306 and pro-Si306 could be considered in GBM treatment alone or in combination with other chemotherapeutics.

Keywords: glioblastoma, multidrug resistance, primary cells, invasion, migration

P30

Biological activity of new ruthenium(II)-arene complexes coordinated to the structural analogs of PARP inhibitor 3-aminobenzamide in HCC1937 human breast cancer cells

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Background: Search for the new effective antitumor therapeutics lead to the DNA damage repair proteins, PARP (poly (ADP-ribose) polymerase), as promising targets for drug optimization. Combining the metal-based drug and PARP inhibitor could increase their antitumor efficacy in synthetic lethality approach. Here we report *in vitro* biological activity of four new ruthenium(II)-arene complexes (C1-C4) coordinated to the structural analogs of PARP inhibitor 3-aminobenzamide (3-AB). **Material and methods:** Colorimetric PARP inhibition assay was conducted to investigate potential of C1-C4 for PARP inhibition. Cytotoxic activity of C1-C4 was evaluated on five different types of human breast cancer cell lines, by MTT assay. Analyses of impact of C1-C4 treatment on HCC1937 cell cycle was performed by flow cytometry. Influence of C1-C4 treatment on the tertiary structure of plasmid DNA was investigated by agarose gel electrophoresis. **Results:** C1, C2 and C4 exhibited IC₅₀ values for PARP inhibition in the line or better than the referent compound 3-AB. MTT assay disclosed modest cytotoxicity of C1-C4, with IC₅₀ values varying from 147-581 μM. While MDA-MB-453 and MDA-MB-361 cells displayed low sensitivity to C1-C4 treatment, HCC1937, MDA-MB-231 and MCF-7 cells showed higher and similar sensitivity pattern toward all four complexes, with C1 being the most active in HCC1937 cells. Flow cytometry results showed moderate cell cycle alterations in HCC1937 cells, most noticeable under the C1 treatment, and characterized by slight increase in S, G2-M and Sub-G1 region. C1-C4 caused various concentration-dependent changes in the plasmid DNA mobility, possibly by forming DNA adducts. **Conclusion:** These results provide the basis for further biological studies and optimization of this type of combo-molecules for potential application as antitumor agents.

Keywords: antitumor agents, HCC1937, PARP inhibitor, ruthenium(II)-arene complexes

P31

Pathohistological changes in experimental fibrosarcoma tumors of hamsters treated with established non-oncologic anti folate drugs

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Background: The anticancer effects of metformin, caffeine, itraconazole and nitroglycerin which have anti folate properties were investigated. The immunohistochemistry of experimental fibrosarcoma tumors was investigated in hamsters treated with metformin, caffeine, itraconazole and and nitroglycerin. **Material and Methods:** The hamsters were injected with BHK-21/C13 cells in order to induce fibrosarcoma, and the animals were treated daily with metformin, caffeine, itraconazole, nitroglycerin or the combination of the two drugs. Subsequently, blood samples were obtained for biochemical analyses and the tumors were excised. The tumor samples were pathohistologically and immunohistochemically assessed for proliferation marker protein Ki-67, hematopoietic progenitor cell antigen CD34, cytochrome c oxidase subunit 4 (COX4), glucose transporter 1 (GLUT1) and inducible nitric oxide synthase (iNOS), and vital organs were toxicologically tested. Ki-67-positivity and cytoplasmic marker (CD34, COX4, GLUT1, iNOS) immunoexpression in the tumor samples were quantified. **Results:** The results revealed that the combination of metformin with caffeine, metformin with itraconazole and metformin with nitroglycerin significantly altered the pathohistological characteristics of the hamster fibrosarcoma tumors, including Ki-67-positivity and the immunoexpression of cytoplasmic markers, without indications of toxicity. **Conclusion:** In conclusion, the administration of metformin in combination with caffeine, itraconazole or nitroglycerin may inhibit fibrosarcoma proliferation in vivo, suggesting that this may be an effective and safe approach as a nontoxic anticancer adjuvant and relapse prevention therapy.

