International Conference

Translational Research in Oncology in New Member State Economies

21-22 MAY 2015

www.**tron**.wum.edu.pl

BASTION

CONTENT

CONTENT

TRON | 21-22.05.2015

CONTENT

BASTION abstracts:

CONTENT

TRON | 21-22.05.2015

BASTION is a multidisciplinary science project co-financed by the European Commission. The objective of the project is **to build up the research potential of the Medical University of Warsaw** (MUW) in experimental oncology, and also to reduce the time from scientific discovery to clinical application.

The research is focused in particular on **personalized oncology** and development of diagnostic and therapeutic methods customized to patients' individual needs. **The project involves eleven research teams of MUW, represented by more than 100 researchers in oncology, cooperating with the university hospitals that also provide training for future medical doctors as well as other research institutes within the Ochota Campus.**

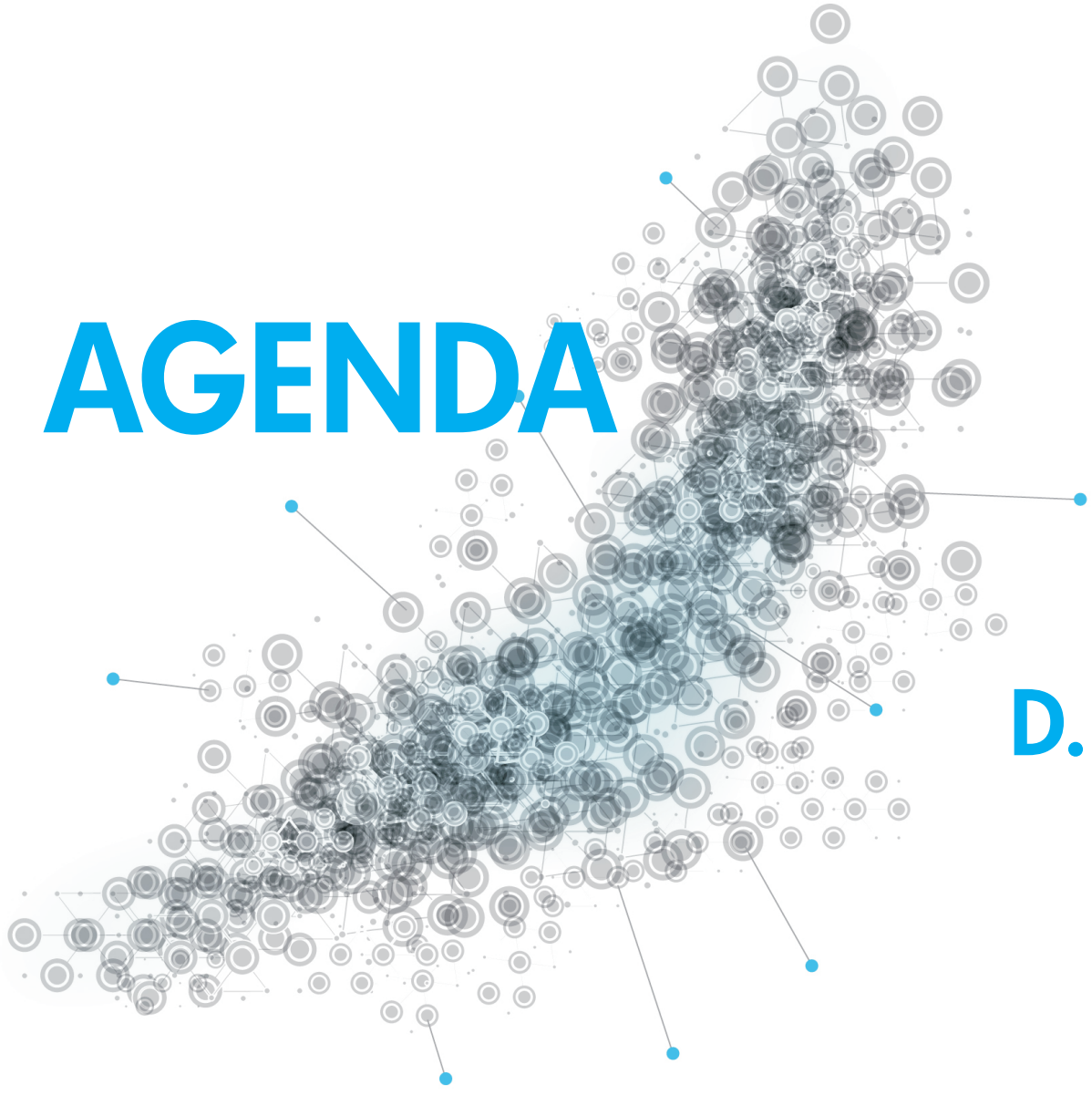
The project aims at technology transfer, acquiring knowledge and establishing closer or new research-oriented cooperation with 11 science centers and two companies operating in the commercialization of science research. Their research efforts will be supported by institutes and companies from eight EU countries as partner organizations. The tasks identified in the project programme will not only strengthen the research potential of the MUW, but they will also help to improve research project management.

BASTION aims at developing the innovation capacity and promoting cooperation with the EU leading scientific centers, and achieving integration with the European Research Area by improving the quality of scientific research.

Project funded by the Ministry of Science and Higher Education and the European Commission under the 7th Framework Programme.

Implementation period: September 2012 – February 2016
Total cost: € 5,309,400
EC funding: € 4,449,500
Ministry funding: PLN 1,885,042
Instrument: Coordination and Support Action

FP7-REGPOT-2012-CT2012-316254-BASTION



AGENDA

D.1-2

AGENDA

DAY 1 | 21.05.2015

7:30	Registration/coffee/breakfast
8:45	Introduction: Prof. Jakub Gołąb

Immunooncology

Chair: Prof. Jakub Gołąb/Dr. Radosław Zagożdżon

9:00	Dr Takanori Kitamura CCL2-induced chemokine cascade promotes breast cancer metastasis by enhancing retention of metastasis-associated macrophages
9:30	Dr Sven Brandau Neutrophils and myeloid-derived suppressor cells in head and neck cancer
10:00	Dr Munitta Muthana The Trojan horse cancer treatment
10:30	Dr Seth Coffelt Cancer-associated inflammation in metastasis and response to chemotherapy
11:00	Discussion
11:15-11:35	Coffee break

Genomics

Chair: Prof. Rafał Płoski/ Dr. Dominika Nowis

11:35	Prof. Lars Bullinger Precision medicine in AML - fact or fiction?
12:05	Dr Roderick Beijersbergen A Shortcut to Accelerate Personalized Cancer Therapy?
12:35	Dr David Laszlo Tarnoki Epigenetic twin studies in cancer
13:05	Dr Adam Domonkos Tarnoki Cancer genetics: findings of twin studies
13:35	Discussion
13:50-14:15	Lunch break

Hematooncology

Chair: Prof. Przemysław Juszczyński/Dr. Tomasz Stokłosa

14:15	Prof. Jacques Nunes Dok proteins in leukocyte signaling
14:45	Dr Josée Golay Cell-mediated activities of first and new generation anti-CD20 antibodies
15:15	Dr Charles Dumontet Therapeutic monoclonal antibodies in hematological malignancies
15:45	Dr Frank Beurskens Enhanced IgG hexamerization mediates efficient C1q docking and more rapid and substantial complement-dependent cytotoxicity (CDC); preclinical proof of concept
16:15	Prof. Tadeusz Robak Cladribine in the treatment of hematologic malignancies - contribution of the Polish investigators
16:45-18:00	Coffee/ Cocktail party
18:00	Jagodziński Trio "Chopin les Brillantes" – Chopin interpreted by Jazz band

DAY 2 | 22.05.2015

8:00	Registration/coffee/breakfast
9:00	Introduction: Prof. Sławomir Majewski
	Chair: Prof. Sławomir Majewski/Richard Hudson

Translational research – International case studies

9:10	Prof. William Gallagher Bridging the Gaps in Translational Cancer Research: An Irish Perspective
9:30	Tim Kievits Personalised medicine in practice in The Netherlands, a SME's view
9:50	Magda Chlebus Public private collaboration to optimise personalised medicine research, development, and pathways to patients: the example of the Innovative Medicines Initiative 2
10:10	Round Table discussion
10:30-11:00	Coffee break

Incentives for translation – evaluation, IP and enterprise

11:00	Marcin Kapczyński Using Web of Science and InCites for research evaluation, strategic planning and research monitoring
11:20	Dr Ali Gure Technology transfer – comparison of American and Turkish reality and scientific issues in intellectual property rights
11:40	Prof. Bruno Botta From bench to Spin-off: How to reach results able to be patented
12:00	Dr Agnieszka Turowska Spin-off in practice – German experience from Polish perspective
12:20	Round Table discussion
12:40-13:20	Lunch

Getting the money

13:20	Danuta Mossakowska A new approach to industry academic collaborations: Discovery Partnerships with Academia
13:40	Prof. Michał Karoński A role of the National Science Centre in financial support of research
14:00	Ioana Ispas Setting up health research as national priority for Romania under the New Research Development and Innovation Strategy for 2014-2020, implications and consequences
14:20	Round Table discussion
14:50	Closing remarks

ABSTRACTS

invited speakers

A.1-22

CCL2-induced chemokine cascade promotes breast cancer metastasis by enhancing retention of metastasis-associated macrophages

Takanori Kitamura,[1] and Jeffrey W. Pollard [1], [2]

1)MRC Centre for Reproductive Health, Queen's Medical Research Institute, The University of Edinburgh, Edinburgh, UK

2) Department of Developmental and Molecular Biology, Center for the Study of Reproductive Biology and Women's Health, Albert Einstein College of Medicine, New York, USA

Pulmonary metastasis of breast cancer cells is promoted by a distinct population of macrophages, metastasis-associated macrophages (MAMs), which originate from inflammatory monocytes (IMs) recruited by the CC-chemokine ligand 2 (CCL2). We have recently demonstrated that, through activation of the CCL2 receptor CCR2, the recruited MAMs secrete another chemokine ligand CCL3. Genetic deletion of CCL3 or its receptor CCR1 in macrophages reduces the number of lung metastasis foci, as well as the number of MAMs accumulated in tumor-challenged lung in mice. Adoptive transfer of wild type IMs increases the reduced number of lung metastasis foci in Ccl3 deficient mice. Mechanistically, Ccr1 deficiency prevents MAM retention in the lung by reducing MAM-cancer cell interactions. These findings collectively indicate that the CCL2-triggered chemokine cascade in macrophages promotes metastatic seeding of breast cancer cells and is a potential target to prevent metastatic disease.

Neutrophils and MDSC in head and neck cancer

Sven Brandau

University of Duisburg-Essen, Essen, Germany

The role of neutrophil granulocytes in tumor host interaction is characterized by a striking dichotomy. In certain therapeutic settings, such as antibody immunotherapy or immunotherapy with BCG mycobacteria, granulocytes exert direct and indirect anti-tumor activity. However, in the majority of tumor types, a high neutrophil-lymphocyte ratio in the blood and/or high numbers of tumor-infiltrating neutrophils are associated with disease progression and poor survival.

We have identified and characterized pathways of recruitment and activation of tumor-associated neutrophils in head and neck cancer. Subsequently, we have elucidated feedback mechanisms exerted by neutrophils on tumor cells, which resulted in the generation of a metastatic tumor cell phenotype.

Myeloid-derived suppressor cells (MDSC) are a heterogeneous population of immature and mature myeloid cells expanded in the tumor host. MDSC were first identified in mice and comprise monocytic and granulocytic subsets. Immunosuppression, best demonstrated for T cells, is a hallmark of MDSC function. We have developed immunomonitoring protocols for human MDSC and have characterized cell biological functions and clinical relevance of granulocytic MDSC isolated from the peripheral blood of patients with head and neck cancer.

Collectively, these studies suggest that tumors exploit the inflammatory and immunoregulatory activity of neutrophils and MDSC to promote tumor progression and metastatic spread.

The Trojan horse cancer treatment

Munitta Muthana

University of Sheffield, UK

Frontline anticancer therapies such as chemotherapy and irradiation often slow tumor growth, but tumor regrowth and spread to distant sites usually occurs after the conclusion of treatment. We recently showed that macrophages could be used to deliver large quantities of a prostate-specific oncolytic virus (OV) to prostate tumors1. When we combined our macrophage virotherapy with conventional therapies this significantly reduced primary tumor growth and metastatic spread as well as increasing the lifespan of tumor-bearing mice compared with those given docetaxel or irradiation alone2. These findings suggest that such macrophage-based virotherapy could be used to markedly increase the efficacy of chemotherapy and irradiation in patients with prostate cancer.

We also set out to improve targeted delivery of our macrophage therapy to specific tissues using magnetic resonance targeting (MRT). This exciting approach uses the magnetic field gradient coils inherent to all MRI systems; to non-invasively steer ferromagnetic particles (or cells containing them) towards specific sites in the body (Fig. 1).

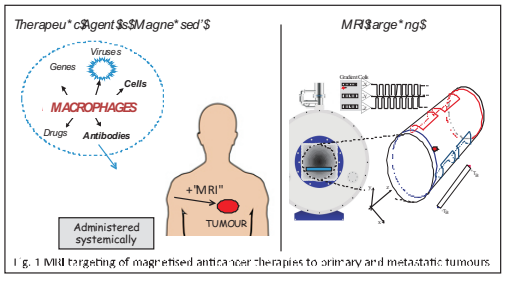


Fig. 1 MRI targeting of magnetised anticancer therapies to primary and metastatic tumours

We demonstrate that macrophages armed with an OV, can be magnetically labeled using super-paramagnetic iron oxide nanoparticles (SPIOs) and then steered from the bloodstream into deep target tissues (primary and metastatic tumors) using pulsed magnetic-field gradients.

This MR targeting caused many more macrophages to infiltrate tumors than would have 'homed' there naturally from the circulation leading to a significant reduction in tumor burden and metastasis. Our study, therefore, shows that clinical MRI scanners could be used, not only to image such magnetically labeled cells after their administration, but also to non-invasively steer them specifically to one or more tumours within the body.

References: Muthana, M. et al. Use of macrophages to target therapeutic adenovirus to human prostate tumors. Cancer Res 71, (2011); Muthana, M. et al. Macrophage Delivery of an Oncolytic Virus Abolishes Tumor Regrowth and Metastasis After Chemotherapy or Irradiation. Cancer Res, (2013).

Cancer-associated inflammation in metastasis and response to chemotherapy

Seth Coffelt

The Netherlands Cancer Institute, Amsterdam, The Netherlands

Over 90% of breast cancer deaths are due to complications of metastasis. Much progress has been made in understanding primary breast cancer formation; however, metastatic disease is still largely unexplored, poorly understood and incurable. Clearly, there is an urgent need for novel therapies with efficacious anti-metastatic activity. The different steps of the metastatic cascade are largely regulated by reciprocal interactions between cancer cells and immune cells in the tumor microenvironment. In addition to this local communication, we recently showed that systemic inflammation is also a major driver of metastasis formation. Our data unraveled how IL1b-expressing myeloid cells within the mammary tumor microenvironment activate gd T cells to produce IL17, leading to the upregulation of G-CSF and subsequently neutrophil expansion. These neutrophils acquired immunosuppressive abilities to dampen anti-tumor CD8+ T cell activity so that disseminated cancer cells could establish themselves in visceral organs. Currently, we trying to understand and test different approaches to block this inflammatory cascade from promoting metastasis. One such approach includes the combination of chemotherapy with immunomodulatory drugs, which will be discussed in this lecture. Through mechanistic understanding of the crosstalk between the immune system and cancer, we aim to fight metastatic breast cancer and to increase the efficacy of conventional anti-cancer therapies. Ultimately, the outcome of these studies may shift therapeutic focus from a cancer cell-intrinsic point of view towards a more combined cancer cell-intrinsic and -extrinsic point of view.

Molecular genetics in AML: clinical implications

Lars Bullinger

Department of Internal Medicine III, University of Ulm, Germany

In the past cytogenetic markers have significantly improved the risk stratification of acute myeloid leukemia (AML), and recently the development of genomics technologies, such as single nucleotide polymorphism (SNP) microarray analysis and next generation sequencing (NGS), has tremendously contributed to decipher the AML associated genetic changes. For example, small genomic losses pointed to a relevant role of the TET2 gene and NGS helped to identify IDH1 and DNMT3A mutations in AML. In accordance, a growing number of genomic aberrations and gene mutations have been identified, which are associated with epigenetic changes and deregulated gene expression. These findings further highlight the molecular heterogeneity of AML and show that individual patients present with a distinct and almost unique combination of somatically acquired genetic aberrations.

Importantly, this growing genetic information has started to translate into the clinical routine, and AML cases are already characterized on the basis of the underlying genetic defects that define distinct entities of clinical importance. First, cytogenetic and molecular genetic changes represent powerful prognostic markers, and second some genetic and epigenetic aberrations can be targeted by novel therapeutic approaches, such as tyrosine kinase inhibitors and demethylating agents. However, there are still limitations regarding the use of prognostic biomarkers in clinical practice as for many novel markers the prognostic impact so far has only been evaluated in retrospective studies, and thus several new markers still need to be interpreted cautiously. Furthermore, for an improved clinical decision making there is an unmet need for predictive markers that can be attributed to the clinical benefit of novel treatment approaches.

Here, I will provide a brief summary of the genetic markers that have already entered clinical practice and are important for diagnosis and guidance of therapeutic decisions in adult AML. In addition, the prognostic value and potential clinical impact of novel markers that remain investigational will be discussed.

A6 | 21-12.05.2015

A shortcut to accelerate personalized cancer therapy

Roderick L. Beijersbergen

Division of Molecular Carcinogenesis, The Netherlands Cancer Institute, Amsterdam, The Netherlands

The complexity and heterogeneity of cancer poses an enormous challenge for the identification and selection of effective cancer therapies. Genomic alterations identified in human cancers commonly affect components of signaling networks representing potential targets for cancer therapy. However, the complex structure of these networks, the extensive crosstalk between pathways and unanticipated feedback control are underlying the limited long-term success in the clinic. To improve the use and outcome of pathway targeted drugs it is crucial to identify biomarkers to stratify patients, and to discover more effective combination therapies. We apply large scale functional genomic screening technologies including RNAi screening (shRNA and siRNA) and CRISPR-based gene editing in combination with (clinically) relevant screening models for the identification of specific dependencies in the context of specific genetic alterations. Using this platform we have identified novel effective drug combinations that are currently tested in the clinic. The results of this work and the clinical implications will be discussed.

Epigenetic twin studies

David Laszlo Tarnoki1), Adam Domonkos Tarnoki1)*

1) Hungarian Twin Registry, Department of Radiology and Oncotherapy, Semmelweis University, Budapest, Hungary

Investigation of monozygotic twins, who share almost 100% of their genes is of the increasing interest of current twin research. Disturbance of the DNA sequence leading to aberrant gene expression has been implicated in the etiology of many diseases. Since monozygotic twins share a common DNA sequence, their study represents an ideal design for investigating the contribution of environmental, such as epigenetic factors to a disease etiology. In the recent years, therefore, twin researchers have been started to focus on monozygotic twin pairs discordant for a phenotype (eg., obesity, cancer, cardiovascular diseases) in order to assess the influence of epigenetic factors which are responsible for discordance between the members of monozygotic twin pairs. These epigenetic factors mean cellular modifications (usually comprise DNA methylation and histone modifications) that can be heritable to the next generation (roughly in 30%), but appear unrelated to DNA sequence changes, and can be modified by environmental stimuli. Recent studies have focused on the investigation of DNA methylation patterns in twins, and reported increasing differences in epigenetic profile by aging due to common or unique environmental exposures. The most interesting first study assessed the epigenetic profile at multiple genomic regions in 3-year-old and 50-year-old monozygotic twins and reported highly similar intrapair epigenetic profiles, whose epigenetic variability increased with age across multiple tissues especially in twins with greater difference in lifestyle. Accordingly, the older twin pairs were and the more different their lifestiles were, the more differences were observed between their DNAs. A recent twin study reported that identical twins, who work in different shifts (eg., one twin daytime and the twin brother/sister works in nightshift) have different gene expressions, therefore, they are more reliable to develop different diseases. In conclusion, monozygotic discordant twin study design combined with sequencing technologies will be possible to explore the complexity of the gene–environment relationships and individual variability to provide important insights into the pathogenesis of various chronic diseases in the future.