Keywords: hamsters, fibrosarcoma, metformin, caffeine, itraconazole, nitroglycerin

P32

Physicochemical changes in experimental fibrosarcoma tumors of hamsters treated with established non-oncologic anti folate drugs

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Background: The anticancer effects of metformin, caffeine, itraconazole and nitroglycerin, which are established non-oncologic drugs with anti folate effects, were investigated in the present study. The weight, diameter, volume, density, surface and surface to volume ratio of experimental fibrosarcoma tumors were investigated in hamsters treated with metformin, caffeine, itraconazole and nitroglycerin. **Material and Methods:** The hamsters were injected with BHK-21/C13 cells in order to induce fibrosarcoma, and the animals were treated daily with metformin, caffeine, itraconazole, nitroglycerin or the combination of the two drugs. Subsequently, blood samples were obtained for biochemical analyses and the tumors were excised, weighed and measured. Vital organs were toxicologically tested. **Results:** The results revealed that the combination of metformin with caffeine, metformin with itraconazole and metformin with nitroglycerin and nitroglycerin significantly altered the physicochemical characteristics of the hamster fibrosarcoma tumors, including absolute and relative weight, volume, density, length, surface area and surface to volume ratio, without indications of toxicity. **Conclusion:** In conclusion, the administration of metformin in combination with caffeine, itraconazole or nitroglycerin may inhibit the growth of fibrosarcoma tumors in vivo, suggesting that this may be an effective and safe approach as a nontoxic anticancer adjuvant and relapse prevention therapy.

Keywords: hamsters, fibrosarcoma, metformin, caffeine, itraconazole, nitroglycerin

P33

Expression analysis of *SMAD7* in human colorectal cancer

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Background: *SMAD7* gene encodes an intracellular antagonist of TGF- β signaling which is involved in malignant progression, invasiveness and metastatic dissemination of cancer. Since it has been reported that disturbed *SMAD7* expression affects the progression of colorectal cancer, the aim of this study was to investigate expression of *SMAD7* gene in tumor tissue, treatment response and normal mucosa. **Material and Methods:** The expression of *SMAD7* gene was analyzed in eleven samples obtained from colorectal cancer patients, as well as in colon cancer cell lines Caco-2, HT-29 and SW620. Additionally, metastatic SW620 cells were treated with chemotherapeutics 5-FU, oxaliplatin, irinotecan and their combinations. Relative quantification of gene expression was performed using quantitative real-time PCR method (qRT-PCR). The expression levels of *SMAD7* were normalized to housekeeping gene *GAPDH*, and compared with healthy mucosal tissue samples as control. **Results:** Decreased expression of *SMAD7* gene was observed in all tumor tissue samples and cell lines. The lowest expression was recorded in Caco-2 cells. In SW620 cells treated with chemotherapeutic drugs elevated expression was observed when compared with both healthy tissue and non-treated SW620 cells, with the most prominent increase of *SMAD7* gene expression observed in the treatment with oxaliplatin. **Conclusion:** Observed downregulation of *SMAD7* gene in tumor tissue and its upregulation in response to chemotherapeutic drugs indicate its potential as suitable target for therapeutic modulation. Expression pattern of *SMAD7* should be further investigated in a larger cohort of patients. **Keywords:** Colorectal Cancer, Cancer Chemotherapy Drugs, Gene Expression Profiling, *SMAD7*

P34

Mechanism of action assessment of highly cytotoxic organotin(IV) complexes with 2-(5-arylidene-2,4-dioxothiazolidin-3-yl)propanoic acid derivatives

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Background: Although cisplatin is a widely used anticancer drug, its application is limited due to intrinsic and/or acquired resistance and many of side effects. Numerous organotin(IV), complexes, in particular carboxylate derivatives, have shown outstanding antiproliferative action.