A8 | 21-22.05.2015

Cancer genetics: findings of twin studies

Adam Domonkos Tarnoki[1)], David Laszlo Tarnoki[1)]

1). Hungarian Twin Registry, Department of Radiology and Oncotherapy, Semmelweis University, Budapest, Hungary

Familial clustering has been observed for cancers that occur at specific sites, such as breast, colon, prostate, lung and stomach. To study the influence of genetic and environmental factors in a cancer epidemiology, the study of twins should be of value because twins either are genetically identical or share half of their segregating genes. Thus, if heritable factors play a role in the origin of a disease, disease concordance should be greater in monozygotic twin pairs than in dizygotic twin pairs. Concordance in both monozygotic and dizygotic twin pairs of a similar magnitude or larger resemblance in dizygotic pairs suggests the contribution of environmental, lifestyle factors. Large, population based twin cohorts are necessary to find enough number of twin pairs with cancers, especially monozygotic twins discordant for a cancer. Accordingly, most such studies originate from the Scandinavian twin registries with thousands of twin pairs. A Swedish twin study involving over 20k twin pairs found that individuals may possess a genetic susceptibility to cancer in general. In addition, a heritable component could be distinguished for colon, prostate and rectum cancer in males, for female breast cancer, for in situ cervical cancer. In general, all cancers are regarded familial to approximately the same degree, with only a few exceptions (both high and low heritability). Studies suggest that most cancers have a multifactorial (polygenic) origin. Studies on disease-discordant monozygotic twins now offer an opportunity to study epigenetic variation as a dynamic quantitative trait, since high monozygotic twin discordance rates for cancers indicate the influence of environmental or epigenetic factors. Large-scale epigenetic studies in twins across different ages and tissues will improve our understanding of the etiology and mechanisms of a wide range of cancers in the future.

DOK proteins in leukocyte signaling

Jacques A. Nunes, Javier Celis-Gutierrez and Emilie Coppin

Centre de Recherche en Cancérologie de Marseille, Inserm U1068, CNRS UMR7258, Institut Paoli-Calmettes – Aix-Marseille Université – Marseille, FRANCE.

Downstream of tyrosine kinase (DOK) proteins, DOK1 and DOK2 are expressed in lymphoid and myeloid lineage. These adaptor molecules regulated negatively PI3K and RAS-dependent signaling pathways. Using cell lines, genetic modified mouse strains and biological samples from leukemia patients, we investigate the role of these proteins both in cancer cells and in immune cells acting as anti-tumor effectors.

We identified DOK gene variations in chronic myelomonocytic leukemia (CMML) patients that could participate to the development of a myeloproliferative neoplasm (1). Further investigations are performed to evaluate the impact of DOK genes ablation in hematopoietic stem cell compartment. Moreover, these genes are highly expressed in natural killer (NK) cells that are able to kill tumor cells. We showed that DOK1 and DOK2 proteins act as negative feedback loops downstream of NK cell activating receptors (2).

These findings highlight the role of these signaling molecules in the control of the tumorogenesis.

(1) Mutational analysis of the DOK2 haploinsufficient tumor suppressor gene in chronic myelomonocytic leukemia (CMML). Coppin E, Gelsi-Boyer V, Morelli X, Cervera N, Murati A, Pandolfi PP, Birnbaum D, Nunès JA. Leukemia. 2015 Feb;29(2):500-2

(2) Dok1 and Dok2 proteins regulate natural killer cell development and function. Celis-Gutierrez J, Boyron M, Walzer T, Pandolfi PP, Jonjić S, Olive D, Dalod M, Vivier E, Nunès JA. EMBO J. 2014 Sep 1;33(17):1928-40.

Cell-mediated activities of first and new generation ANTI-CD20 antibodies

Josée Golay

Center of Cellular Therapy "G. Lanzani", Division of Haematology, Azienda Ospedaliera Papa Giovanni XXIII, Bergamo, Italy.

Monoclonal antibodies (MAbs) have become an important therapeutic tools in a variety of clinical contexts and in particular for cancer treatment. The anti-CD20 antibody Rituximab has been approved by the FDA for treatment of B-Non Hodgkin's lymphoma (B-NHL) and later chronic lymphocytic leukemia (CLL), alone and in combination with chemotherapeutic agents. Nonetheless resistance or relapse still occur, raising the necessity to improve the activity of this drug. Rituximab is thought to act through immune-mediated mechanisms, in particular complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC) by natural killer cells (NK) and antibody-dependent phagocytosis (ADCP) by macrophages; more recent evidence indicates this MAb can also promote activation of polymorphonuclear neutrophils (PMN) and phagocytosis. Thus novel anti-CD20 monoclonal antibodies have been selected for their improved biological activities with respect to rituximab: ofatumumab (OFA) shows enhanced CDC whereas and obinutuzumab (OBZ, GA101), a glycoengineered anti-CD20, shows more potent ADCC and activated neutrophils more efficiently. OBZ also shows enhanced direct cell death induction of B cell targets, through a different modality of binding to CD20 with respect to rituximab. Despite improved activities in vitro and in vivo of anti-CD20, and the design of successful combined treatment of these antibodies with chemotherapeutic agents, the following questions still need to be fully answered: 1) What are the most important resistance factors and how to overcome them? 2) Which are the most effective chemotherapeutic agents to be combined with anti-CD20 and according to which schedule? 3) What is the relative role of different effector mechanisms (CDC, ADCC, direct cell death) in different diseases or different tissues? 4) Is it possible to induce or enhance a vaccine effect of anti-CD20 antibodies that would lead to long term response in treated patients? The major mechanism of anti-CD20 antibodies will be reviewed and put in the context of these important questions.

Antibody drug conjugates: currently available molecules and further developments

Charles Dumontet

Anticancer Antibody Team, Cancer Research Center of Lyon

Antibody drug conjugates (ADC) combine the specificity of monoclonal antibody therapy with the potency of cytotoxic chemotherapy. An ADC is composed of an antigen-binding moiety, a cleavable linker and an active agent. These "magic bullets" have needed to overcome several hurdles including linker instability and systemic toxicity or immunogenicity (depending on the nature of the payload). While the first approved ADC contain cytotoxic agents with conventional mechanisms of action (DNA binding agents such as calicheamycin, tubulin binding agents such as maytansin or auristatin) novel conjugates are currently being evaluated. An important issue currently being addressed is the standardization of the number of conjugates per antibody molecule. Toxicity and off-target effects will be the subject of close scrutiny, in particular in the context of combination regimens. The most recent generation of ADC, including recently approved therapies for CD30 + lymphoid malignancies and Her2 + breast cancers have paved the way for the rapid development of this family, with scores of other molecules currently in early phase evaluation. A key topic will be the positioning of these novel agents in the global therapeutic strategy of patients with neoplasia.

Enhanced IgG Hexamerization Mediates Efficient C1q Docking and More Rapid and Substantial Complement-Dependent Cytotoxicity (CDC): Preclinical Proof of Concept

Frank Beurskens

Genmab, The Netherlands

We revealed that IgG antibodies form hexamers on the cell surface following antigen binding. These hexamers are critical for optimal C1q binding and CDC. IgG hexamerization occurs through specific non-covalent interactions between Fc-segments. We now identified mutations that enhanced IgG clustering after antigen binding to cells which led to an increase in C1q binding and CDC. Our data represent a promising novel approach for improving the efficacy of therapeutic antibodies.

Cladribine in the treatment of hematologic malignancies - contribution of the Polish ivetigators

Tadeusz Robak

Department of Hematology, Medical University of Łódź, Poland

Cladribine (2-CdA, 2-chlorodeoxyadenosine) is synthesized by a simple substitution of a chlorine atom with a hydrogen atom at the position 2 of the purine ring of deoxyadenosine. 2-CdA has high efficacy in lymphoid and myeloid malignancies. It is the drug of choice in the treatment of hairy cell leukemia (HCL) but is also highly active in low-grade B-cell lymphoid malignancies, including chronic lymphocytic leukemia (CLL), Waldenström macroglobulinemia and non-Hodgkin lymphoma. Moreover, several investigations have revealed that these agent is also active in other hematologic malignancies including advanced cutaneous T-cell lymphoma, acute myeloid leukemia (AML), Langerhans cell histiocytosis, and systemic mastocytosis. Polish ivetigators performed several preclinical and clinical studies with 2-CdA, used alone and in combination with other drugs.

2-CdA induces durable and unmaintained complete response (CR) in about 80% of patients with HCL after a single course of therapy. It is usually administered as a continuous i.v. infusion at a dose of 0.09 mg/kg over 5-7 days or as a two-hour i.v. infusion at a dose of 0.12 mg/kg also for 5-7 days. Preliminary observations implied that a CR following 2-CdA administration was durable even without maintenance therapy, so this drug was considered to be potentially curative against HCL [1]. However, weekly administration may be less toxic and reduces the risk of infection complications in comparison with standard 2-CdA daily regimens. In our randomized study we compared weekly administration of 2-CdA (0.12 mg/kg in two-hour i.v. infusion once a week for 6 weeks) with daily administration (0.12 mg/kg in two-hour i.v. infusion for 5 consecutive days) [2]. The results of this study indicate that both CR and overall response (OR) rates were similar in the compared groups. There was also no statistically significant difference in toxicity between groups.

Several clinical trials have confirmed the value of 2-CdA in CLL. 2-CdA used alone or in combination with other cytotoxic drugs showed good efficacy and acceptable toxicity profile in CLL. In the largest study of 184 pretreated patients, reported by our group the OR rate was 48.4% including CR in 23 (12.5%) and partial responses (PR) in 66 (35.9%) [3]. In other study in previously untreated CLL patients, the use of 2-CdA as first-line

treatment of progressive CLL result in significantly longer PFS, but similar overall survival compared to 2-CdA monotherapy [5].

Efficacy and safety of 2-CdA combined with cytarabine (Ara-C) and daunorubicin (DNR) (DAC-7) has been recently compared with the standard 3+7 (DA-7) chemotherapy in previously untreated AML patients in randomized multicenter studies in Poland [6]. This study proves that addition of 2-CdA increases antileukemic potency of DNR+Ara-C regimen in patients with AML.

References

1. Robak T, Błasińska-Morawiec M, Błoński J, et al. 2-chlorodeoxyadenosine: (cladribine) in the treatment of hairy cell leukemia and hairy cell leukemia variant 7-year experience in Poland. Eur J Haematol. 1999; 62: 49-56.
2. Robak T, Jamroziak K, Gora-Tybor J, Blonski JZ, et al. Cladribine in a weekly versus daily schedule for untreated active hairy cell leukemia: final report from the Polish Adult Leukemia Group (PALG) of a prospective, randomized, multicenter trial. Blood. 2007 ;109:3672-5.
3. Robak T, Bloński JZ, Kasznicki M, et al. Cladribine with or without prednisone in the treatment of previously treated and untreated B-cell chronic lymphocytic leukemia- updated results of the multicentre study of 378 patients. Br J Haematol. 2000; 108: 357-68.
4. Robak T, Bloński JZ, Kasznicki M, , Blasińska-Morawiec M, Krykowski E, Dmoszyńska A, et al.Cladribine with prednisone versus chlorambucil with prednisone as first-line therapy in chronic lymphocytic leukemia: report of a prospective, randomized, multicenter trial. Blood 2000; 96: 2723-9.
5. Robak T, Blonski JZ, Gora-Tybor J, et al. Cladribine alone and in combination with cyclophosphamide or cyclophosphamide plus mitoxantrone in the treatment of progressive chronic lymphocytic leukemia: report of a prospective, multicenter, randomized trial of the Polish Adult Leukemia Group (PALG CLL2). Blood. 2006;108:473-9.
6. Holowiecki J, Grosicki S, Giebel S, et al.. Cladribine, but not fludarabine, added to daunorubicin and cytarabine during induction prolongs survival of patients with acute myeloid leukemia: a multicenter, randomized phase III study. J Clin Oncol. 2012 10;30:2441-8.

Personalised Medicine in practice in The Netherlands – a SME's view

Tim Kievits

Vitromics, The Netherlands

The presentation will cover on-going projects that are examples of the added-value Personalised Medicine approaches can bring to patients, payers, physicians and innovator. This is achieved by aiming at reducing overtreatment and limiting healthcare budget spillage while introducing innovative tools. In one example Dutch healthcare insurer CZ, the Center for Personalized Cancer Treatment (CPCT) and VitrOmics, a company specialised in personalized medicine, have started a joint project to address the overtreatment issue within a certain group of patients with breast cancer. The goal set by the collaborators is to provide therapy to this group much more selective than currently is done in practice.

Public private collaboration to optimise personalised medicine research, development, and pathways to patients: the example of the Innovative Medicines Initiative 2

Magda Chlebus

European Federation of Pharmaceutical Industries and Associations, Brussels

The challenges facing healthcare systems and the scientific community cannot be addressed by one stakeholder group alone, we must look to translate new scientific and technological opportunities into integrated healthcare solutions – we have change the way we engage and collaborate.

EFPIA believes that the Innovative Medicines Initiative (IMI), Europe's public private partnership for health between the European Union and the European pharmaceutical industry, provides an effective vehicle to test the boundaries of open collaboration and to integrate different perspectives to reduce attrition, speed up patient access to innovative healthcare solutions and to improve outcomes.

It does this by facilitating collaboration between the key players involved in healthcare research, including universities, the pharmaceutical and other industries, small and medium-sized enterprises (SMEs), patient organisations, and medicines regulators.

Using Web of Science and InCites for research evaluation, strategic planning and research monitoring

Marcin Kapczyński

Thomson Routers, Poland

The presentation shows the ways of using research analytics tools such as Web of Science and InCites for determining research impact, identifying prospective research areas, choosing organizations and scientists for collaboration and monitoring the results of research activities. Also it touches interesting aspect of using bibliometrical data for creating the publication strategy. Both Web of Science and InCites are citation based discovery and research analytic resources. Citation analysis itself is an established quantitative methodology for the assessment of the contribution, dissemination and influence of knowledge-exchange within a research area, where the citations to the work of a researcher act an indicator of scientific impact. It has proved to be a powerful and popular method of examining and mapping intellectual impact at various organizational levels.

Technology transfer – comparison of American and Turkish reality and scientific issues in intellectual property rights

Ali Gure

Bilkent University, Turkey

It has been now 35 years since the first patent in biotechnology was issued. The idea of assigning the intellectual property rights to the inventor was intended as a "retrospective reward" to the scientists involved in the discovery and a "prospective reward" for the companies that would obtain the licenses for them. Despite these facts, and that many scientific institutions promote "number of patents" as a measure of scientific output, only few scientists aim for a patent as the end point of their work, and most don't even have the impression that this is what should be aimed for; for reasons I will try to summarize in this talk. I will argue that each scientist needs to know the basic instruments available to him in this area and to actively participate in the shaping of the pertinent laws which can be considered to be still at their youth if not infancy. I will also explain how I favor the "non-exclusive" licensing option over some others as the most feasible solution in the interim.

From Bench to Spin-Off: How to reach results able to be patented

Bruno Botta, Deborah Quaglio, A)* Cinzia Ingallinaa, B)* Francesca Ghirga A)* and Mattia Mori, B)*

A) Dipartimento di Chimica e Tecnologie del Farmaco, Università "La Sapienza" di Roma, Italy
B) IIT Center for Life Nano Sciences, CLNS@Sapienza, Rome, Italy

During the past seven years Computer Modeling Techniques have been widely applied by Botta's group following both structure-based and ligand-based drug design approaches have been applied in the research of novel antiviral, anti-TB and anticancer agents.
In some cases automatic docking simulations has performed to find the orientation(s) of each ligand among the 850 natural compounds of chemical libraries belonging to Botta's unit leading to the most profitable interactions with the corresponding binding site on the target proteins/receptors. These studies allowed to select just a few compounds (e.g. Antraquinones, Ferruginines and Iboga-alkaloids all of them containing different alkyl side chains) as possible lead compounds.

The selected compounds have been submitted to the very innovative computational methodology (very often applied in the last three years) of the virtual library design technique (VLD) that allowed to generate virtual focused libraries around previously identified hit compounds. These were then submitted to virtual screening, second generation hit compounds with improved pharmacological profile were identified. VLD also allowed to manipulate a large number of compounds from databases of virtual or available compounds. On this basis, VLD allowed also, applied in conjunction with pharmacophoric and other ligand-based models, either to predict activity of virtually generated compounds (i.e., from focused libraries) or as screening tools for databases of available compounds (i.e., compounds from natural sources) to find new hits to be directly submitted to the biological tests, completely avoiding the synthesis step.

Among selected compounds, it turned out that one product named Glabrescione B (GlaB, an isoflavone naturally found in the seeds of Derris glabrescens – Leguminosae) showed a robust inhibitory effect on Gli1 activity. Its interaction with Gli1 was characterized by NMR spectroscopy and a good agreement with molecular modeling predictions was observed.1)*
Moreover, Glabrescione B inhibited the growth of Hedgehog-dependent tumor cells in vitro and in vivo as well as the self-renewal ability and clonogenicity of tumor-derived stem cells, thus becoming a profitable pre-clinical candidate. These results were then Patented.2)*

Diel-Alder adducts belonging to kuwanon family showed an interesting Mtb PtpB inhibition activity.3)*

Botta B, Infante P, Mori M, et al. EMBO J, 2014, 34,200-217; Botta B, Gulino A, Botta M, Mori M, et al. PCT Int. Appl. 2014, WO 2014207069; Botta B, Mascarello et al. PLoS One. 2013;8(10):e77081

Spin-off in practice – German experience from Polish perspective

Agnieszka Turowska

Sterna Biologicals GmbH, Germany

The development of a new drug costs from one to three billion euros, and takes 10 to 15 years. Moreover, only one out 10,000 active substances will succeed in completing the journey from initial discovery to a place on the market (source: Report: The German Biotechnology Sector 2014, biotechnologie.de). Each ongoing stage of drug development is frequently associated with larger investments and higher risk. This is especially true for biotech spin-off companies, formed to commercialize inventions generated from the research work from a parent institution. Spin-off is not a new research project; it is a business project that can generate a return on investment for the investors. Cooperation with investors is of crucial importance for the high-cost development of biotech products and therefore, preparing for such an interaction should be inscribed in the spin-off strategy at its foundation stage already. This lecture discusses drug development stages with regard to identification of optimal time points for external investments. It contains practical tips how to succeed in preparation of an academic spin-off company for interaction with external business partners and thus to secure seed financing.

A new approach to industry academic collaborations: Discovery Partnerships with Academia

Danuta Mossakowska

Glaxo Smith Klein, UK

Discovery Partnerships with Academia (DPAc) is a unique global GlaxoSmithKline initiative that establishes integrated partnerships with academic groups to undertake early drug discovery and translate innovative research into medicines that benefit patients. DPAc was initiated just over four years ago, with the first collaborations in the UK before the initiative was expanded globally. Multiple projects are now up and running with academics in major European and North American institutions, bringing together the deep biology and disease knowledge of academia with the drug discovery expertise of GSK to research and develop new medicines. These partnerships are focused from start to finish on delivery of a specific project with a joint team working together towards agreed common goals. This presentation will describe the DPAc model, highlighting examples of collaborations, and discussing the potential as well as the challenges of working across the industry – academia interface.

The role of the National Science Centre in financial support of research

Michał Karoński
Chairman of the Council of the National Science Centre, Poland

The presentation shows the main aims and mission of the National Science Centre (Narodowe Centrum Nauki, NCN) as an executive agency for funding basic research in Poland. Thanks to this institution, researchers themselves can decide how a substantial portion of research funds is allocated from the state budget. 24 distinguished researchers selected from amongst candidates appointed by Polish scientific institutions are the Council of the NCN. The Council sets priority areas in basic research, decides on the type of programmes and specifies call regulations. Its range of competence also includes electing members of expert teams responsible for proposal evaluation, as well as appointing the Director and Discipline Coordinators.

The Centre funds research projects carried out by a wide range of applicants from pre-doctoral researchers to advanced researchers. One of the priorities of the Centre is to support and develop the scientific careers of pre-doctoral and doctoral scientists. The Centre allocates more than 20% of its budget towards grants for this group of researchers.

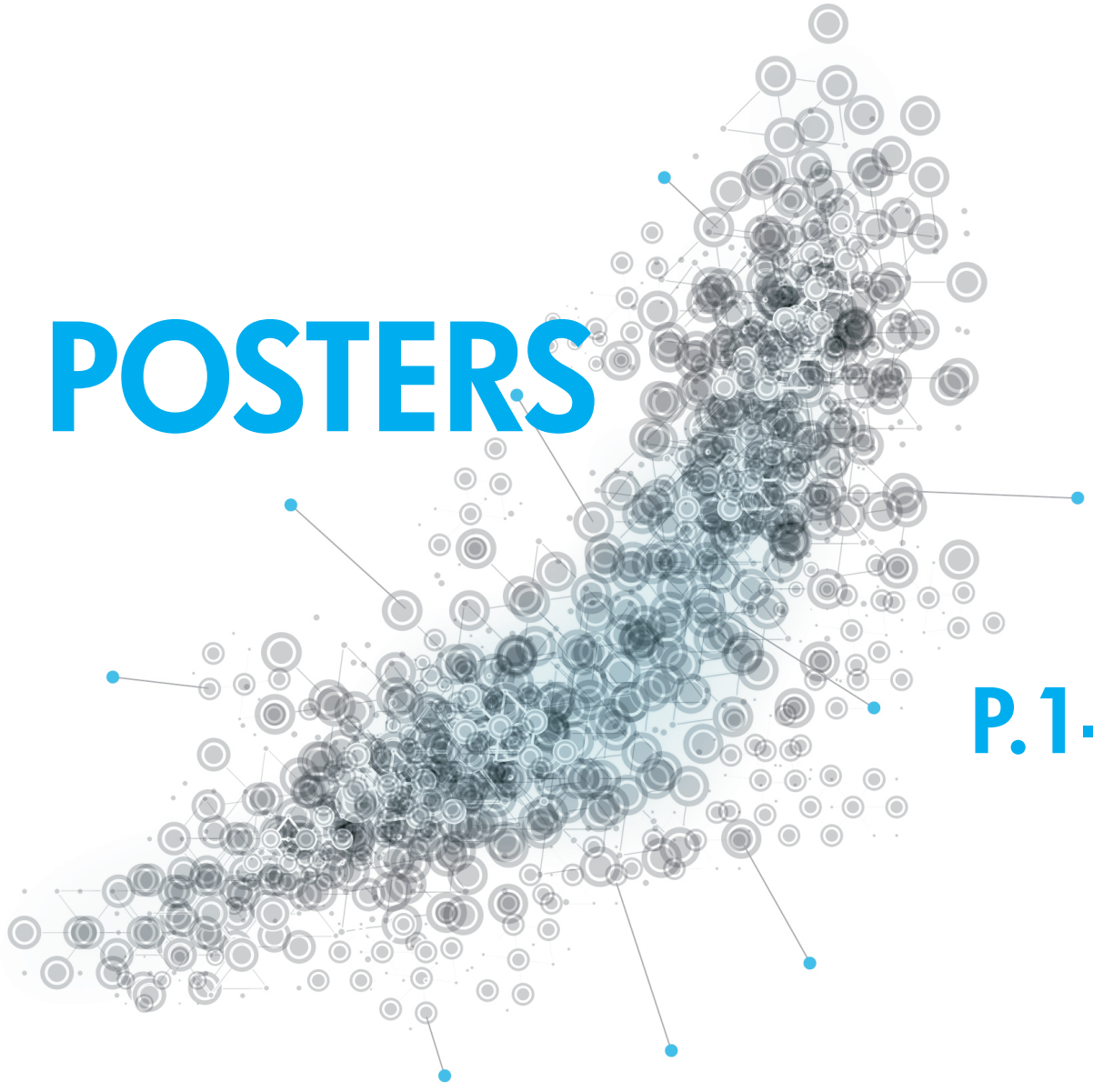
The next part of the presentation is about proposal evaluation. As the National Science Centre seeks to select the very best proposals for funding, it employs an evaluation procedure based on a two-stage peer review process similar to that used by the European Research Council. The NCN Council has adopted the general principle of carefully weighing up the quality of the project against the achievements of its authors. The eligibility of research projects submitted to the NCN is examined by NCN Coordinators. The projects are afterwards peer reviewed by members of the NCN's Expert Teams (excellent Polish researchers appointed by the NCN Council and the Director as reviewers of research proposals) and External Reviewers, among whom are also international researchers.
The last part of the presentation includes some statistics about the National Science Centre's activity, especially in the Life Sciences area.

Setting up health research as national priority for Romania under the New Research Development and Innovation Strategy for 2014-2020, implications and consequences

Ioana Ispas

Ministry of National Education, Romania

The New Research Development and Innovation Strategy for 2014-2020 is the result of the broad public consultation of scientific community. The presentation underlines the Romanian research landscape in area of cancer research involving not only 10 Medical Universities but also public and private research institutes and hospitals dedicated to oncology. Also from 47 national RD activities and 65 research institutes and centers of the Romanian Academy, the relevant organizations for cancer research will be detailed: Oncology Institute from Bucharest, Victor Babes Research Institute for Pathology and Biomedical Sciences, Virology Institute Stefan S. Nicolau together with some relevant scientific results. The previous National Research and Development (2007-2014) will be briefly presented, indicating that 14% from the total budget were dedicated to health research without any restrictions for oncology research. Funding tools were: projects in partnership with SMEs, fellowship for young scientists, ERC like projects, vouchers for innovations, projects for fundamental research, support for research infrastructures. Defining health as national priority is expected that this will open the very good prospective for funding an increasing number of research activities based on national problems identified.



POSTERS

P.1-19

Liposomal drug forms used in cancer treatment

Beata Sarecka-Hujar, Aneta Ostróżka-Cieślik, Anna Banyś, Joanna Wysocka-Jędruszek, Andrzej Jankowski

Medical University of Silesia in Katowice, School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Department of Drug From Technology, Chair of Applied Pharmacy, Kasztanowa Str 3, 41-200 Sosnowiec, Poland; e-mail: beatasarecka@poczta.onet.pl

Aqueous reservoir in liposomes allows to encapsulate many hydrophilic agents, including anticancer agents which improved its delivery to the cells. Cytostatics in liposomal forms are used in the treatment of various cancers. Placing toxic chemotherapeutic agents in liposomes enables also to avoid a number of side effects and the controlled release of cytostatics in cancer cells, it can toxicity based on the modified biodistribution of the drug. Pegylation of liposomes further improves the pharmacological advantages of this drug form. PEGylated liposomal formulation of doxorubicin was approved by FDA for the treatment of Kaposi's sarcoma in 1995 [1].

There is a lot of data showing advantages of cancer treatment using liposomal drug forms [2-3]. Previous studies concerning liposomal form of a specific enzyme - T4 endonuclease V, which repairs cyclobutane dimers pyrimidin (CPD) damages in DNA - demonstrated that mice treated with such form of the enzyme showed dose-dependent reduction in the number of changes squamous cell carcinoma compared to the controls. When liposomal T4 endonuclease V was applied to the mammalian skin cells, the removal of CPD damage from DNA was increased and the number of neoplastic lesions in mice irradiated with UV rays were reduced [2].

Methotrexate inhibits dihydrofolate reductase (DHFR), which causes defective nucleic acid synthesis in fast-growing cancer cells. Additionally, it has low immunosuppressive activity. It is used to treat various types of cancers: leukemia, lymphoma, malignant cell and neck, breast, ovarian and head cancer. In the studies of breast cancer treatment a liposomal form of a new inhibitor of dihydrofolate reductase (MV-05) was developed [3]. This form was more effective than the standard methotrexate, and its activity was comparable to liposomal doxorubicin. Liposomal MV-05 appears to be a promising therapeutic agent and may be used in the adjuvant therapy of breast cancer [3].

1. Gabizon AA. Pegylated liposomal doxorubicin: metamorphosis of an old drug into a new form of chemotherapy. Cancer Invest 2001; 19: 424–36; 2. Yarosh D, Alas LG, Yee V, Oberyszyn A, Kibitel JT, Mitchell D, Rosenstein R, Spinowitz A, Citron M. Pyrimidine dimer removal enhanced by DNA repair liposomes reduces the incidence of UV skin cancer in mice. Cancer Res. 1992 Aug 1; 52(15): 4227-4231; 3. Tan BJ, Quek KS, Wong MY, Chui WK, Chiu GN. Liposomal M-V-05: formulation development and activity testing of a novel dihydrofolate reductase inhibitor for breast cancer therapy. Int J Oncol. 2010 Jul; 37(1): 211-218

Targeted exome sequencing of the gliosarcoma revealed new variants in tumorigenesis related genes

Bartlomiej Gielniewski[1], Bartosz Wojtas[1], Marta Maleszewska[1], Pawel Nauman[2], Tomasz Tykocki[2], Marcin Machnicki[3], Tomasz Stoklosa[3], Rafal Ploski[4], Bozena Kaminska[1]

1). Laboratory of Molecular Neurobiology, Neurobiology Center, Nencki Institute of Experimental Biology, Warsaw; 2). Dept. Neurosurgery, Institute of Psychiatry and Neurology, Warsaw; 3). Depts. Immunology and Medical Genetics, Medical University of Warsaw

Gliosarcoma (GSM) is an extremely rare primary tumor of the central nervous system (WHO grade IV), composed of a glioblastoma multiforme mixed with a sarcomatous component. The incidence of GSM is between 2% and 8% of all malignant gliomas and mean survival is 13 months (6.9-19.4 months). Gliosarcomas have a distinct genetic profile, similar to glioblastoma except for the amplification of EGFR, recent studies suggest amplification of genes on proximal 12q. To date, little is known about the molecular genetics of GSM and the attempts to characterize adult GSM have been limited by the rarity of this malignancy. Next generation sequencing has become a powerful tool in dissecting and identifying mutations and genomic structural variants that accompany tumorigenesis. We applied targeted exome sequencing of a primary GSM (obtained from 2nd and 3rd resections) and blood DNA to look for somatic alterations that may "drive" tumorigenesis. Library preparation for sequencing was performed with NimbleGenSeqCap EZ Choice Libraries that enables enrichment of cancer-related genes (approximately 600 genes). Next-generation sequencing was performed with a paired end technology on the HiSeq 1500 Illumina platform. The analysis has revealed new somatic variants in genes ABCB1, ABCA5 and SORCS2, coding for proteins that are implicated in processes related to cancer progression, for example multidrug resistance. Detected genetic alterations were confirmed using Sanger sequencing. Those findings extend our understanding of GSM pathobiology and may have importance for the future diagnostics and GSM treatment.

Transcriptional activity of angiogenic genes stimulated by hypoxia in cervical cancer

Anna Banyś1), Bogdan Michalski2), Urszula Mazurek3), Joanna Orchel3), Ewa Długosz4), Beata Sarecka-Hujar1), Andrzej Jankowski1)

1). Department of Drug Form Technology, Chair of Applied Pharmacy, School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia, Poland 2). Sub-Faculty of Women's Health, Medical University of Silesia, School of Health Sciences in Katowice, Poland 3). Department of Molecular Biology, Medical University of Silesia, School of Pharmacy with the Division of Laboratory Medicine, Sosnowiec, Poland 4). Department of Retail Pharmacy, Chair of Applied Pharmacy, School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Medical University of Silesia, Poland

Background: The development of cervical cancer begins when a cell, unable to realize apoptosis, starts an uncontrollable division. Oxygen, nutrients and growth factors needed for proliferation reach neighboring blood vessels using the phenomenon of diffusion. The next phase of the neoplasm development is dependent on acquiring an angiogenic phenotype and the growth of new blood vessels – angiogenesis.

Aim: The aim of the study was to evaluate the transcriptional activity of angiogenic genes stimulated by hypoxia in cervical cancer in clinical stages I-III detected through QRT-PCR.
Methods: The researched tissue samples of cervix were obtained from 42 women with squamous cell cervical cancer in clinical stages I-III according to FIGO classification and from 20 women operated because of a different gynecological indication. The transcriptional activity of HIF-1a, VEGF, VEGFR1, VEGFR2, sVEGFR1-coding genes was determined. Next, a gene copy number in control samples was compared to a gene copy number obtained from the cervical cancer patients.

Results:
The transcriptional activity of all analyzed genes was significantly increased in a group of cervical cancer patients when compared to the controls. A significant increase of VEGF, VEGFR2 and sVEGFR1 gene expression was observed after the estimation of the relation between gene expression and a stage of cancer. During the analysis of VEGF and VEGFR2 genes a significant difference between groups in I and II clinical stage and groups in I and III clinical stage was observed. There is a statistical increase of sVEGFR1 gene expression between I and III clinical stage.

Conclusions: An increased transcriptional activity of HIF-1a-coding gene, VEGF-coding gene and VEGF receptor-coding genes (receptors such as VEGFR1, VEGFR2, sVEGFR1) indicates new treatment targets in cervical cancer treatment. Significantly increased transcriptional activity of VEGF among women in II and III clinical stage when compared to women in I clinical stage may indicate the necessity of implementing a therapy directed against VEGF protein or its receptors in order to block the angiogenic signal.

The A118G polymorphism in MOR gene in patients with breast cancer

Matysiewicz Michał, Cieślińska Anna, Fiedorowicz Ewa, Sienkiewicz-Szłapka Edyta, Kostyra Elżbieta

Medical Biochemistry Peptides and Proteins Research Team; Faculty of Biology & Biotechnology; University of Warmia and Mazury in Olsztyn, Oczapowskiego 1a Street, 10-719 Olsztyn

In recent years, more and more attention is paid to the role of the opioid system in various disease processes. The most important components of this system are opioid receptors.
Structure-dependent μ-receptor (MOR) activity is an important element in cancer opioid analgesic effectiveness. The most commonly occurring MOR polymorphism is A118G variant (rs1799971). This is located in the first exon of gene, and consists of non-synonymous substitution of guanine (G) for adenine (A), resulting in the exchange of aspartic acid for asparagine at MOR protein position 40 (N40D). This is associated with decreased binding ability in both exogenous and endogenous opioids, resulting in increased human pain resistance. The endogenous opioid system's function in body homeostasis maintenance is considered mainly regulatory, so its participation in breast tumor formation and progression is identified herein. Further studies suggest that G-allele carriers exhibit decreased breast cancer-specific mortality and significantly lowered risk of esophageal carcinoma development.

We examine the association of the most frequent MOR (A118G) gene polymorphism on breast cancer risk in a North-Eastern Polish population by PCR-RFLP comparison of A and G allele frequency at OPRM1 gene A118G polymorphic site in breast cancer diagnosed patients with healthy control group frequencies.

The obtained preliminary results suggest a strong association between A118G polymorphism in MOR receptor, with coexisted increase in occurrence of breast cancer (OR = 3.3, 95% CI: 2.2-5.0, P <0.0001) and female gender (OR = 2.0, 95% CI: 1.4-2.9, P = 0.0004). Consequently, OPRM1 G allele presence at that site is a highly significant risk factor in breast cancer development.

Influence of anti-depression drugs at human breast cancer cells proliferation and epithelial to mesenchymal transition

Chojnowska K 1), Lin KW 2), Souchelnytskyi S 2)

1).Department of Animal Phisiology, University of Warmia and Mazury, Olsztyn, Poland

2).Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden

Breast cancer is the most common type of cancer found in women globally. It accounts for one fourth of all new malignancies in the female population and about half a million deaths annually. The main cause of lethality for most cancer types, including breast cancer, is metastases. The first step in development of metastasis is acquiring of invasive phenotype.

We describe here a 21-protein signature associated with acquisition of invasiveness by the human breast epithelial cells. This signature was built by using intact-protein proteomics of MCF7, MCF7-c46 (invasive clone of MCF7) and MDA-MB-231 cells. Systemic analysis of the 21-protein signature highlighted novel mechanisms regulating invasiveness, and confirmed already known mechanisms. Regulators of cell adhesion, membrane trafficking, proliferation, apoptosis and scaffold proteins have been identified. Correlation analysis of the 21-protein signature with available mRNA profiling data also confirmed the value of the 21-protein signature. Systemic analysis pointed to dependencies that may explain impact of treatment of collateral diseases, e.g. depression, on aggressive tumorigenic features of cells, and indicate regulatory mechanisms previously not associated with invasiveness.

We will present the 21-protein signature and the validation studies, e.g. the cross-Talks with antidepressant drugs. We observed that Prozac and Amitriptyline may have inhibitory effect on transformation features of non-aggressive tumor cells (clonal growth of MCF7 and MCF7-c46 cells was inhibited). However, no significant change in motility using wound healing assays was observed. Moreover, our study showed that for transformation assays, e.g. clonogenic, the antidepressants may be not beneficial for inhibition of highly aggressive and metastatic cells. Our studies showed that antidepressant drugs Fluoxetine (Prozac) and Amitriptyline had a direct impact on proliferation and invasiveness of human breast epithelial cells. Our study suggests that these antidepressants may be used in their other role of anti-cancer drugs.

*This work has been supported by the "Oves Minnesfond", the Swedish Cancer Foundation, the Swedish Research Council, the Swedish Institutet, KID/KI, Radiumhemmets Research Funds, and ERASMUS program to S.S.

Expression of AQP1 and AQP5 gene expression in human endometrial cancer

Młotkowska P.1), Skowrońska A.2), Frąckowiak L.3), Tański D.1), Bocian A.D.2), Jaśkiewicz Ł.2), Skowronski M.T.1)

1). Department of Animal Physiology, Faculty of Biology and Biotechnology, University of Warmia and Mazury, Olsztyn

2). Department of Human Physiology, Faculty of Medical Sciences, University of Warmia and Mazury, Olsztyn

3). Department of Public Health and Epidemiology, Faculty of Medical Sciences, University of Warmia and Mazury, Olsztyn

Incidence rates of endometrial carcinoma have been increasing worldwide in recent years. Endometrial carcinoma (EC) is the most common gynecological cancer in developed countries. Accumulating evidence for overexpression of AQP1 and AQP5 in various types of human cancer suggests that their play a key role in tumor biology. However, little is known about the function of AQP 1 and 5 in human endometrial cancer. Aquaporins (AQPs) are a family of membrane channel proteins that facilitate bulk water transport. To date, 11 isoforms of AQPs have been reported to be expressed in the female and male reproductive systems. Aquaporins are present in all cells of the body and in the reproductive organs of women fullness of many functions. Earlier studies of cancer have shown that increased expression of the aquaporin promotes proliferation of blood vessels supplying the tumor cells with nutrients through this contributes to tumor growth. Previously, we demonstrated for the first time the protein expression of AQP1, AQP5 and AQP9 in uterine leiomyomata and in the adjacent normal endometrium and myometrium.

In this study, we evaluated the expression profile of AQP1 and AQP5 mRNA in endometrial cancer tissue and in corresponding normal tissue by real-time PCR. All data were analyzed by one-way ANOVA and least significant difference (LSD) post hoc test and were reported as the means ± SEM.

The Real Time PCR analysis revealed variable expression of AQP1 and AQP5 in the examined tissues. Expression of AQP1 was higher in myometrium while expression of AQP5 increased in endometrium. Our findings showed that AQPs might play important roles in uterine carcinogenesis and tumor progression.
In addition, immunohistochemistry results regarding AQP1, AQP5 and E-cadherin protein will be presented on the poster.