Material and Methods: The mechanism of action of two novel triphenyltin(IV) compounds, with 2-(5-arylidene-2,4-dioxothiazolidin-3-yl)propanoic acid derivatives, **Ph₃SnL1** and **Ph₃SnL2**, was investigated through the morphology of PC3 cell death (AO and DAPI staining) and cell cycle analysis as well as metal uptake in PC3 cells by ICP-OES analysis. **Results:** The obtained results suggest that both tested complexes induced morphological changes in treated cells in terms of nuclear condensation and cell structure loss indicating apoptosis as the mode of cell death. Additionally, triphenyltin(IV) complexes inhibited PC3 cell cycle in mitosis. Drug accumulation studies of prepared compounds in PC3 cells showed that the total tin uptake is 3 fold lower than that of platinum, indicating that the mechanism of action of tested organotin(IV) compounds may be different in comparison to cisplatin. **Conclusion:** All of these findings are leading to a conclusion that both newly synthesized organotin(IV) compounds may be considered as good candidates for some further *in vitro* and/or *in vivo* investigations.

Keywords: Apoptosis, Cell cycle, Metal uptake, Organotin(IV) complexes

P35 - O

Repurposing of pantoprazole as anticancer drug through induction of apoptosis, modulation of autophagy and enhancement of the effect of vincristine in cancer cells

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Background: Cancer cells show an increased number and activity of proton pumps, leading to acidic tumor microenvironment responsible for higher tumor invasiveness and resistance, especially to pH dependent drugs such as vinca alkaloids. Therefore, proton pump inhibitors, including pantoprazole commonly used to reduce gastric acidity, are being tested as possible anticancer drugs alone or in combination with cytostatics. Autophagy, a process of controlled self-digestion, can be enhanced and express cytoprotective effect in tumors, hence also represents a novel target of anticancer therapy. Considering the above, we investigated the effects of pantoprazole alone on survival, apoptosis and autophagy induction in mouse melanoma (B16) and human glioma (U251) cells, and in combination with vincristine on survival of B16 and non-small-cell lung cancer cells (H460). **Material and Methods:** Cell viability was examined using MTT and crystal violet tests. Cell cycle analysis, reactive oxygen production (ROS), caspase activation, and cell death type were determined by flow cytometry. Induction of autophagy was investigated by immunoblot analysis of autophagic flux. Role of autophagy in cell death was determined using pharmacological inhibitors of this process. **Results and conclusion:** Pantoprazole caused dose and time-dependent decrease in survival of B16 and U251 cells, increased DNA fragmentation, ROS production and caspase activation, finally causing apoptotic cell death, and induced cytoprotective autophagy. In B16 and H460 cells pantoprazole enhanced the killing effect of vincristine. These data suggests anticancer properties of pantoprazole alone and in combination, but because the induction of cytoprotective autophagy, additional studies are needed to improve this therapeutic approach.

Keywords: apoptosis, autophagy, pantoprazole

P36

Anti-cancer Drug Discovery Based on Marine Natural Products

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Background: Many marine natural products possess outstanding anti-proliferative properties to cell growth, making them valuable molecular probes for the investigation of biochemical pathways and promising lead compounds for the development of new antitumor chemotherapeutic agents. Unfortunately, the amounts of these compounds isolated is usually quite low, and further biological evaluation, including elucidation of the mechanism of action, is often precluded. Total synthesis of marine natural products plays a critical role in structure verification and also provides solutions to the supply problem. **Materials & Methods:** All the biologically active marine natural products were prepared by total chemical synthesis. Anti-cancer drug discovery includes selection of the marine natural products and usage of various computer aided drug design and medicinal chemistry tools to develop novel potential drug candidates for cancer treatment. **Results and Conclusion:** We have completed the total synthesis of 56 marine natural products including some most potent lead compounds. The presentation will focus on total synthesis of representative marine natural products with low nanomolar potency, and the development of anticancer agents based on the core structure of the natural products as well as biological evaluation of synthetic derivatives.

Keywords: Anti-Cancer drugs, Medicinal Chemistry, Natural Products

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