Photochemical internalization (PCI): a novel technology for site-specific drug delivery

Kristian Berg[1)], Anette Weyergang[1)], Colin Hopper[2)], Anders Høgset[3)], Michael G. Rosenblum[4)], Pål K. Selbo[1)]

1). Oslo University Hospital, Oslo, Norway; 2). UCLH Oral and Maxillofacial/Head and Neck Centre, London, United Kingdom; 3). PCI Biotech, Oslo, Norway, M.D. Anderson Cancer Center, Houston, Texas

The utilization of macromolecules in therapy of cancer and other diseases is becoming increasingly important. Recent advances in molecular biology and biotechnology have made it possible to improve targeting and design of cytotoxic agents, DNA complexes and other macromolecules for clinical applications. To achieve the expected biological effect of these macromolecules in many cases internalisation to the cell cytosol is crucial. At an intracellular level, the most fundamental obstruction for cytosolic delivery of therapeutic macromolecule is the membrane-barrier of the endocytic vesicles. Photochemical internalisation (PCI) is a novel technology for release of endocytosed macromolecules into the cytosol.

The technology is based on the use of photosensitizers located in endocytic vesicles that upon activation by light induces rupture of the endocytic vesicles and thereby release of the macromolecules into the cytosol. PCI has been shown to enhance the biological activity of a large variety of macromolecules and other molecules that do not readily penetrate the plasma membrane, including type I ribosome-inactivating proteins (RIPs), gene-encoding plasmids, adenovirus, oligonucleotides and the chemotherapeutic agent bleomycin. For clinical utilization a novel photosensitizer has been developed and evaluated for PCI of bleomycin. A phase I dose-escalating clinical trial has recently been finalized and followed up by a phase II clinical trial on recurrent head & neck squamous cell carcinoma and a phase I/II trial on cholangiocarcinoma.

Sesquiterpene lactones – natural active compounds with anticancer properties

Ewa Długosz[1)], Kazimiera Klementys[1)], Beata Szulc-Musioł[2)], Wioletta Siemiradzka[2)]

1). School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Department of Retail Pharmacy, Medical University of Silesia in Katowice, Poland, e-mail:ewa.dlugosz@sum.edu.pl
2). School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Department of Drug Form Technology, Medical University of Silesia in Katowice, Poland

Introduction: Sesquiterpene lactones are oxygen derivatives of hydrocarbons with a chain or cyclical construction. They are liquids isolated from essential oils, resins and plant saps, mostly from plants of the Asteraceae family. They are also present in plant families such as: Apiaceae, Magnoliaceae, Lauraceae, Winteraceae, Aristolochiaceae, Merispermaceae. Sesquiterpene lactones include: guaianolides, pseudoguaianolides, eudesmanolides, vernolepin, melampodinin A, eupatoriopikrin, germacranolides, arglabin, ridentin, canin, a-santonin, vulgarin, ludovicin [1,2].

Aim of thesis: In the present thesis the current state of knowledge regarding biological pharmacological properties of sesquiterpene lactones and their usage in medicine, are presented.
Materials and methods: Overview and analysis of selected texts concerning sesquiterpene lactones. The following medical bases were used: PubMed, Embase, Web of Science, Medline, Science Direct (Elsevier), from the resources of medical bases of the Main Library of the Silesian Medical University.

Results: Sesquiterpene lactones are created out of acetyl coenzyme A through a stage of mevalonic acid. Through trans cyclization and trans farnesyl pyrophosphate, sesquiterpenes with a germacranolid skeleton are created. The joining of the carboxyl and hydroxyl group and lactonization leads to the creation of sesquiterpene lactones of various carbon skeletons.

Guaianolide type lactones constitute the largest group from among the lactones in the Asteraceae family. The main pharmacological activities of sesquiterpenes are: antibacterial, anti-inflammatory, anticancer, anti-haemorrhagic, antifungal, immunomodulating. The anticancer activity occurs thanks to the particle structure – the presence of the a-methylene-γ-lactone group. DMA-arglabin is an organophosphorus derivative of arglabin. In in vitro tests of DMA-arglabin, a high cytotoxic activity and inhibition of farnesyltransferase, were shown. This enzyme participates in the binding of Ras proteins with the cytoplasmic membrane [2].

Conclusions: The few available sesquiterpene lactone studies, show that these types of compounds are potentially effective anticancer substances. The possibilities of using biotechnology in the process of obtaining these compounds may pave the way to using sesquiterpene lactones in both medicine and pharmacy.

Bibliography:

1. http://rozanski.ch/cancer2000.htm
2. Grech-Baran M., Pietrosiuk A. Arglabina-lakton seskwiterpenowy o właściwościach przeciwnowotworowych, Biul. Wydz. Farma. WUM, 2010, 3, 22-26

The role of pegylation process of anthracycline antibiotics in preventing damage to the heart muscle

Ewa Długosz[1)], Kazimiera Klementys[1)], Wioletta Siemiradzka[2)], Beata Szulc-Musioł[2)]

1). School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Department of Retail Pharmacy, Medical University of Silesia in Katowice, Poland
2). School of Pharmacy with the Division of Laboratory Medicine in Sosnowiec, Department of Drug Form Technology, Medical University of Silesia in Katowice, Poland, e-mail: bszulc@sum.edu.pl

Introduction: Anthracycline antibiotics exhibit the strongest cardiotoxic activity. Their application can lead to the occurrence of post-anthracycline cardiomyopathy, which occurs in about 30% of patients. Modifying the drug particle in pegylation process is based on covalent binding of polyethylene glycol do the drug particle. It influences the supply speed, half-life period and a decrease in the immunological response. Increasing the size of the active particle by adding recurring polyethylene oxide units from which polyethylene glycol (PEG) is made, lowers the filtration level as well as renal excretion which contributes to increasing the bioavailability of the preparation.

Aim: The aim of the present work is researching the influence of the pegylation process on lowering toxicity of anthracycline antibiotics, especially doxorubicin hydrochloride.
Materials and methods: Overview and analysis of the literature concerning the PEGylation process of anthracycline antibiotics. The following medical bases were used: PubMed, Embase, Web of Science, Medline, Science Direct (Elsevier), from the resources of medical bases of the Main Library of the Silesian Medical University.

Results: Pegylated liposomal doxorubicin is a III generation anthracycline. Pegylated drug carriers have better pharmacokinetic properties – increasing accumulation in pathologically changed tissue, extending blood circulation period. The increased accumulation is caused by a decrease in the uptake of liposomes by the reticuloendothelial system, blocking interaction with opsonin and binding water on the surface of the modified carrier, which causes a halt in the binding of plasma proteins.

Conclusions: Using pegylated liposomal doxorubicin causes a greater saturation of the tumor tissue, a better treatment tolerance, possibility of safe combining with other cytostatics, comfortable application for patient – once every 28 days. Cardiotoxicity of pegylated doxorubicin in comparison with the conventional was up to seven times lower. No deaths were observed in connection with the treatment and the therapy was not stopped due to cardiotoxicity.

Bibliography:

1. Pasut G., Veronese FM, Polymer-drug conjugation, recent achievements and general strategies, Prog. Polym. Sci. 2007, 32, 933-961.
2. O'Brien ME, Single-agent treatment with pegylated liposomal doxorubicin for metastatic breast cancer Anticancer Drugs 2008, 19, 1-7.

Synthesis and characterization of melamine-formaldehyde resins with magnetic nanoparticles – medicine carriers

Marta Bartel[1)], Maciej Mazur[1)]

1). Faculty of Chemistry, University of Warsaw (UW), Warsaw, Poland;
martabartel@chem.uw.edu.pl

Delivery of drugs into the body in order to improve the therapeutic efficacy of new and existing pharmaceuticals is one of the main problems, which scientist are facing nowadays. The existing methods of supplying drugs underuse the therapeutic potential of available medicines. The way of delivering drugs by the oral route reduces the chance of reaching the intended dose at the destination, and as a result, a large dose of drug is taken by the patient. On the other hand, there is a problem of instability of therapeutic agents in biological environment and therefore ineffective transport across cell membranes. The use of appropriate drug carries can possibly solve these problems and increase the therapeutic efficacy of drugs used.

This paper reports on synthesis and characterization of melamine – formaldehyde (MF) resins with embedded magnetic nanoparticles that could be potentially used in cancer therapy. Commercially available MF microspheres having the diameter of 1 μm were coated with a thin layer of polypyrrole. This layer is used as a matrix to immobilize previously synthesized nickel zinc ferrite nanoparticles. The modification of the beads with magnetic nanoparticles makes it possible to use them as a contrast agent in magnetic resonance imaging (MRI). Moreover, by immobilization of magnetic nanoparticles, the melamine-formaldehyde microspheres can be directed to their final location in the body by using external magnetic field.

The second part of this study is concerned with loading the microspheres with a fluorescein-labelled mRNA cap analog (GMP – EDA – Flu I2). mRNA cap analog is a new potential anti – cancer pharmaceutical, thus the microspheres with incorporated magnetic nanoparticles and loaded with the cap analog are an interesting model of a drug delivery system.

The bead structures were investigated using transmission electron microscopy, scanning electron microscopy and energy dispersive spectroscopy, SQUID magnetometry, confocal microscopy and IR spectroscopy.

Diagnostic method for occult hepatitis B based on ultra sensitive quantitative real-time PCR

Kurantowicz N.1), Windyga J.2), Kopacz A.3), Brojer E.4), Grabarczyk P.3)

1). Division of Nanobiotechnology, Warsaw University of Life Sciences - SGGW
2). Department of Disorders of Hemostasis and Internal Medicine, Institute of Hematology and Transfusion Medicine
3). Department of Virology, Institute of Hematology and Transfusion Medicine
4). Department of Immunohematology, Institute of Hematology and Transfusion Medicine

Hepatitis B virus (HBV) is a species of the genus Orthohepadnavirus, which is likewise a part of the Hepadnaviridae family of viruses and causes the disease hepatitis B. In addition to causing hepatitis, infection with HBV can lead to cirrhosis and hepatocellular carcinoma and increase the risk of pancreatic cancer. The detection and quantification of hepatitis B virus DNA play an important role in diagnostic and monitoring HBV infection. The main goal of this research was to design a specific diagnostic method for occult HBV infection based on ultra sensitive real-time-PCR to detect low level of HBV DNA during occult HBV infection. Method of isolation was modified, first stage was preincubation of serum with buffer contains protease, then it was isolating in EasyMag by Biomerieux and finally HBV DNA were detected by Real-time-PCR in Rotor Gene 3000 by Corbett Research. Method sensitivity has been checked on standard ratio of HBV DNA from World Health Organization, HBV DNA 100% detection limit of the new method was 5 IU/ml. DNA HBV positive results were testing to determine HBV genotype with line probe assay (INNO-LiPA HBV Genotyping assay; Innogenetics N.V., Ghent, Belgium). Among examined probes HBV genotypes from A to G were performed. In conclusion, it is a great method to detect and quantify occult HBV infection as well as mutants of HBV, it could be used to examine health status among liver transplant patient and to control efficiency of their treatment before transplantation. This diagnostic method could be used by hematologist to evaluate efficiency of treatment and disease monitoring.

Composite microspheres PLGA/AuNPs/FA – model theranostics

Paulina Głowala, Maciej Mazur
University of Warsaw, Faculty of Chemistry, Pasteura 1, 02-093 Warsaw, Poland
pglowala@chem.uw.edu.pl

Multifunctional polymer colloidal structures are the subject of worldwide conducted intensive research. [1] This is due to their exceptional properties which are used inter alia in chemical analysis, catalysis and medicine. [2] The medical applications are especially interesting because multifunctional polymer microspheres may be used in medical diagnosis as well as therapy. [3]

A model theranostic consists of PLGA microspheres, gold nanoparticles and folic acid. The PLGA, which is a biocompatible and biodegradable polymer, constitutes a matrix where the other components are embedded. The gold nanoparticles deposited on the surface of PLGA microspheres are intended to provide contrast in computed tomography (CT), which is a 3D imaging medical diagnostic tool. On the other hand, the adsorption of folic acid onto gold nanoparticles is believed to facilitate penetration of the carriers into the tumor cells. Moreover, the folic acid molecule is modified with polyethylene glycol moiety which prevents removal of the carriers from the circulatory system by phagocytosis. Such a designed drug vehicle is capable to carry pharmaceuticals, e.g. doxorubicin.

Composite microspheres PLGA/AuNPs/FA have been thoroughly characterized with transmission electron microscopy, scanning electron microscopy, confocal microscopy, thermogravimetry, energy-dispersive X-ray spectroscopy, X-ray photoelectron spectroscopy and Fourier transform infrared spectroscopy.

Biological research on composite PLGA/AuNPs/FA showed that it is biocompatible and non-toxic (the carriers were tested in vivo on male Wistar rats). The drug carriers loaded with doxorubicin have been also used in in vitro studies on cultured breast cancer cells MCF-7. The study confirmed that the carriers can penetrate into tumor cells, and thus are effective for drug delivery.

1). F. Caruso, Colloids and Colloid Assemblies: Synthesis, Modification, Organization and Utilization of Colloid Particles, Wiley-VCH 2004.
2). H. Kawaguchi, Progress in Polymer Science 2000, 25, 1171-1210.
3). F. Danhier, E. Ansorena, J.M. Silva, R. Coco, A. Le Breton, V. Préat, Journal of Controlled Release 2012, 161, 505-522.

Magnetoliposomes as Potential Carriers of Doxorubicin to Tumours

Aleksandra Joniec, Paweł Krysiński

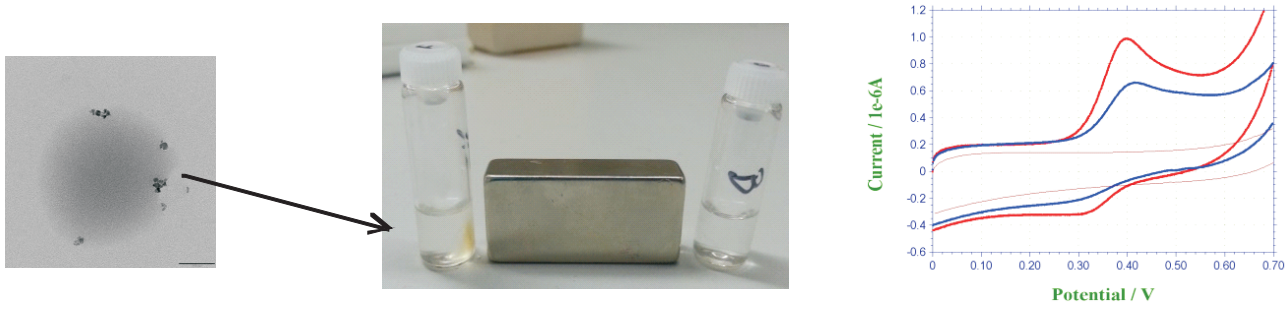
Faculty of Chemistry, University of Warsaw , Pasteura 1, Warsaw 02-093, Poland
aleksandra.joniec@student.uw.edu.pl

Although doxorubicin (DOX) is highly effective anticancer drug, its usage is limited due to side-effects it causes – probably the most serious of them is heart failure. In conventional therapies the patient is treated with doxorubicin solutions, administered intravenously. This route results in the drug acting not only on cancer tissues, but also on healthy ones. One method of avoiding the side-effects of this drug is to use carriers that would transport medicine directly to malignant tissues with it subsequent, controlled release. Here we report the design and synthesis of magnetoliposomes loaded with DOX.

The first step to obtain such structures was synthesis of small hydrophobic magnetic iron oxide nanoparticles (MNP), that were meant to be a vector for DOX molecules in external magnetic field. These structures consisted of a hydrophilic superparamagnetic core and a protective hydrophobic layer of oleic acid, bound to the core surface. They were characterised with SEM, EDS, SQUID, TG and TEM techniques. Due to their small size (diameter around 4-12 nm) and hydrophobic properties, they could be then incorporated into the bilayer membrane of liposomes, thus forming magnetoliposomes.

Next, doxorubicin was loaded inside such magnetoliposomes using two methods – passive and pH-gradient loading (remote loading). The presence of MNP inside the liposome membrane enabled the drug release by using controlled low frequency alternating magnetic field. The kinetics of drug release and its amount was studied using electrochemical and spectroscopic methods.

We believe magnetoliposomes will be useful in anticancer therapies.



Left: TEM picture of magnetoliposome – dark spots are MNP agglomerates. Scale bar is 100 nm

Middle: Magnetoliposomes with DOX accumulated with a magnet at the wall of a vial (B – liposomes without MNP).

Right: Cyclic voltammogram confirming, that DOX is released from magnetoliposomes under temperature stress.

Acknowledgments: A. Joniec thanks the Polish Ministry of Sciences and Higher Education for support through the project "Diamond Grant" no. 0172/DIA/2014/43 and Warsaw Consortium of Academic Chemistry for KNOW scholarship.

Tumor-derived osteopontin and lactadherin shape tumor-associated macrophages polarization and immune response in rat model of glioma

Pszczolkowska Dominika, Kijewska Magdalena, Ellert-Miklaszewska Aleksandra, Wisniewski Pawel, Kaminska Bozena

Laboratory of Molecular Neurobiology, Neurobiology Center, The Nencki Institute of Experimental Biology, Warsaw, Poland

Malignant gliomas are fast-growing, heterogeneous and invasive brain tumors strongly infiltrated by non-tumor cells. Glioma attracts variety of immune cells, in particular microglia/macrophages and re-program these cells into immunosupresive, tumor-supporting cells. Factors responsible for pro-invasive macrophage polarization and shaping tumor microenvironment in tumor-supporting manner are poorly known. We analysed glioma secretome using proteomical approach and identified lactadherin (Mfge8) and osteopontin (Spp1) in microglia-activating fractions. Both osteopontin and lactadherin are avβ3/avβ5 integrin ligands able to interact with receptors present on microglia and macrophages and thus could be involved in pro-invasive polarization of microglia/macrophages. Moreover, both Spp1 and Mfge8 are overexpressed in glioma cells, but not in non-transformed astrocytes.

C6 glioma cells stably expressing shRNA specific to lactadherin (shMfge8), osteopontin (shSpp1) and negative shRNA (shNeg) were implanted into striatum of Wistar rats. There was no difference in proliferation and viability of C6 glioma cells, cells stably expressing shRNA specific to lactadherin, ostopontin and negative shRNA in vitro, that demonstrates the negligible effect of autocrine production of these protein on tumor cell growth. Knockdown of Spp1 and Mfge8 resulted in reduction of tumor volume in rat model of glioma. Immunochemical analysis of brain sections revealed similar numbers of infiltrating microglia/macrophages (Iba1 staining), but the reduced number of amoeboid, arginase 1 expressing cells in Mfge8- nad Spp1-depleated tumors. We also investigated blood vessels density in lactadherin and osteopontin-depleted tumors. Our results suggest that glioma-derived integrin ligands are important factor in polarization of glioma infiltrating microglia/macrophages into the pro-invasive phenotype and its targeting could be a new therapeutic strategy.

Studies supported by a grant 2012/04/A/NZ3/00630 from the National Science Center.

Macrophages interact with mammary cancer cells via non-canonical WNT pathway

Bartlomiej Taciak, Magdalena Krol

Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Poland; E-mail: bartlomiej_taciak@sggw.pl

Macrophages can constitute even 50% of the tumor mass and in mammary metastatic cancer the number of tumor-associated macrophages is significantly higher than in non-metastatic tumors [1]. Recent results [2] showed, for the first time, that macrophages could mediate `switch` between canonical and non-canonical Wnt signaling pathway in cancer cell. The increasing number of macrophages migrating to the tumor mass due to tumor growth and development inhibits Wnt/β-catenin signaling leading to a decreased cancer cell proliferation and survival, but as a `side effect`, they activate the non-canonical Wnt pathway and induce metastasis. Our results showed that co-culture of macrophages with canine and murine mammary cancer cells induced in cancer cells expression of genes belonging to non-canonical Wnt pathway. Especially, we observed high expression of Wnt/PCP pathway genes (e.g. Ephb2, Fzd10, Dvl2, Rac1). Thus, based on our results it can be definitely concluded that macrophages increase cancer cells malignancy, changing their phenotype to mesenchymal by switching between canonical and non-canonical Wnt signaling pathway.

Acknowledgments: work supported by the National Science Centre (UMO-2013/09/D/NZ5/02496)

References: [1]. Król M, Pawłowski KM, Majchrzak K, Dolka I, Abramowicz A, Szyszko K, Motyl T (2011). Pol J Vet Sci 2011, 14:3-10.; [2]. Król M, Majchrzak K, Mucha J, Homa A, Bulkowska M, Majewska A, Gajewska M, Abramowicz A, Musielak O, Dolka I, Pawłowski KM, Pietrzak M, Perszko M, Romanowska K, Motyl T (2014) PLoS One. 2014 Jan 3;9(1):e83995.

P16 | 21-22.05.2015

Profiling of microRNA expression in canine mammary cancer

Malgorzata Bulkowska[1], Agata Rybicka[1], Kerem Mert Senses[2], Katarzyna Ulewicz[1], Katarzyna Witt[1], Joanna Szymanska[1], Robert Klopfleisch[3], Eva Hellmen[4], Izabella Dolka[5], Ali O. Gure[2], Joanna Mucha[1], Magdalena Krol[1]

1). Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Nowoursynowska 159, 02-776 Warsaw, Poland
2). Department of Molecular Biology and Genetics, Faculty of Science, SB Building, Bilkent University, 06800 Ankara, Turkey
3). Institute of Veterinary Pathology, Freie Universitaet Berlin, Robert-von-Ostertag-Strasse 15, Building 31, 14163 Berlin, Germany
4). Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, Ulls vag 6, 756 51 Uppsala, Sweden
5). Department of Pathology and Veterinary Diagnostics, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Nowoursynowska 159, 02-776 Warsaw, Poland

Background: There were found several common oncogenic microRNAs for canine mammary cancer and human breast cancer. microRNAs may act as oncogenes or tumour suppressor genes, which make these small molecules the potential diagnostic/prognostic factors and the targets for anticancer therapies. On account of this, large-scale profiling of microRNA expression in canine mammary cancer seems to be important for both dogs and humans.

Methods: The expression profiles of 317 microRNAs in 146 canine mammary tumours of different histological type, grade and clinical history (presence of metastases) and in 25 control group samples were identified. The profiling was performed using microarrays (miRCURY LNATM microRNA Array 7th generation, Exiqon). Benjamini and Hochberg adjustment method, one-way ANOVA test and Tukey's 'Honest Significant Difference' test were applied in the analysis of data.

Both unsupervised and supervised data analyses were performed. Validation of obtained results was performed using real-time qPCR. Predicted targets for microRNAs were searched in miRBase.

Results: The results of the unsupervised analysis show that the primary factor separating the samples is the metastatic status. In the supervised analysis 83, 178 and 171 microRNAs were identified that are significantly differentially expressed in different groups for tumour type, malignancy grade and metastasis factor, respectively. Predicted targets for microRNAs differentially expressed in metastatic vs. non-metastatic group are mostly engaged in cell cycle regulation, cell differentiation and DNA-damage repair.

Conclusions: The most significant difference in microRNA expression was observed between metastatic and non-metastatic group, which indicates a greater role of miRNAs in the metastasis process than in the malignant transformation. This main result also makes the differentially expressed miRNAs the potential metastasis markers.

These results were supported by the grant No. 2011/03/B/NZ5/05299 from National Science Centre.

P17 | 21-22.05.2015

The influence of IL-28/IL-28RA protein-protein interaction on angiogenesis in mouse mammary cancer

Katarzyna Witt[1], Joanna Mucha[1], Bartłomiej Taciak[1], Katarzyna Ulewicz[1], Tomasz Rygiel[2], Mattia Mori[3], Bruno Botta[4], Magdalena Król[1]

1). Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Nowoursynowska 159, 02-776 Warsaw, Poland

2). Department of Immunology, Medical University of Warsaw, Poland

3). IIT Center for Life Nano Sciences, CLNS@Sapienza, Roma, Italia

4). Dipartimento di Chimica e Tecnologie del Farmaco, Università "La Sapienza" di Roma, Roma, Italia

Recent studies showed that IL-28 is secreted by Myeloid-Derived Suppressor Cells (MDSCs) which are associated with tumor progression and metastasis (Mucha et al. 2015). Transplantation of MDSCs into tumor-bearing mice significantly promotes tumor growth whereas anti-MDSC treatment has been successfully used as a part of anti-cancer therapy.

We showed, that in mice in CD11b cells isolated from lung metastases expression of IL-28 was significantly higher than in CD11b cells isolated from primary tumor with no detectable metastases. The expression of IL-28 correlated with the number of MDSC.

We showed that that in EMT6 and 4T1 cell lines IL-28 binds to its receptor (IL-28RA) and triggers STAT3 signaling cascade leading to VEGF overexpression. Therefore, tumor cells treated with IL-28 induced endothelial cells proliferation and their 3D vessel formation in HUVEC 3D angiogenesis test. Interestingly, co-culture of endothelial cells with control cancer cells or with IL-28 alone induced only slight endothelial cell branching. Moreover, IL-28 promotes cancer cell migration and invasion in Boyden chambers.

Knowing 3D structure of IL-28/IL-28RA complex, we were able to select among the 850 natural compounds of chemical libraries belonging to Botta's unit a few small molecule inhibitors that disturb IL-28/IL-28RA protein-protein interaction.

Our research shows that IL-28/IL-28RA protein-protein interaction may play an important role in MDSCs-related cancer development and might by a new therapeutic target.

Acknowledgements: This work was supported by the grant from National Science Centre UMO-2013/09/D/NZ5/02496.

Morphological and histopathological characterization of feline fibrosarcoma growth on CAM - a new model in veterinary oncological studies

K. Zabielska[1)*], A. Wojtkowska[1)], I. Dolka[2)], A. Żbikowski[2)], R Lechowski[1)]

1). Department of Small Animal Diseases with Clinic, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Poland

2). Dpartment of Pathology and Veterinary Diagnostics, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Poland

*Corresponding author: katarzyna_zabielska@sggw.pl

Introduction

Chick embryo chorio-allantoic membrane (CAM) model is a cheap, easy to perform model, which does not need the Bioethics Commision approval. It has been utilized in studies for human glioma, osteosarcoma and colon cancer. In veterinary medicine, the use of CAM model was only described for feline vaccine-associated fibrosarcoma growth from FFS1 WAW cell line. The main objective of this study was to assess the ability of three feline fibrosarcoma cell lines (FFS1, FFS3, FFS5) to form solid tumors on CAM and describe their morphological and histopathological features. The proliferative activity by monoclonal antibodies against PCNA and Ki-67 were evaluated. The PCNA and Ki-67 index are helpful for diagnosis and prognosis in various tumors.

Materials and methods

Cell lines were cultivated under standard aseptic conditions (5% CO2, 95% humidity and temperature 370C) in DMEM enriched with glucose (4500mg/L), 10% fetal bovine serum and antibiotics (Penicillin with Streptomycin, Amphotericin B). The medium was changed every 48–72 hours. On the 6th day of incubation the FFS1, FFS3, FFS5 cells were inoculated into sterile silicon rings placed on CAM (5x106 cells in 25ul of medium per egg) according to the procedure described previously (Zabielska et al., 2012). After 10 days tumors growth was observed and immunohistochemical nuclear staining for Ki-67 (clone MIB-1, 1:75 Dako) and PCNA (clone PC10, 1:200, Dako,) was performed. Histological grading was calculated according to scale proposed by Couto et al. (2002).

Results and conclusions

1. All tested feline fibrosarcoma cell lines (FFS1, FFS3, FFS5) can form solid tumors on CAM.
2. No correlation between Ki-67 index and tumors grade was observed.
3. The PCNA index revealed a good correlation with the grading of feline fibrosarcomas, what indicates that it may be a better prognostic marker than Ki-67.

Acknowledgments

We would like to thank to prof. Manfred Reinacher (Justus Liebig University, Germany) for kind providing of FFS1, FFS3 and FFS5 cell lines. The authors would like to acknowledge support by the Polish National Science Centre Project No. DEC-2012/07/N/NZ4/02413.

Quercetin promotes migration of mouse mammary cancer cells EMT6 and 4T1 via the switch between canonical and non-canonical Wnt pathway

Katarzyna Ulewicz[1], Bartłomej Taciak[1], Katarzyna Witt[1], Joanna Mucha[1], Mattia Mori[2], Bruno Botta[3], Magdalena Król[1]

1). Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences - WULS, Warsaw, Poland
2). IIT Center for Life Nano Sciences, CLNS@Sapienza, Roma, Italia
3). Dipartimento di Chimica e Tecnologie del Farmaco, Università "La Sapienza" di Roma, Roma, Italia

Quercetin is considered as a potent anticancer drug which acts by inhibition of canonical Wnt pathway via β-catenin/Tcf signalling. However, in cancer cell, that inhibition of β-catenin may lead to `switch` between canonical and non-canonical Wnt signalling pathway.

Our results showed that treatment of EMT6 and 4T1 mammary cancer cells with quercetin increases in cancer cells expression of genes belonging to non-canonical Wnt pathway, in particular Wnt/PCP (e.g. Ephb2, Fzd10, Dvl2, Rac1). The same was confirmed at protein level. Moreover, we showed that quercetin enhances migration of EMT6 and 4T1 cancer cells exalmined in Boyden chambers in vitro.

Inhibition of canonical Wnt pathway leads to decrease in cancer proliferation and survival, but the `side effect` of this function, ipso facto, is activation of non-canonical Wnt pathway, which promotes cancer metastasis. Quercetin inhibits transcriptional activity of β-catenin/Tcf and inhibits the canonical Wnt pathway, although it activates Wnt non-canonical pathway and promotes migration and invasion of cancer cells. Therefore, relevance of patient treatment with canonical Wnt inhibitors should be discussed and further investigated.

Acknowledgments: work supported by the National Science Centre (UMO-2013/09/D/NZ5/02496)

BASTION abstracts

B.1-12

Peroxiredoxins-1 and 2 affect proliferation and survival of lymphoma cells

Małgorzata Firczuk[1], Anna Trzeciecka[1], Małgorzata Bajor[1], Angelika Muchowicz[1], Radosław Zagożdżon[1], Joanna Barankiewicz[1], Antoni Domagala[1], Szymon Klossowski[2], Agata Malinowska[3], Justyna Chlebowska[1], Ryszard Ostaszewski[2], Jakub Golab[1], Dominika Nowis[4],[5]

1). Department of Immunology, Medical University of Warsaw, Poland
2). Institute of Organic Chemistry, Polish Academy of Sciences, Warsaw, Poland
3). Laboratory of Mass Spectrometry, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Warsaw, Poland
4). Genomic Medicine, Department of General, Transplant and Liver Surgery, Medical University of Warsaw, Poland
5). Laboratory of Experimental Medicine, Center of New Technologies, Warsaw University, Poland

Introduction: Peroxiredoxins (PRDXs) are abundant antioxidant enzymes. In mammals there are six PRDXs. Among them, four (PRDXs 1-4) are functional dimers. PRDXs are oxidoreductases that scavenge peroxides with reducing equivalents from thioredoxin-thioredoxin reductase system. PRDXs function as a defense against redox stress but their role far exceeds peroxides removal. They directly support the activation of some protein tyrosine phosphatases, suppressing kinase-mediated signaling. PRDXs are usually highly expressed in tumor cells, but there are cases of epigenetic downregulation. Depending on a particular model and the stage of tumor development, PRDXs can either suppress or support tumor cell growth. The role of peroxiredoxins in B-cell derived tumors has not been studied so far.

Methods: We have used two Burkitt's lymphoma cell lines: Raji and Ramos. To isolate protein targets for a novel small molecule peptidomimetic inhibitor1 with antitumor activity we have used biotin affinity probe labelling approach and mass spectrometry. Covalent crosslinking of PRDXs dimers as well as the activation of MAPK signaling were investigated by Western blotting.

We employed quantitative real time PCR and Western blotting techniques to analyze the expression of PRDX1 and PRDX2 in a range of human leukemia and lymphoma cell lines at the mRNA and the protein levels, respectively. To study the role of PRDX1 and PRDX2 in lymphoma cells, we have employed the shRNA-mediated knockdown approach. To evaluate cell viability and proliferation rate, the number of viable cells were counted in a hemocytometer or, alternatively, propidium iodide-negative cells were counted using BD Accuri flow cytometer. To study cell cycle we analyzed DNA content by propidium iodide staining followed by flow cytometry and evaluated the levels of cell cycle associated markers, e.g. p21, cyclin E2 using Western blotting. To detect apoptosis we evaluated the levels of cleaved caspase-3, caspase-9, and PARP with Western blotting.

Results: We have found that in human Burkitt's lymphoma cell lines, a novel peptidomimetic inhibitor with antitumor activity targets dimeric 2-cystein peroxiredoxins. In Raji and Ramos cells, the inhibitor covalently crosslinks PRDX dimers, triggers MAPK activation, cell cycle arrest in G1 phase and apoptosis. Next, we have analyzed the levels of PRDX1 and PRDX2 in a range of human leukemia and lymphoma cell lines, and found them upregulated, as compared to normal B cells.
To further study the role of PRDX1 and PRDX2 in lymphoma cells, we have reduced the level of PRDX1 in Raji cells expressing significant levels of PRDX2 and in a sub-clone of Raji cells, which does not express PRDX2. The effects of PRDX1 downregulation strongly depend on the level of PRDX2 expression. In cells, which express PRDX2, a decrease in PRDX1 expression results in the suppression of cell proliferation and cell cycle arrest in the G1 phase. In Raji cells, which do not express PRDX2, the downregulation of PRDX1 entirely inhibits cell growth and induces apoptosis.

Conclusions: We show that in lymphoma cell lines dimeric peroxiredoxins are targets for a small molecule peptidomimetic inhibitor. Our findings indicate that PRDX1 and PRDX2 support lymphoma cell proliferation and survival. Moreover, they can substitute each other, at least to some extent, suggesting overlapping functions of these two cytoplasmic PRDXs in lymphoma cells.

References:

1. Kłossowski S, Muchowicz A, Firczuk M, Swiech M, Redzej A, Golab J, Ostaszewski RJ. 2012. Studies towards novel peptidomimetic inhibitors of thioredoxin-thioredoxin reductase system. J Med Chem. Jan 12;55(1):55-67.

Comparison of whole-exome and custom-gene sequencing as tools for analysis of chronic myeloid leukemia progression

Iwona Solarska 1),4), Marcin M. Machnicki1),2), Joanna Niesiobedzka-Krezel3), Piotr Stawinski5), Rafal Ploski5) i Tomasz Stoklosa1)*

1). Department of Immunology, Center of Biostructure Research, Medical University of Warsaw, Poland.
2).Postgraduate School of Molecular Medicine, Warsaw, Poland.
3). Department of Hematology, Oncology and Internal Diseases, Medical University of Warsaw, Poland.
4). Department of Diagnostic Hematology, Institute of Hematology and Transfusion Medicine, Warsaw, Poland.
5). Department of Medical Genetics, Medical University of Warsaw, Poland.
* e-mail: tomasz.stoklosa@wum.edu.pl

Research in the field of cancer genetics accelerated rapidly after the introduction of next-generation sequencing (NGS). One of the model human neoplasms is chronic myeloid leukemia (CML), the first human malignancy in which a genetic abnormality was linked to an oncogenic transformation and a successful targeted treatment was developed. The BCR-ABL1 fusion gene resulting from t(9;22)(q34;q11) reciprocal translocation, encodes constitutively active tyrosine kinase that can be effectively inhibited by tyrosine kinase inhibitors (TKI). While TKI therapy induces durable remission in majority of patients, leukemic stem cells are never fully eradicated and significant number of patients experience relapse of the disease.

We decided to investigate mechanism of CML progression by sequencing paired diagnosis-progression samples using two different NGS-based approaches in order to find new genetic abnormalities responsible for disease progression and/or TKI-resistance. Here we show a pilot analysis of paired samples from patients who progressed to advanced phases by whole-exome (44.1 Mb) versus custom-gene sequencing (7MB).

DNA was isolated from leukemic cells from peripheral blood collected at two time points: at diagnosis and at acute phase (either accelerated phase or blast crisis). Whole-exome / custom sequences were acquired by solution-based capture (Roche NimbleGen) and high-throughput sequencing (Illumina HiSeq1500). Mutational differences between samples were analyzed by Mutect.

In the exome-sequenced patient AA samples, 19 variants were detected exclusively in the diagnostic sample and 15 in the sample from progression by Mutect. In the custom-sequenced patient BB samples, 9 variants were exclusive for diagnostic sample and 13 for the sample from acute phase. Most of these variants were rare according to NHLBI Exome Sequencing Project (absent in database), suggesting an ongoing clonal evolution of the leukemic clones.

The most biologically significant mutation in patient AA (present only in the progression sample) was a G>C transversion in ABL1 kinase domain of BCR-ABL1, which results in p. Q252H (NM_005157) substitution in the P-loop and not only causes resistance to imatinib, but is also a negative prognostic factor. In patient 2, the most significant mutation was a RUNX1 G>T transversion, resulting in p.R166L substitution. Various aberrations of RUNX1 were previously shown to be a recurring event in CML blast crisis. Both mutations were completely absent at diagnosis, which was additionally confirmed for BCR-ABL1 mutation by using targeted amplicon sequencing.

We also analyzed copy number variation data in both patients using CEQer. No significant changes were detected at covered regions between samples from different time points.

In conclusion, we were able to detect mutations in chronic myeloid leukemia of two patients that arose during progression of the disease by using whole-exome and custom-gene sequencing. We were also able to determine with high sensitivity if patients already harbored leukemic clones with mutations at the time of diagnosis, proving that NGS is a promising and valuable tool for investigation of the genetic background of CML progression.

Adenanthin, a new peroxiredoxin inhibitor, induces a switch between estrogen receptor alpha-mediated and Akt-driven signaling in breast cancer cells

Malgorzata Bajor[1], Agata O. Zych[1], Patrick C. O'Leary[2],[3], Anna Czekalska[1], William M. Gallagher[3], Jakub Golab[1], Radoslaw Zagozdzon[1]

1). Department of Immunology, Center of Biostructure Research, Medical University of Warsaw, Warsaw, Poland
2). Helen Diller Family Comprehensive Cancer Center & Dept. of Cell and Molecular Pharmacology, University of California San Francisco, San Francisco, USA
3). Cancer Biology & Therapeutics Laboratory, UCD Conway Institute, UCD School of Biomolecular and Biomedical Science, Dublin 4, Ireland

Increasing evidence indicates that oxidative stress is involved in the progression of estrogen receptor (ER)-positive breast cancer. A moderate increase in cellular oxidants contributes to the genomic instability and to the change in cellular growth pattern, which in turn can facilitate progressive transformation of normal cells into cancer cells. Accordingly, the oxidative stress-related gene expression signature has been suggested to correlate with therapy resistance and poorer outcome in breast cancer. Therefore, it is crucial to determine the antioxidant defense mechanisms that are utilized by breast cancer cells to regulate oxidative stress.

Peroxiredoxin 1 (PRDX1) is one of the most prevalent hydrogen peroxide scavenging enzymes in mammalian cells. Our recent studies indicated that PRDX1 is an independent biomarker of favorable prognosis in ER-positive breast cancer. Our results indicate the mechanistic link between PRDX1 and ERa in breast cancer and suggest a role for PRDX1 in mammary carcinogenesis. We provide a molecular explanation for this phenomenon in the current project.

To evaluate the importance of PRDX1 activity in ER-positive breast cancer, we have used adenanthin, a newly described PRDX1/2 inhibitor. In our studies, we have shown that adenanthin

strongly inhibits metabolism of exogenous hydrogen peroxide by breast cancer cells. This phenomenon is accompanied by a shift from H2O2-degrading PRDX1 dimers into enzymatically inactive monomers and by a dramatic decrease of ERa protein presence in the cells. Moreover, we have observed that incubation of ER-positive breast cancer cells with adenanthin leads to a marked increase in phosphorylation status of proteins associated with Akt-driven signaling in breast cancer. Thus, our results suggest that PRDX1 can play an important role in controlling the switch between estrogen receptor- and growth factor-driven signaling in breast cancer.

In summary, in our studies we describe for the first time molecular consequences of rapid dysfunction of PRDX-related system in ER-positive breast cancer. The deeper knowledge on the mechanisms of PRDX1 functioning can change our understanding of the events leading to the progression of ER-positive breast cancer and provide new opportunities for pharmacological interventions in this disease, especially in the context of recent observations connecting the oxidative stress and resistance to endocrine therapy.

Immunophenotypic identities of clinical samples have the potential to correlate with overall survival in cytogenetically normal AML patients

Pawel Gaj[1),2)], Dominika Nowis[3),4)] , Stefano Volinia[5),]

1). Department of Immunology, Center of Biostructure Research, Medical University of Warsaw, Banacha 1A, 02-097 Warsaw, Poland
2). Laboratory of Human Cancer Genetics, Centre of New Technologies, CENT, University of Warsaw, Banacha 2C, 02-097 Warsaw, Poland
3). Genomic Medicine, Department of General, Transplant and Liver Surgery, Medical University of Warsaw, Banacha 1A, 02-097 Warsaw, Poland
4). Laboratory of Experimental Medicine, Center of New Technologies, CENT, University of Warsaw, Banacha 2C, 02-097 Warsaw, Poland
5). Department of Morphology, Surgery and Experimental Medicine, Human Anatomy Branch, University of Ferrara, 44121 Ferrara, Italy

Tumor infiltrating lymphocytes (TILs) have been described to have great potential to modulate course of the disease. Capacity to eliminate cancer cells primarily depends on the interplay between tumor and its microenvironment. Despite the great interest which this topic gathered over last decades the role of immune cells interaction with cancer has hardly been studied in acute myeloid leukemia patients.

Multitude of patient-specific factors, as well as a great repertoire of different kinds of tumor-infiltrating immune cells, potentially affecting disease progression should be the motivation to investigate implication of tumor immune cells interplay in possibly maximized cohorts of patients. Due to technical reasons the traditional microscopy-based techniques do not allow for the desired high-throughput approach.

In the present study we demonstrate an inter-platform-compatible method which allowed us to predict the gene expression component involvement of 37 cell populations representing different

stages of hematopoietic linage differentiation. The predictions were done for an extensive set of peripheral blood mononuclear cells (PBMC) and bone marrow aspirate material sampled from cytogenetically normal (CN) AML cases.

Our results nicely confirmed poor survival prognosis observed for the patients demonstrating high contribution of the CD34+, CD38- hematopoietic stem cells in their bone marrow samples HR=2.05, 95%CI=1.22-3.43, p-value=6.73E-03. We also show that a specific Mature NK cell_CD56+ CD16+ CD3- component found in gene expression signatures of bone marrow samples correlated independently with significantly worse survival in CN AML patients HR=2.56, 95%CI=1.49-4.42, p-value=7.03E-04.

Same analysis carried for the CN AML PBMC samples indicated independent positive effects for the predicted involvement of the Erythroid_CD34+ CD71+ GlyA- and Hematopoietic stem cell_CD133+ CD34dim cell populations in The Cancer Genome Atlas (TCGA) CN AML cohort.

Acknowledgements

The research was supported by a grant from European Commission 7th Framework Programme: FP7-REGPOT-2012-CT2012-316254-BASTION and a grant from the National Science Centre Poland [grant no 2013/10/E/NZ5/00778 (DN)].

The role of ATM-CHEK2-BRCA1 axis in determination of genetic predisposition and clinical presentation of papillary thyroid carcinoma

Anna Wójcicka, Małgorzata Czetwertyńska, Michał Świerniak, Joanna Długosińska, Monika Maciąg, Agnieszka Czajka, Kinga Dymecka, Adam Kot, Rafał Płoski, and Krystian Jażdżewski

Genomic Medicine; Department of General, Transplant and Liver Surgery; Medical University of Warsaw (AW, MCz, MŚ, MM, AC, KD, KJ); Department of Endocrine Oncology and Nuclear Medicine, Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland (MCz, JD); Department of Nuclear Medicine and Endocrine Oncology, Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology, Gliwice, Poland (MŚ); Department of Medical Genetics, Medical University of Warsaw, Poland (RP); Centre of New Technologies, CENT, University of Warsaw (AW, AK, KJ).

Background: Risk of developing papillary thyroid carcinoma (PTC), the most frequent thyroid malignancy, is elevated up to 8.6-fold in the first-degree relatives of PTC patients, what could be explained by polygenic action of low-penetrance alleles. Since the DNA-damaging exposure to ionizing radiation is a known risk factor for thyroid cancer, polymorphisms in DNA repair genes are likely to affect this risk. Among the DNA repair proteins, the ATM-CHEK2-BRCA1 axis seems to be of particular interest. In response to double-strand DNA breaks, ATM is recruited to DNA damage sites, phosphorylating BRCA1 and CHEK2 and initiating a signaling cascade of DNA damage response and cell-cycle control proteins.

Aim of the study: The aim of this study was to identify low-penetrance susceptibility alleles for PTC by genotyping deleterious SNPs in genes involved in DNA damage-response and cell-cycle pathways.

Methods and results: Sequenom iPLEX technology was employed to genotype polymorphisms: rs1801516 in ataxia telangiectasia mutated (ATM), rs17879961 in CHEK2 checkpoint yeast homolog (CHEK2), and rs16941 in breast cancer 1 gene (BRCA1) in 1781 PTC patients and 2081 healthy controls. We identified BRCA1 rs16941 (odds ratio [OR]=1.16, P=0.005) and CHEK2 rs17879961 (OR=2.2, P=2.37e-10) as the risk alleles for PTC. ATM rs1801516 variant modifies the risk associated with BRCA1 variant by 0.78 (P=0.02). Both ATM and BRCA1 variants modify the impact of male gender on clinical variables: T status (P=0.007), N status (P=0.05), and stage (P.=0.035).

Conclusion: This is the first study showing the complex association between genetic variants of ATM-CHEK2-BRCA1 axis and the predisposition to PTC. The study supports previous findings on the importance of age and gender on the clinical outcome of the disease and showed that this effect is significantly altered by the minor alleles of the analysed genes, emphasizing their importance in the pathogenesis of PTC.

Funding: This work was supported by Foundation for Polish Science, Programme FOCUS and Programme TEAM, co-financed by the European Union European Regional Development Fund. The Laboratory of Genomic Medicine of the Medical University of Warsaw participates in Bastion, a Programme financed by the European Union (FP7-REGPOT-2012-CT2012-316254-BASTION).

Functional analysis of SMAD4 mutants in an in vitro system reveals upregulation of SMAD2, SMAD3 and SMAD4 by Myhre syndrome-associated variants.

Lech Trzeciak 1, Paweł Gaj 2, Agata Skórka 3, Paulina Nadkowska 4, Agnieszka Pollak 5, Joanna Kosińska 1, Rafał Płoski 1 , Radosław Zagożdżon 2

1 – Department of Medical Genetics, Medical University of Warsaw, Poland
2 – Department of Immunology, Institute of Biostructure, Medical University of Warsaw, Poland
3 – Department of Pediatrics, Warsaw Medical University, Warsaw, Poland
4 – graduate student of Medical University of Warsaw, Poland
5 – Department of Genetics, Institute of Physiology and Pathology of Hearing, Kajetany/Warsaw, Poland

Background and aim: SMAD4 protein is a crucial element of TGFb signaling pathway, trimerizing with SMAD2 and/or SMAD3, translocating to a nucleus and (co)regulating transcription. Disturbances of TGFb pathway are often oncogenic. Somatic SMAD4 mutations occur in pancreatic, gastrointestinal and skin cancers, while inherited SMAD4 mutations lead to juvenile polypopsis syndrome with ~20% risk of malignancy. In contrast, de novo mutations altering I500 in SMAD4 were in 2012 identified as a cause of non-cancerous, developmental Myhre syndrome (MIM#139210). We have recently discovered another mutation of SMAD4 (p.R496C) in an atypical case of Myhre syndrome. Little is known about the molecular function of Myhre-associated mutants and similarly located oncogenic mutations (such as R496H). Here we report an attempt to create a framework for detailed studies of these differences.

Methods: The 3D structure of SMAD4 homotrimer (PDB 1DD1) was analysed and effects of 496C versus 500M mutations were modeled in silico. Human Flag-tagged SMAD4 cDNA (wild-type) in pBabe-puro mammalian expression vector was obtained from Addgene and site-directed PCR-based

mutagenesis was used to create derivative R496C and I500M constructs. These constructs were transfected into HEK293 cells. Puromycin-resistant cell pools were stimulated with TGFb for 0, 2 and 5 hours. Expression of SMAD2, SMAD3 and SMAD4 protein as well as phosphorylation of SMAD2 and SMAD3 was studied by Western blotting. Transcript levels of several TGFb-dependent genes were compared by SybrGreen-based quantitative PCR (qPCR).

Results and conclusions: 3D modeling indicated that R496 and I500 are located very close to the intermolecular binding interface within homotrimer, with R496 having far larger impact on other interface aminoacids. Simulated mutations in I500 caused a very pronounced free energy increase of the D493 location, suggesting a strong involvement in maintenance of the multimer structure of the SMAD4 active complex. Additionally, I500 is spatially adjacent to ubiquitinylated L519. Western blotting technique revealed that all three SMAD4 fusions were expressed in HEK cells on a similar level; surprisingly, presence of 496C (and, to less extent, 500M) led to a disproportional increase of endogenous SMAD4 level accompanied by an increase of SMAD2 and SMAD3. SMAD2 phosphorylation after TGFb treatment was however similar in all 3 lines. Also RT-qPCR did not detect significant differences in a steady-state expression of COL1A1, SERPINE1 and SMAD6. Further studies should address gene expression after TGFb stimulation and expand the list of studied genes (transcriptome analysis); analysis of SMAD4 ubiquitination and SMAD4 mRNA level would address possible mechanisms of SMAD4 upregulation.

Financing: LT, PG, RP and RZ are supported by Bastion under 7th Framework Programme (FP7-REGPOT-2012-CT2012-316254-BASTION). PN research was sponsored by a minigrant 1M19/NM6/14 from Medical University of Warsaw.

c.449-1G>T mutation of TMC8 gene as an unreported frequent cause of EV in Polish population – a case study and molecular basis

Joanna Sałkowska-Wanat &2, Lech Trzeciak &1, Steffi Silling 3, Agnieszka Pollak 4, Małgorzata Rydzanicz 1, Piotr Stawiński 5, Herbert Pfister 3, Rafał Płoski 1, Sławomir Majewski 2

& - equal contribution

1 – Department of Medical Genetics, Medical University of Warsaw, Poland
2 – Department of Dermatology, Medical University of Warsaw, Poland
3 – Institute of Virology, University of Cologne, Germany; National Reference Center for Papillomaviruses and Polyomaviruses, Germany.
4 – Department of Genetics, Institute of Physiology and Pathology of Hearing, Warsaw, Poland
5 – Department of Immunology, Institute of Biostructure, Medical University of Warsaw, Poland

Background and aim: Epidermodysplasia verruciformis (EV; MIM#226400) is an inherited susceptibility to skin infection with Human Papillomavirus (HPV) that is caused by a recessive mutation of an autosomal TMC6 or TMC8 gene and often leads to skin carcinoma. A female with EV and an unusual involvement of genitals was referred to our centre for genomic sequencing. Results of clinical and genetic examinations of a proband and her family are presented and confronted with data in public and in-house databases.

Methods: DNA for analyses was isolated from formalin-fixed paraffin-embedded (FFPE) biopsies of diseased skin, fresh whole blood and/or buccal swabs. RNA was extracted from blood nuclear cells using Trizol-based method. Genotyping of HPV strains employed PCR-based reference methods (BSGP5+6+, EV and A6/A8) followed by agarose electrophoresis, Luminex detection and/or LineBlot hybridization, where appropriate. Whole exome sequencing (WES) was performed on Illumina HiSeq 1500 platform; the presence of mutation was verified by Sanger sequencing. Aberrant

splicing was predicted in silico and verified in RNA from white blood cells by reverse-transcription – polymerase chain reaction (RT-PCR) followed by agarose electrophoresis and capillary sequencing of products.

Results: Genitals of patient were presistently infected with HPV51 and only transiently with HPV16. In skin subtypes HPV5, HPV9 and HPV20 were continuously present, with intriguing increase of HPV20 load in vulva after skin transplantation to vulvar area for cosmetic reasons. WES of proband DNA revealed homozygous c.449-1G>T mutation in TMC8 gene, confirmed by Sanger sequencing. Both parents, one of 3 siblings and a daughter of proband were heterozygous for the mutation, while remaining 2 siblings were wild type homozygotes. Only the proband had symptoms of EV, consistent with an autosomal recessive inheritance mode. Mutation was predicted to alter splicing, resulting in a frame shift and premature termination of the protein translation. Sequencing of RT-PCR product confirmed the presence of predicted abnormal transcript in white blood cells of the proband and of one heterozygous family member (but not in wild type homozygotes) and the absence of normal transcript in the proband. Incidentally, while the discovered variant was extremely rare in public databases (8×10^-5) and not linked to EV by Clinvar or HMGD, we found two more occurences of it in our database of ~200 WES (one being a heterozygous person without EV, the other being a compound heterozygote with evident EV).

Conclusions: The c.449-1G>T mutation in TMC8 was identified, which in a homozygotic form cosegregated with EV in a 3-generation family and seems to be relatively common in Polish population. Population genotyping is warranted to establish the real frequency of the described variant.

Financing:
LT, PS, SM and RP are supported by Bastion grant from the European Commission 7th Framework Programme (FP7-REGPOT-2012-CT2012-316254-BASTION)

AKT and PTEN, two important players in the regulation of CD20 expression, affect the sensitivity of lymphoma malignancies to rituximab-based therapy

Beata Pyrzynska, Kamil Bojarczuk, Michal Dwojak, Malgorzata Bobrowicz, Marta Siernicka, Nina Miazek, Piotr Zapala, Agnieszka Zagozdzon, Jacek Bil, Jakub Golab, Magdalena Winiarska

Department of Immunology, Institute of Biostructure, Medical University of Warsaw, Poland

The therapeutic strategies currently used in B cell malignancies include the treatment with monoclonal antibodies (rituximab or ofatumumab) directed against CD20 antigen. These antibodies specifically eliminate B cells by triggering indirect effector mechanisms of the immune system, such as complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), or immunophagocytosis. In many patients, the reduced level of CD20 antigen on the surface of tumor B cells leads to the resistance to anti-CD20 therapy. The aim of this study was to explore the molecular mechanisms governing the regulation of CD20 expression in lymphoma cells as a potential explanation of the resistance to rituximab/ofatumumab therapy.

We previously observed that CD20 mRNA expression is significantly affected by the SRC family inhibitors. Here, we report that AKT, the downstream target of SRC kinases, positively influences CD20 expression. Not surprisingly, PTEN tumor suppressor is in turn a negative regulator of CD20 expression. To uncover the transcriptional mechanisms governing the CD20 expression we employed the construct encoding the promoter region of CD20 cloned upstream of the firefly luciferase gene. Overexpression of wild-type PTEN (but not the phosphatase-deficient mutant) strongly reduced the promoter activity and the expression of CD20, leading to decreased binding of rituximab and ofatumumab and increased resistance of tumor cells to complement-dependent cytotoxicity. Using the truncated versions of the CD20 promoter we identified a particular region (-313/-198) as the major region sensitive to AKT or PTEN overexpression. We found that the negative regulation of CD20 promoter activity by PTEN was dependent on inhibition of AKT signaling. We observed that the overexpression of constitutively active AKT1 (CA-AKT1) overcame the negative effect of PTEN and sensitized cells to rituximab/ofatumumab treatment.

The application of either AKT inhibitors or SRC inhibitors for the treatment of B cell malignancies should be therefore carefully considered when combined with anti-CD20 therapies, such as rituximab or ofatumumab.

The results of our studies indicate that PTEN status in tumor cells should therefore be considered when analyzing the mechanisms of resistance of B cell malignancies to anti-CD20 therapies.

Anti-cancer effector functions of human natural killer cells are hampered by adenanthin, a new inhibitor of thiol-dependent antioxidant enzymes

Radoslaw Zagozdzon[1),2),*], Marta Siernicka[1),3),*], Malgorzata Bajor[1)], Malgorzata Firczuk[1)], Angelika Muchowicz[1)], Malgorzata Bobrowicz[1),2)], Cyril Fauriat[3)], Jakub Golab[1)], Daniel Olive[3)], Magdalena Winiarska[1)]

1 - Department of Immunology, Center of Biostructure Research, Medical University of Warsaw, Banacha 1A, 02-097 Warsaw, Poland
2 - Department of Bioinformatics, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Pawińskiego 5a, 02-106 Warsaw, Poland
3 - Postgraduate School of Molecular Medicine, Medical University of Warsaw, Zwirki i Wigury 61, 02-091 Warsaw, Poland
4 - IBiSA Cancer Immunomonitoring Platform, Institut Paoli Calmettes, Inserm, U1068, CRCM, Institut Paoli-Calmettes, CNRS, UMR7258, Aix-Marseille Université, Marseille, France
*RZ and MS: equal first-author contribution to the work

Objectives: Natural killer (NK) cells are considered essential components of the innate and adaptive immune responses. Deficiencies in NK cells functions are common in cancer patients and such deficiencies underlie dysfunctional immune surveillance. Persistent oxidative stress is intrinsic to many malignant tumors and numerous studies have focused on the effects of reactive oxygen species on the antitumor activity of NK cells. It is being regarded as one of the mechanisms of tumor escape from the immune system. Indeed, investigations in animal models suggested that thiol-dependent antioxidant enzymes, e.g. peroxiredoxins (PRDX), are crucial for NK cells functions. There is little data regarding the importance of this phenomenon in human NK cells.
Methods: In order to study the role of thiol-dependent antioxidants in more detail in humans, we applied a novel thiol-targeted compound, adenanthin. Then, we assessed a range of effector functions of NK cells using flow cytometry and Western blotting-based methods.

Results: Following adenanthin treatment, in human primary NK cells we have observed profound alterations in redox balance parameters, changes in spontaneous and antibody-dependent NK cell cytotoxicity against cancer cells, impairment of degranulation and cytokine expression, and a decreased expression of activation markers.

Conclusions: Collectively, our study pinpoints the unique role for the antioxidant activity of PRDX-related enzymatic chain in human NK cell functions. Further understanding of this phenomenon will prospectively lead to fine-tuning of novel NK-targeted therapeutic approaches in human disease.

Financing: RZ, MB, MF and MW are supported by BASTION under 7th Framework Programme (FP7-REGPOT-2012-CT2012-316254-BASTION). Funding has been also obtained from Polish National Science Center (2012/07/B/NZ7/04183; RZ, 2012/07/B/NZ6/03498, MW; 2014/13/N/NZ6/02081, MS) and Polish Ministry of Science and Higher Education (IP2011 012671, MF). MS and MB are supported by the stipend from the Postgraduate School of Molecular Medicine (Warsaw, Poland). DO and CF are supported by the Institut National du Cancer (InCa) and the Fondation pour la recherche médicale (FRM).

The adenosine pathway in ovarian carcinoma: tumor cells and tumor-derived exosomes express CD39 and CD73 ectonucleotidases, produce adenosine and mediate immune suppression

M. Czystowska-Kuźmicz, Marta Szajnik[1,2], Marcin Michalak[1], Hanna Drzewiecka[1], M. Glura[1], Theresa L. Whiteside[4]

1.Poznan University of Medical Sciences Poznan, Poland
2.Military Institute of Medicine, Warsaw, Poland
3.Poznan University of Life Sciences Poznan, Poland
4.University of Pittsburgh, Pittsburgh, USA

Background: Ectonucleotidases CD39/CD73 have been reported to play an important role in functional supression of various immune cells via adenosine that is generated locally in the tumor microenvironment. In patients with ovarian cancer (OvCa) exosomes released by tumor cells (TEX) are abundant in body fluids, including the plasma or ascites and may be involved in tumor progression. Based on the observations suggesting that TEX carry proteins that are expressed on tumor cells from which TEX originate, we hypothesized that CD39+ and CD73+ TEX could deliver these enzymes to distant immune cells. Adenosine produced fom ATP in the presence of TEX could suppress functions of these cells or elevate suppressor activity of regulatory T cells (Treg)

The aim of the study was to: (1) investigate the expression and clinical significance of CD39, CD73, adenosine deaminase (ADA) and CD26 in OvCa tissues and in TEX isolated from OvCa body fluids; (2) determine whether OvCa TEX metabolize exogeneous ATP to adenosine. (3) characterize the molecular profile of TEX; and (4) test whether OvCa TEX can suppress activities of NK cells and up-regulate suppressive activity of Treg.

Methods: The expression of CD39, CD73, ADA and CD26 in OvCa tissues was determined by immunohistochemistry (IHC). The relationship between expression of these enzymes and clinicopathological characteristics was analyzed. TEX were isolated from supernatant of two OvCa

cell lines (A2780, SKOV-3) and from the patients' plasma by exclusion chromatpgraphy followed by ultracentrifugation, as previously described.. Western blots were used for molecular characterization of TEX. NK cells and Treg were separated from the PMBC of normal donors and co-cultured with TEX. The phenotype of NK cells and Treg was evaluated by flow cytometry. ATP hydrolysis was measured using a luciferase detection assay.

Results: By IHC in tissue sections, 70% of tumor cells were CD39+, 77% were CD73+ and 100% were CD26+ADA+.Expression levels of the ectonucleotidases varied from strong to moderate, and patients with a more advanced disease stage had tumors showing strongest CD73 expression ($p<0,05$). Exosomes isolated from plasma of OvCa patients were enriched in TEX which carried LAMP-1, CD63, TGF-β1, MAGE3/6, CD39, CD73, ADA and Ep-CAM. In contrast, exosomes isolated from the plasma of healthy donors carried LAMP-1 and CD63. TEX obtained from OvCa patients hydrolyzed more exogeneous ATP than did TEX from OvCa cell line supernatants ($p<0.05$) and produced more adenosine ($p<0,05$). After co-incubation with TEX, normal NK cells downregulated expression of NKG2D, NKp44 and NKp46 ($p<0,05$) and Treg up-regulated expression of Perforin, FasL, CCR7 ($p<0,05$). Co-incubation of Treg with TEX resulted by increased suppression of responder cells ($p.<0,01$).

Conclusion: Similar to OvCa tumor cells in tissue exosomes isolated from the plasma of OvCa patients were found to carry enzymatically-active ectonucleotidases and to produce extracellular adenosine. These exosomes were capable of down-regulating NK cell functions and up-regulating Treg activity in vitro. The same TEX-mesiated mechanisms could contribute to tumor-induced immune suppression characteristic of OvCa and resulting in tumor immune escape and OvCa progression.

Evaluation of the role of CMV infection in colorectal cancer progression

Oksana Kovtonyuk[1], Sharan Ananthaseshan[2], Joanna Soin[1], Piotr Religa[1]

1 - Department of Internal Medicine, Hypertension and Vascular Diseases, Medical University of Warsaw, Poland
2 - Department of Medicine, Karolinska Institutet, Sweden

Colorectal cancer is the third most common malignant neoplasm and the third leading cause of cancer death worldwide. The major cause of death is metastasis. Recent evidence suggests that Cytomegalovirus (CMV) has been detected in several human cancers, and CMV may affect tumor cell progression and metastasis formation.

PURPOSE: The goal of the study was to evaluate the role of CMV in colorectal cancer progression.

METHODS: The research consisted of a series of in vitro (CT26.CL25 colon carcinoma cells) and in vivo (murine colon carcinoma CT26 cells, syngeneic to BALB/c mice) experiments. In the first step of the experiment, the effect of CMV infection on tumorigenic properties (such as proliferation, migration and invasion) of CT26.CL25 colon carcinoma cells was estimated. Further, assessment of CMV influence on EMT modulation and tumor progression was studied in CT26-bearing mice (CMV infected and uninfected tumors). Tumor cells were infected with mCMV (0.5 MOI) three days prior subcutaneous injection into the mice. Tumors were measured every second day to observe the dynamics of tumor growth. After primary tumors reached 1,5 cm3 in volume, they were removed surgically, embedded into paraffin blocks and used subsequently for immunohistochemical staining in order to analyze tumor morphology (hematoxylin and eosin staining) and estimate expression of epithelial-mesenchyimal transition (EMT) markers: E-cadherin, N-cadherin. Eight weeks after tumor surgery, the mice were sacrificed and their organs collected for immunohistochemical analysis and evaluation of metastasis.

RESULTS AND CONCLUSIONS:

In vitro MTT, invasion and migration assays have revealed increased proliferation and tumorigenic properties of CMV infected CT26.CL25 colon carcinoma cells, compared with uninfected control. Analysis of tumor samples obtained from BALB/c mice have shown the acquisition of mesenchymal marker N-cadherin in CMV infected colon carcinoma tissues. N-cadherin up-regulation was associated with concomitant increase of metastatic potential of CMV infected tumors. However, we have not observed any significant difference in the levels of expression of the epithelial marker E-cadherin between CMV infected and uninfected tumor tissues. The obtained results suggest that CMV may influence on colon cancer progression and metastasis through regulation of EMT phenotype. A new set of experiments has been initiated to further elucidate the association of CMV with EMT process at colorectal cancer progression.

C/EBPβ expression is an independent predictor of overall survival in breast cancer patients by MHCII/CD4-dependent mechanism of metastasis formation

Kurzejamska E(1), Johansson J(2), Jirström K(3), Prakash V(1), Ananthaseshan S(4), Boon L(5), Fuxe J(2), Religa P(1).

(1)1] Department of Medicine, Centre for Molecular Medicine, Karolinska Institute, Stockholm, Sweden [2] Postgraduate School of Molecular Medicine, Department of Internal Medicine and Hypertension, Medical University of Warsaw, Warsaw, Poland. (2)Division of Vascular Biology, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden. (3) Division of Pathology, Department of Clinical Sciences, Lund University, Skåne University Hospital, Lund, Sweden. (4)Department of Medicine, Centre for Molecular Medicine, Karolinska Institute, Stockholm, Sweden. (5)Bioceros Holding BV, Utrecht, The Netherlands.

CCAAT-enhancer binding protein β (C/EBPβ) is a transcription factor that has a critical role in mammary gland development and breast cancer progression. Loss of C/EBPβ increases metastatic dissemination of mouse mammary tumor cells. However, the mechanism by which C/EBPβ expression affects metastasis formation remains unknown. This study aims at determining the relationship between C/EBPβ and survival of breast cancer patients, and elucidating C/EBPβ's link with metastasis formation. C/EBPβ expression was evaluated in 137 cases of human breast cancer, and the correlation with overall survival was estimated by Kaplan-Meier analysis.

Additionally, the mouse 4T1 tumor model was used for in vivo studies. Decreased C/EBPβ expression was found to be associated with shorter overall survival of breast cancer patients. In the murine 4T1 model, loss of C/EBPβ affects tumor growth, morphology and promotes metastatic spread to the lungs.

Immunohistochemical analyses showed that C/EBPβ inhibition leads to increased major histocompatibility complex II (MHCII) expression, followed by the accumulation of CD45-, CD3- and CD4-positive (CD4+) lymphocytes in the tumors.

Inflammation involvement in C/EBPβ-mediated metastasis formation was confirmed by DNA microarray and by experiments on CD4+ cell-deprived nude mice. Additionally, anti-CD3 and anti-CD4 treatments of C/EBPβ-silenced tumor-bearing mice resulted in reverting the C/EBPβ effect on tumor growth and metastasis. Altogether, C/EBPβ is a predictor of overall survival in breast cancer patients, and affects tumor growth, morphology and lung metastasis formation in murine 4T1 model. The mechanism of metastasis formation involves immunologic response depending on C/EBPβ-mediated activation of MHCII and accumulation of CD4+ lymphocytes in the tumor.

SPEAKERS

S.1-23

Roderick Beijersbergen

Dr. Roderick Beijersbergen is group leader at the Netherlands Cancer Institute and heads the NKI Robotics and Screening Center. His work evolves around the development and application of large-scale functional genomic technologies with the goal to identify more effective cancer treatments. This work has led to the identification of novel targets for cancer therapy, to the understanding of the mechanisms of action of novel drugs and the identification of novel mechanisms of acquired resistance to pathway targeted therapeutics. With this approach novel treatment combinations have been identified that are currently in several clinical trials with promising results. Recently his work has extended into the use of primary cancer derived organoid cultures from colorectal cancer patients to study the individual patient's tumor response and characteristics to improve treatment strategies.

S2 | 21-22.05.2015

Frank Beurskens

Dr. Frank Beurskens studied biology at the Utrecht University, in 1999 he received a PhD in immunology at the same university. During his PhD and his postdoctoral fellowship at Harvard Medical School he specialized in the complement system and inflammation. In 2002 he started to work at Genmab and has been involved in the development of several therapeutic antibodies, like ofatumumab. He was involved in unravelling the mechanism of action of this antibody. Furthermore he was the driven force to unravel the mechanism of complement activation by antibodies. He found that specific non-covalent interactions between Fc segments of immunoglobulin G antibodies resulted in the formation of ordered antibody hexamers after antigen binding on cells. These hexamers recruited and activated C1, the first component of complement, thereby triggering the complement cascade. These findings were recently published in Science. With the knowledge of the basic understanding of natural behaviour antibodies he co-developed Genmab's proprietary HexaBodyTM platform, which creates effector function enhanced antibodies

Bruno Botta

Prof. Bruno Botta is full professor and he has been nominated Deputy Rector for the Internationalization in November 2014. He is Head of the Dipartimento di Chimica e Tecnologie del Farmaco at Sapienza University of Rome since 2011. His interest during the years has been focused on the structural elucidation and synthesis of biologically active compounds derived from living plants. Since 20 years, he has been working on the field of plant tissue cultures in combination with chemistry directed toward the understanding of biosynthetic pathways of the compounds under investigation. During the years, some plant enzymes (such as those belonging to the peroxidases family) derived from the above-mentioned cell cultures have been isolated and purified and some kinetic studies have been performed too. During the last 15 years, Prof. Botta focused his attention also on both the synthesis and host-guest studies of artificial receptors of the resorcarenes family. During the last 7 years he focused his attention on the Hedgehog (Hh) signalling pathway which plays a pivotal role in the initiation, proliferation, invasion and metastasis of various cancers (inter alia medulloblastoma). He is author of 130 publications, including 5 patents (3 national and 2 international – USA and Europe), and, besides that, he is author and co-author of 10 books. For the Italian publishing house Edi-Ermes he edited 2 text books of Organic Chemistry.

S4 | 21-22.05.2015

Sven Brandau

Prof. Sven Brandau studied biology in Hamburg and Los Angeles and performed his PhD work from 1993-1996 at the Bernhard-Nocht Institute for Tropical Medicine in Hamburg. From 1996 until 2007 he worked as a post-doc and later as senior scientist and independent group leader at the Research Center Borstel. During his habilitation period he started to focus on cancer immunotherapy and obtained his venia legendi in Immunology and Cell Biology at the University of Lübeck in 2003. In 2007 he moved to the University Duisburg-Essen, where he became Associate Professor in 2009. Prof. Brandau is the Head of the Experimental Research Division of the Department of Otorhinolarnygology, University Hospital Essen, and co-chairman of the BIOME graduate school. He received several awards for his work on experimental and translational aspects of tumor immunology. Sven Brandau´s main research area is the immunological tumor-host interaction with a focus on myeloid cells. Additional research projects aim at developing novel immunotherapies for head and neck cancer and investigate the role of mesenchymal stromal cells in cancer and infection.

Lars Bullinger

Lars Bullinger is a hematologist/oncologist of the University of Ulm, Germany, who is focusing on the dissection of the molecular pathogenesis of leukemias by using "omics" approaches. His work contributed to the discovery of novel clinically relevant AML subclasses and currently he is also working on the implementation of "omics" and NGS findings into the clinic.

Magda Chlebus

Magda Chlebus, Director Science Policy at the European Federation of Pharmaceutical Industries and Associations is in charge of policy and legislative debates which shape research environment in Europe. This includes public private collaborations (incl. the Innovative Medicines Initiative) and enabling and sensitive technologies. After a Master Degree in Applied Linguistics at University of Warsaw in 1992 and a carrier as translator and teacher, in 1995 she joined EFPIA, the representative voice of R&D-based pharmaceutical industry in Europe. Her experience covers public and government affairs with focus on Brussels Village, including designing and implementing advocacy campaigns on EU legislation as well as implementation of the pharmaceutical legislation in new Member States.

Seth Coffelt

Dr. Seth B. Coffelt completed his Ph.D. studies in Molecular & Cellular Biology at Tulane University (New Orleans, Louisiana, USA) in 2006 under the direction of Dr. Aline Betancourt. During this time, Seth was awarded a Cancer Association of Greater New Orleans student grant for his work on mesenchymal stem cells, antimicrobial peptides/alarmin molecules, and their role in ovarian cancer progression. From 2008-2011, Seth was a postdoc in Dr. Claire Lewis's lab at the University of Sheffield (Sheffield, UK). His project focused on the endothelial cell-derived molecule, Angiopoietin-2, and its influence on pro-angiogenic and immunosuppressive functions of TIE2-expressing monocytes/macrophages (TEMs). The University of Sheffield gave him an Exceptional Contribution award in 2010. Seth was awarded a Marie Curie Intra-European fellowship in 2011 and joined Dr. Karin de Visser's group at the Netherlands Cancer Institute (Amsterdam, The Netherlands) to study the role of neutrophils and gamma delta T cells in breast cancer metastasis

Charles Dumontet

Prof. Charles Dumontet graduated Alexis Carrel Medical School, Université Lyon (France). He did his PhD in Immunology at Université Claude Bernard, Lyon (France) and postdoc at Stanford Medical Center (USA). He spent a Freshman year at Yale University (USA). Currently he works as a Professor in Hematology at University of Lyon and as a Senior Physician at Hospices Civils de Lyon. He is P.I. of "Anticancer Antibodies" team. He is also head of INSERM then INSERM/CNRS team since 1998. Prof. Dumontet is a Vice President for Health & Sciences, Claude Bernard Lyon 1 University, Head of Scientific Council of the Hospices Civils de Lyon, Head CLARA Pharmacogenomics Platform, Member of the Administrative Council of the Intergroupe Francophone du Myélome (IFM), Member of the Scientific Group of the French Cooperative Group on Chronic Lymphocytic Leukemia.

William M. Gallagher

Prof. William M. Gallagher is a Director of BREAST-PREDICT (Irish Cancer Society Collaborative Cancer Research Centre) and Professor of Cancer Biology at UCD School of Biomolecular & Biomedical Science, Conway Fellow at UCD Conway Institute and Co-Founder/Chief Scientific Officer of OncoMark Limited. Prof. Gallagher graduated from the Department of Biochemistry, UCD with a 1st Class Joint Honours degree in Molecular Genetics and Biochemistry. Subsequently, he obtained a PhD in Molecular Oncology from the Cancer Research UK Beatson Laboratories in Glasgow. Then, he moved to Paris to undertake a Marie Curie Individual Fellowship at Rhone-Poulenc Rorer (currently Sanofi-Aventis). Afterwards, he returned to Ireland upon receipt of an Enterprise Ireland Post-Doctoral Fellowship and, subsequently, a Marie Curie Return Fellowship. He co-founded OncoMark Ltd., which is a private company centred on the development and application of biomarker panels and associated technologies, on both tissues and biological fluids. Prof. Gallagher is currently the Chief Scientific Officer (CSO) at OncoMark. A major focus of Prof. Gallagher's research work is the identification and validation of candidate biomarkers of breast cancer and melanoma, with particular emphasis on translation of transcriptomic and proteomic datasets into clinically relevant assays. In addition, his team (the Cancer Biology and Therapeutics Lab) investigates the functional relevance of candidate tumour progression-associated genes at both in vitro and in vivo levels, as well as engages in preclinical evaluation of novel anti-cancer agents. Prof. Gallagher is currently Director of BREAST-PREDICT, which is the first Irish Cancer Society Collaborative Cancer Research Centre (CCRC) to be funded. Prof. Gallagher is also co-PI and Deputy Co-ordinator of a major Science Foundation Ireland-funded Strategic Research Cluster, Molecular Therapeutics of Cancer. Prof. Gallagher previously co-ordinated an FP6 Marie Curie Transfer of Knowledge Industry-Academia Partnership Programme, Target-Breast, which was focused on converting omic datasets into clinically relevant diagnostic assays. He also co-ordinated an analogous FP7 Marie Curie Industry-Academia Partnership and Pathways (IAPP) Programme, Target-Melanoma, which was focused on identification and validation of novel molecular determinants of melanoma progression. Prof. Gallagher has received a number of awards based on his research work to date, including the BACR/AstraZeneca Young Scientist Frank Rose Award in 2004, the St. Luke's Silver Medal Award in 2008 and the NovaUCD 2011 Innovation Award. Prof. Gallagher has had productive collaborative interactions with a variety of other industrial partners throughout his research, and has filed/been awarded multiple patents

Josee Golay

Dr. Josee Golay following a B.A. Honours degree in Biochemistry at Oxford University (UK) in 1986 she obtained PhD in Cellular Immunology at University College London, under the direction of PCL Beverley, identifying several new B cell surface molecules, including CD20, involved in B cell proliferation and differentiation. She then moved to the European Molecular Biology Laboratory (EMBL), Heidelberg, Germany, in the group of Dr. Thomas Graf, where she gained experience in molecular biology techniques in the context of leukemogenesis. In 1989, she moved to the Institute of Pharmacological Research Mario Negri (IRFMN) (Milan, Italy) where, she studied the role of the myb family of oncogenes in haematopoietic differentiation. In 1998 she obtained a permanent position at IRFMN, and in 2004 moved as staff scientist to the Haematology Unit of the Bergamo main Hospital (presently Papa Giovanni XXIII). In the last 15 years her attention turned again towards the CD20 molecule, and more specifically the mechanism of action of the therapeutic antibody rituximab as well as new generation anti-CD20 antibodies. In Bergamo, she also played a central role in the set up the new Center of Cellular Therapy "G. Lanzani", a GMP-compliant Cell Factory, fully authorized by the national authorities since 2008, and contributed to the development of new cell therapy strategies, combining antibody and cell therapy and participating in the development of a new bispecific antibody format. She is co-inventor of 2 patents and author of 100 publications in refereed journals

Ali O. Gure

Dr. Ali O. Gure is an Assistant Professor at Bilkent University, Department of Molecular Biology and Genetics, Ankara, Turkey (since 2006). Obtained his MD degree in 1988 from the University of Ankara, and a PhD in immunology from Cornell University Graduate School of Medical Sciences, USA, in 1995. Worked as an Assistant Member at the Ludwig Institute for Cancer Research - NYC branch (2000-2006) after completing a post-doctoral fellowship with Lloyd J. Old at the Memorial Sloan Kettering Cancer Center in New York City (1995-2000). His initial work focused on the discovery of tumor antigens and in particular the utilization of autologous immune responses against these as diagnostic and prognostic markers. He is thus, a co-inventor in 22 international patents related to tumor antigens/biomarkers. His more recent work focuses on those mechanisms leading to expression of cancer-testis antigen genes which function as biomarkers of ectopic hypomethylation in cancer, and the identification of biomarkers of diagnosis, prognosis and chemosensitivity. His work is currently supported by 3 grants from the Turkish Scientific and Medical Council. He co-authored 40 manuscripts receiving over 5500 citations.

Ioana Ispas

Ioana Rodica Ispas is currently Advisor for European Affairs in Genomics, Bioethics and Health at Romanian Authority for Scientific Research and Innovation. She graduated Biochemistry at University of Bucharest and she continued her studies at the same university where she obtained her first Master's degree in 1994 and has a Master degree in Molecular Biology and a second Master in European Studies and Community Rights at the University of Bucharest –Faculty of Philosophy in 2009. As scientist she was Assistant Professor in Biochemistry and Organic Chemistry at the University of Ecology in Bucharest and scientific researcher (Molecular Biology Department) at University of Bucharest. She has more than 30 papers in molecular biology and bioethics. Current research interests focus on: bioethical issues related primarily with new and emerging fields of science and technology and development of the ethical matrix as a tool for evaluation of research projects. She is devoted to examining the ethical implications of research misconduct and of new and emerging fields of science and technologies, with a special focus on genetically modified organisms. From 2007-2008 and 2011-2012 she worked as General Secretary of Romanian National Research Ethics Council and member of Romania National Ethics Commission for Ethics in Life Sciences where she was actively involved in drafting regulations for research ethics and code of conduct for research in life sciences. She specialises in applied ethics. She worked for European Commission – Brussels, between 2004-2006, and took a second appointment between 2008-2011 as National Expert for three DGs: DG RTD, DG SANCO and DG ENV, being in charge with monitoring of Framework Programme contracts, policy briefings and foresight in biotechnology, ethics, gender research and environmental risk assessment for genetically modified organisms. Starting with 2001 she is Romanian Governmental representative in Programme Committee of European Medical Research Programme (FP5, FP6, FP7 and Horizon 2020), Innovative Medicine Initiative, Joint Programming Initiative Neurodegenerative Diseases, Joint Programming Initiative of Antimicrobial Resistance.

She has extensive experience in EU project management (more than 16 years), currently being scientific officer for 6 FP7 and H2020 projects in: neurosciences, nanomedicine, infectious diseases (NEURON II, JPI AMR, EURONANOMED II, ERASYSAPP, HIVERA, INFECT-ERA, JPND COFUND) on behalf of Romanian Ministry of National Education.

Marcin Kapczyński

Marcin Kapczyński is a Strategic Business Manager at Thomson Reuters Intellectual Property & Science. He has started his career at Cardinal Wyszynski University in Warsaw, Psychology Department. His primary area of interest was measurement of individual differences, intelligence, cognitive styles, interpersonal competence and intellect diagnosis.
In Thomson Reuters since 2010. He is involved in shaping research evaluation process in Poland. He provides support for research analytical and discovery tools such as Web of Science, InCites or Journal Citation Reports. He enthusiastically shares understanding of scientometry and bibliometric approach. Kapczynski works with researchers, academics and librarians at all levels and offers consultancy services to government and corporate institutions.

Michał Karoński

Prof. Michał Karoński is a head of the Department of Discrete Mathematics at the Faculty of Mathematics and Computer Science at Adam Mickiewicz University in Poznań. He does research in discrete mathematics (random discrete structures, graph theory) and theoretical computer science (graph algorithms). In the mid-1970s, Professor Karoński established a research group working on the theory of random structures, which quickly achieved international renown, becoming one of the leading teams in the world on this subject. He has authored over 50 publications and delivered over 30 plenary lectures and guest speaker talks at international conferences. During his academic career he has held several positions including a postdoctoral fellowship at the University of Florida and visiting professorships at Southern Methodist University, Purdue University and the Johns Hopkins University. He has also conducted research in many academic centres abroad, including universities in Moscow, Lund, Bielefeld, Pittsburgh (CMU) and Singapore, as well as at research centres in the USA (Bellcore, Microsoft Research), Denmark (BRICS), South Korea (Com2Math), England (Isaac Newton Institute) and Sweden (Mittag-Leffler Institute). Since 1992, he has been a visiting professor at Emory University in Atlanta. He is editor-in-chief of the journal Random Structures & Algorithms (Wiley, USA). Since 2010 he has been the Chairman of the Council of the National Science Centre.

Tim Kievits

Tim Kievits started his career as a biochemist in the department of Human Genetics at the University of Leiden in 1985. In 1990 he joined Organon Teknika's nucleic acid diagnostics R&D group that specialized in HIV, CMV and HCV detection and held various positions at this company. He left his last position as Director Technology of the Nucleic Acid Diagnostics Business Area to spin-out and develop an innovative microarray technology. In 2000, he founded PamGene, a science and technology driven company in the multi marker testing area with a specialization in protein activity scans in clinical samples. As CEO of PamGene, he organized several finance rounds for Pamgene, securing investments from international venture capital funds, corporate, local and private funds. He built up an experienced management team that has developed, under his leadership, the novel technology into a robust CE marked system (PamStation® and PamChips®). This system is now in use by pharmaceutical companies such as MSD, Johnson&Johnson and Amgen as well as national and international academic institutes (Europe, US, Japan). From January 2013 on, he is director of Healthcare Innovation at Vitromics Healthcare Holding, the parent company of PamGene. He leads the embedding of the PamGene products and services as routine companion diagnostic tests in the clinic. Tim Kievits also chaired the Personalised Medicines Topic Group of the EuropaBio Association in Brussels from 2009-2014

Takanori Kitumura

Dr. Takanori Kitumura graduated Veterinary Medicine at Hokkaido University (Sapporo, Japan) and obtained PhD title in Biochemistry at the same University. He was also a Research Associate at Albert Einstein College of Medicine (New York, USA) in a group of prof. Jeffrey W. Pollard. Currently, he is a Chancellor's Fellow at MRC Centre for Reproductive Health, Queen's Medical Research Institute (University of Edinburgh, UK). He has received many awards: Breast Cancer Postdoctoral Fellowship Award (CDMRP DOD, USA) (2011-2014); Grant-in-Aid for Young Scientists (B) from Japan Society for the Promotion of Science (2008-2010); Postdoctoral Fellowship from the Japan Society for the Promotion of Science (2003-2004). He is an author of many papers published in Nature, Cancer Cell, PNAS, Cancer Res, Nature Genetics.

Danuta Mossakowska

Danuta Mossakowska graduated from University College London with a degree in Biochemistry which was followed by a PhD at the University of Surrey studying the mechanisms of bacterial resistance. It was during these studies she developed a passion for drug discovery. She continued her academic studies with two Post Doctoral Fellowships working on the kinetics of Barnase with Professor Alan Fersht followed by a second fellowship at the Institute of Cancer Research on understanding the biochemical architecture of transcriptional regulation factors involved in the regulation of cancer cell lines. She then moved to SmithKline Beecham Pharmaceuticals working on methods to inhibit the complement system. This research led to a number of publications and patents that in turn formed the basis of a Biotech company; one of the proteins she designed is currently in clinical trials for prevention of acute rejection in renal transplantation. In trying to get to the biochemical mechanisms of proteins, Danuta developed expertise in gene expression, cellular signalling and assay development and has led a number of drug discovery programs bringing together her expertise to a variety of drug targets ranging from nuclear receptors, cell surface receptors as well as a variety of enzymes. She is currently one of the biology leaders in Discovery Partnerships with Academia (DPAc) where she runs projects in collaboration with academic investigators

Munitta Muthana

Dr. Munitta Muthana is lecturer at the University of Sheffield. Her research has focused on the role of innate immune cells like macrophages and dendritic cells in tumour progression, and response to frontline therapies like chemotherapy. Recently, she has used her knowledge of this area to develop innovative cell-based methods to target anticancer therapy to tumours. She has devised a way to use macrophages to deliver large quantities of oncolytic virus to both primary and secondary tumours simultaneously (bbc.co.uk/news/health-20795977).
Moreover, on a recent MRC Discipline-Hopping Award, she showed that MRI could be used to 'steer' large numbers of such macrophages into tumours. Now she wishes to develop this MRI approach to steer anticancer therapies into tumours found deep within the body

Jacque Nunes

Dr. Jacques A. Nunes is working in Immunology and Cell Signaling field. He studied Immunology in Marseille and London and performed his PhD work from 1989 - 1992 at the Cancer Research Centre in Marseille. From 1993 until 1997, he worked as a post-doc at the Cancer Research UK (London Research Institute) then at the Centre d'Immunologie de Marseille-Luminy. Then, he got a permanent position from the French biomedical agency (Institut National de la Sante et de la Recherche Medicale - Inserm) at the Cancer Research Centre in Marseille (CRCM), where he developed his own research as a group leader in the "Immunity & Cancer" team headed by Pr. Daniel Olive. His work contributed to the discovery of new regulatory mechanims for signaling pathways in lymphocytes. Currently he is also working on the impact of T cell costimulatory molecules in cancer biology and developing "signal monitoring" into the clinic.
See his list of publications at http://cvscience.aviesan.fr/cv/97/jacques-nunes

Tadeusz Robak

Prof. Tadeusz Robak is professor of hematology at the Medical University of Lodz, Poland. Professor Robak studied medicine at this institution and after graduating in 1973 went on to specialize in internal medicine, clinical pharmacology and hematology at the Copernicus Memorial Hospital in Lodz. He undertook further postgraduate training at Hammersmith Hospital, London, in 1983-1984. He currently holds the posts of Professor of Hematology at the Medical University of Lodz and Chief of the Department of Hematology at the Copernicus Memorial Hospital in Lodz. Professor Robak is a reviewer for numerous journals including Journal of Clinical Oncology, Blood, Lancet, Lancet Oncology and Leukemia. He is current president of the Polish Society of Hematology and a member of European Hematology Association, European Society for Clinical Oncology and American Society for Hematology. The particular research emphasis has been on the application of new drugs in the treatment of leukemia, multiple myeloma and lymphoma. His special interest is in chronic lymphocytic leukemia, lymphoma and autoimmune disorders with particular emphasis on the use of purine analogues and monoclonal antibodies in the treatment of these diseases. Professor Robak was principal investigator of the Polish multi-centre phase 3 trials of cladribine in CLL and, recently, of the international trial of the first in a new classes of recombinant polyclonal antibodies, rozrolimupab in ITP. His research has been published in New England Journal of Medicine, Journal of Clinical Oncology, Cancer, Lancet Oncology, Blood, Leukemia, European Journal of Hematology, and several other journals. Professor Robak published more than 500 journal articles, 450 abstracts and 25 books or chapters

Adam Tarnoki

Dr. Adam Tarnoki finished his radiology residency training and PhD at Semmelweis University (Budapest, Hungary). In his research he mainly focused on: effect of fibrin structure on fibrinolysis, twin study (Twinsburg, Ohio, USA, 2006), patients with kidney disease in the praxis (research program at Department of Family Medicine, Semmelweis University, 2006-2008), the role of twin studies in the public health (research program at Department of Public Health, Semmelweis University, 2006-2008), contemporary instruction: lectures of drug, life style and smoking prevention for secondary school students (Department of Public Health, Semmelweis University, 2007); heredity of weather changes among twin brothers and sisters (research program at National Center of Epidemiology, Semmelweis University Department of Public Health, Budapest, 2006-2008); effect of early cardiac rehabilitation after biventricular pacemaker implantation (research program at Cardiology center, Semmelweis University, Budapest, 2007-2008); effect of weather changes to aneurysm ruptures (Division of Neurosurgery Memorial Regional Hospital, Hollywood, Florida, USA); global air monitoring tobacco study in Hungary – research with K. Michael Cummings, PhD, MPH, leader of anti-tobacco campaign in the USA (Department of Health Behaviour, Roswell Park Cancer Institute, State University of New York at Buffalo, NY, USA); Role of genetics and environment in evolution of metabolic syndrome (glucometabolic risk) among twins (Bajcsy Zsilinszky Hospital, Semmelweis University Department of Physiology, National Center of Epidemiology); heritability of vein distensibility among twins (twin study, Semmelweis University Department of Diagnostic Radiology and Oncotherapy, Budapest, Hungary; Institute of Behavior Sciences of Semmelweis University, Budapest, Hungary; Methodist DeBakey Heart Center, Cardiovascular Surgery, The Methodist Hospital, Houston, USA; SUNY at Buffalo Roswell Park Cancer Institute, University of Rome La Sapienza, University of Padova, University of Perugia, University of South Wales, Australia etc.); arterial stiffness among patients with coronariasclerosis and valve replacement (State Hospital for Cardiology, Balatonfüred, Hungary); second hand smoke exposure and excess heart disease and lung cancer mortality among a Hungarian hospital's staff; GLOBAL Twin study (2013-2014) and sleep twin study (2014-now).

David Tarnoki

Dr. David Tarnoki completed Radiology residency training and PhD studies at Semmelweis University (Budapest, Hungary). In his research he focused on: effect of fibrin structure on fibrinolysis, twin study (Twinsburg, Ohio, USA, 2006), patients with kidney disease in the praxis (research program at Department of Family Medicine, Semmelweis University, 2006-2008), the role of twin studies in the public health (research program at Department of Public Health, Semmelweis University, 2006-2008), contemporary instruction: lectures of drug, life style and smoking prevention for secondary school students (Department of Public Health, Semmelweis University, 2007); heredity of weather changes among twin brothers and sisters (research program at National Center of Epidemiology, Semmelweis University Department of Public Health, Budapest, 2006-2008); effect of early cardiac rehabilitation after biventricular pacemaker implantation (research program at Cardiology center, Semmelweis University, Budapest, 2007-2008); effect of weather changes to aneurysm ruptures (Division of Neurosurgery Memorial Regional Hospital, Hollywood, Florida, USA); global air monitoring tobacco study in Hungary – research with K. Michael Cummings, PhD, MPH, leader of anti-tobacco campaign in the USA (Department of Health Behaviour, Roswell Park Cancer Institute, State University of New York at Buffalo, NY, USA); Role of genetics and environment in evolution of metabolic syndrome (glucometabolic risk) among twins (Bajcsy Zsilinszky Hospital, Semmelweis University Department of Physiology, National Center of Epidemiology); heritability of vein distensibility among twins (twin study, Semmelweis University Department of Diagnostic Radiology and Oncotherapy, Budapest, Hungary; Institute of Behavior Sciences of Semmelweis University, Budapest, Hungary; Methodist DeBakey Heart Center, Cardiovascular Surgery, The Methodist Hospital, Houston, USA; SUNY at Buffalo Roswell Park Cancer Institute, University of Rome La Sapienza, University of Padova, University of Perugia, University of South Wales, Australia etc.); arterial stiffness among patients with coronariasclerosis and valve replacement (State Hospital for Cardiology, Balatonfüred, Hungary); second hand smoke exposure and excess heart disease and lung cancer mortality among a Hungarian hospital's staff; GLOBAL Twin study (2013-2014) and sleep twin study (2014-now).

Agnieszka Turowska

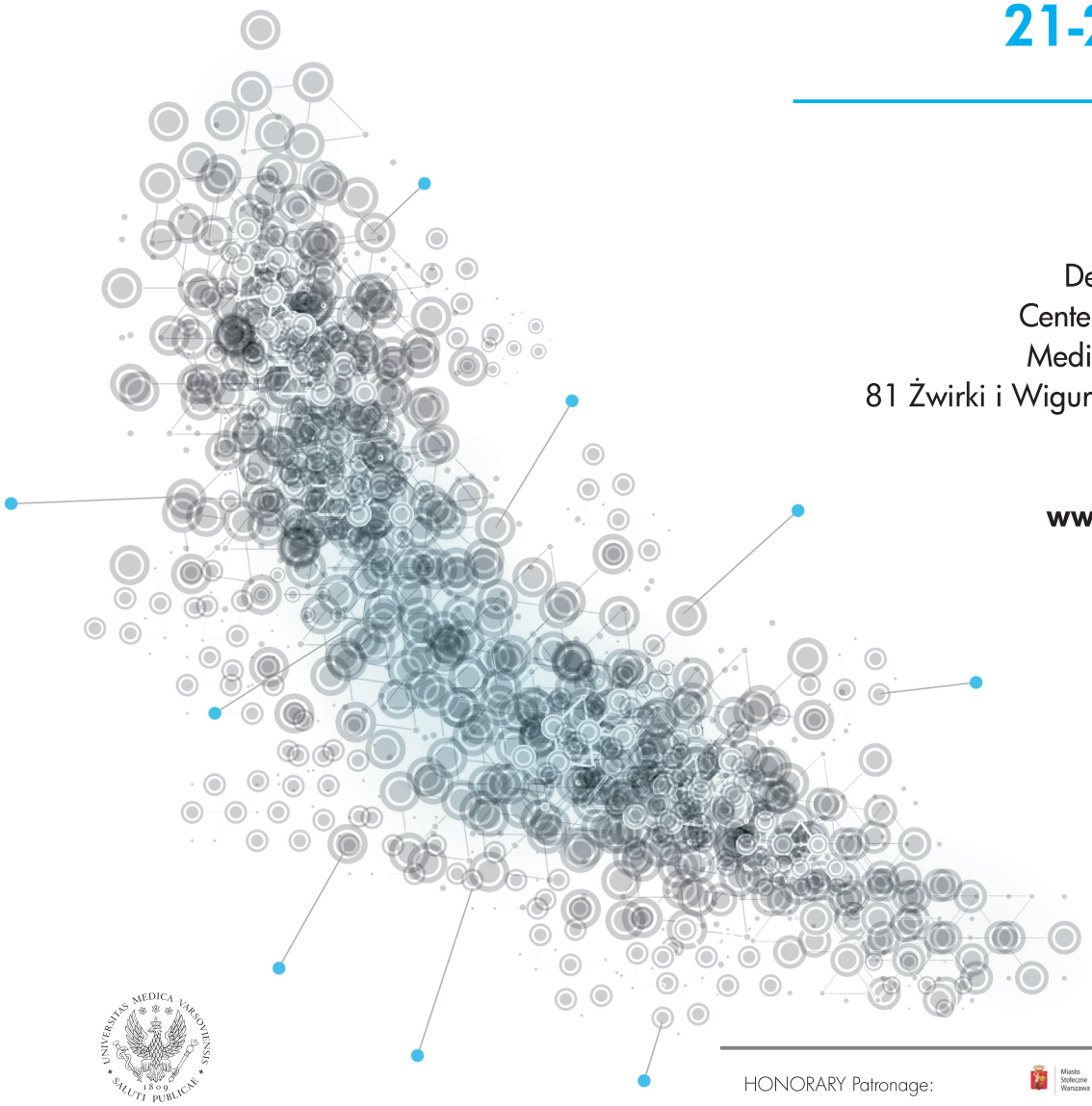
Dr. Agnieszka Turowska is a scientific project manager in biotechnological spin-off company sterna biologicals GmbH in Marburg, Germany. She is involved in all stages of innovative drug development: starting from the design of preclinical experiments to the organization and management of clinical trials. Her work evolves around the development and application of novel treatments for chronic inflammatory diseases of lung and gut such as asthma, COPD and inflammatory bowel disease. Her primary focus is on targeting transcription factors that play a pivotal role in regulating underlying inflammatory mechanisms by antisense molecules DNAzymes. Her major task is to link university and business and to collaborate very closely with academic partners. The results of successful collaboration are published in well recognized journals like New England Journal of Medicine or Cell, where she is a co-author. Dr. Turowska graduated Biotechnology at Warsaw University of Life Sciences and obtained PhD title in Faculty of Veterinary Medicine at the same university. Recently, she completed her executive MBA at University of Applied Sciences in Germany. She was awarded with fellowship from German Academic Exchange Service (DAAD). Before joining the industry she was working as post-doc in the Department of Internal Medicine at Justus Liebig University in Giessen Lung Center in Germany.

BASTION

International Conference

Translational Research in Oncology in New Member State Economies

21-22 MAY 2015



BASTION Project office:
Department of Immunology
Center of Biostructure Research
Medical University of Warsaw
81 Żwirki i Wigury street, 02-091 Warsaw
room 73, 2nd floor

www.BASTION.wum.edu.pl

Venue place:
Medical University of Warsaw
Księcia Trojdena 2a Street , Hall B

HONORARY Patronage:

Conference PARTNERS: