# International Society for Enzymology Annual Conference: Advances in Laboratory Medicine and Pathobiology 2017

## ISE 2017

*June 16-19, Santorini Greece*

*Santorini Palace Hotel, Fira*




## Organizing Committee:

Eleftherios P. Diamandis, Andreas Scorilas

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International Society for Enzymology

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Dear Colleagues

Following the successful International Society for Enzymology Annual Conference 2016 in the island of Syros, Greece, we are now announcing the next ISE Annual Conference 2017. The conference will take place in the beautiful island of Santorini, Greece, between the dates Friday June 16 - Monday June 19, 2017. As usual, the guiding principles of this Annual Conference are: Top science related to all aspects of laboratory medicine, in a wonderful location, with a limited number of participants.

The "International Society for Enzymology Annual Conference 2017" conference will cover a wide range of aspects on Laboratory Medicine and Pathobiology including, but not limited to: New biomarkers for cancer and other diseases, informatics, automation, genomics, proteomics, epigenomics, transcriptomics, other omics, enzymes in health and disease, theranostics, micro RNAs, long non-coding RNA, quality assurance in medical laboratories.

As last year, the meeting will be restricted to a maximum of 50 registrants, on a first-come-first served basis. All participants will be responsible for their expenses and will be invited to present on a topic of their choice, if they prefer.

Please take your time before or after the symposium to visit the old city of Santorini and its surroundings as well as some of the island's wonderful beaches, villages and events. You may also compine your trip with a stop in Athens.

We look forward to seeing you, your colleagues and your families in Santorini for a wonderful scientific meeting and an overall enjoyable experience.

Information: http://ise.biol.uoa.gr

With best wishes

Sincerely,

The Organizers:

Dr. Eleftherios P. Diamandis

Dr. Andreas Scorilas

# *Scientific Programme*

***Invited Speakers' Abstracts***

***Poster Abstracts***

## Day one – Friday, 16th June 2017

***15:00 -17:00*** *Registration*

***17:00 - 18:00*** *Opening Ceremonies*

**Eleftherios P. Diamandis & Andreas Scorilas**

*History of Greek Music and of the Island Santorini (E.P Diamandis)*

***18:00 – 18:30*** *Modern Greece between East and West*

**Sotiris Mitralexis**

***18:30 – 19:00*** *Questions & Discussion*

***19:00 – 20:00*** *Opening Mixer (Dinner on your own)*

## Day two –Saturday, 17th June 2017

***09:30 - 09:45*** *Irreproducibility in science*

**Eleftherios P. Diamandis**

***09:45 - 10:00*** *Managing accreditation performance in Europe*

**Bernard Gouget**

***10:00 - 10:15*** *Updates of Biomarkers in Prostate Cancer*

**Qing Meng**

***10:15 – 10:45*** **Questions & Discussion**

***10:45 -11:30 BREAK*** *(e-poster viewing)*

***11:30 - 11:45*** *The human gut microbiome: its role in some pathological conditions*

**Francesco Salvatore**

***11:45 -12:00*** *Enzymes, life molecules*

**Enrique de la Morena**

***12:00 - 12:30*** **Questions & Discussion**

## Day two –Saturday, 17th June 2017

***12:30 -15:00 Lunch***

***15:00 -15:15*** *The five rights of Laboratory medicine*

**Mario Plebani**

***15:30 -15:45*** *Our last 7-8- years research in diabetes*

**Ivan Brandslund**

***15:45 -16:00*** *Immune based diagnostics for cancer detection*

**Karen Anderson**

***16:00 - 16:15*** *Small-coding RNAs as novel tumor biomarkers in prostate and bladder cancer*

**Margaritis Avgeris**

***16:15 -16:45*** **Questions & Discussion**

## Day three –Sunday, 18th June 2017

***09:30-09:45*** *Diabetic Dyslipidemia: A Major Complication of Obesity and Diabetic States*

**Khosrow Adeli**

***9:45 - 10:00*** *Diagnosis of genetic cardiomyopathies by way of multigene panels*

**Valeria D'Argenio**

***10:00 - 10:15*** *PT or EQA program: A necessary evil or a guardian angel in Laboratory Medicine?*

**Alexander Haliassos**

***10:15 -10:30*** *Long non-coding RNAs in cancer and their potential clinical applications*

**Herbert Yu**

***10:30 - 11:00*** **Questions & Discussion**

***11:00 -11:30 BREAK*** *(e-poster viewing)*

***11:30 -11:45*** *High sensitivity and ultrasensitive cardiac troponin assays in the clinical laboratories*

**Petr Jarolim**

## Day three –Sunday, 18th June 2017

***11:45 -12:00*** *KLK6 proteolysis is implicated in the regulation of extracellular alpha-synuclein species and may represent a novel therapeutic approach..*

**Georgia Sotiropoulou**

***12:00 -12:15*** *Enhanced proteolytic activities in Acral Peeling Skin Syndrome: A role of transglutaminase 5 in epidermal homeostasis".*

**Dimitra Kiritsi**

***12:15 -12:30*** **Questions & Discussion**

***12:30 -15:00 Lunch***

***15:00 -15:15*** *New targeted multimodal therapeutic approaches for type 2 diabetes mellitus: the new kids on the block.*

**Steven C. Boyages**

***15:15 -15:30*** *Therapeutic modulation of BDNF signaling in autism*

**Margaret Fahnestock**

***15:30 -15:45*** *Discovery of novel tumor biomarkers in prostate cancer using high sensitive proteomic methodologies*

**Spiros D. Garbis**

***15:45 -16:30*** **Questions & Discussion**

## Day four –Monday, 19th June 2017

***09:30-09:45*** *GcfDNA as a liquid biopsy in transplantation*

**Michael Oellerich**

***09:45-10:00*** *Tumor cell-free DNA copy number instability (CNI) to early predict and monitor therapeutic response to anticancer therapy*

**Ekkehard Schuetz**

***10:00-10:15*** *Mycobacterium brumae cell wall fractions with potential immunotherapeutic activity for bladder cancer*

**Naciye Leyla Acan**

***10:15-10:30*** *Demonstrating the value of laboratory medicine*

**Howard Morris**

***10:30 – 11:00*** **Questions & Discussion**

***11:00 -11:15 CLOSING REMARKS***

***11:15-15:00 ISE General meeting***

# INVITED SPEAKERS' ABSTRACTS

**Modern Greece between East and West**

Sotiris Mitralexis

In recent years, the European economic crisis--and Greece as one major epicenter thereof since 2009--stimulated Greek public debate on identity, and particularly on the relationship between Greek and European identity. Greece's Byzantine past and heritage, as well as today's predominance of an Eastern Orthodox religious affiliation, emerge as aspects of exceptional importance in this debate, which both directly and indirectly affect society, politics, and the actual relationship of Greece with the European Union and its member-states. In this paper, I will inquire into the deeper assumptions within the Greek public sphere as these are to be traced in the discourse produced by Greek philosophers, public intellectuals and academics who share the following two common traits: (a) a claim that Greece possesses a cultural otherness in comparison to "West" and "East" alike, which (b) is to be traced in its historical, cultural and religious roots. An unbiased inquiry into those currents of thought has been made almost impossible by what I name "Greek Neo-Orientalism." However, such an inquiry should contribute not only to the understanding of the Orthodox and Byzantine roots of modern Greek identity *per se* and of Greece's contemporary ambivalence, but it should also provide any interested scholar with an inner perspective on the causes and mechanisms of contemporary Greek political mentality.

**Irreproducibility in science**

Eleftherios P. Diamandis

Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Canada
Department of Clinical Biochemistry, University Health Network, Toronto, Canada
Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada

In this presentation I will draw attention to the issue of irreproducibility in science. Although this is not a new issue, recently, this problem has attracted a lot of interest. It has now been realized that at least 50% of published science is not reproducible and this is due to many reasons. During my presentation I will analyze the possible reasons for irreproducibility and measures that have been proposed to improve on the situation. More specifically, I will comment on my own suggestion for a "5 or 10 year reflection" which, I believe, is an effective , and cheap way of assessing irreproducibility in science. The advantages and disadvantages of some other suggestions that have been proposed by other will also be discussed.

**Managing accreditation performance in Europe**

Bernard Gouget

Fédération Hospitalière de France

The accreditation is reinforcing confidence as official recognition of competences and is offering a common framework of reference. An update on the EA survey-analysis of questionnaires filled by 36 national accreditation bodies will be presented to establish the state of accreditation process in European countries and to discuss the current situation with voluntary or mandatory accreditation considering that France was the first country to implement mandatory accreditation for the whole activity of the medical labs by order since 2013, confirmed by law since 2013. The accreditation process is under the responsibility of the Healthcare section of the Comité francais d'accréditation (COFRAC). This section is totally dedicated to human healthcare issues with two instances. The section committee participates in the elaboration of the strategy for accreditation of medical laboratories as well as in preparing the documents useful to evaluate and accredit. The current agenda for accreditation is formed by: 1) 50% of medical biology tests by volume which have to be accredited by December 31, 2017, and 2) by 100% of tests have to be accredited by November 1, 2020. In June 2017, 81% of the medical labs are complying with 50% of the tests accredited. In addition, 11 hospital medical labs are accredited for POCT according to the standard NF EN ISO 22870. as well as 15 Pathology labs with NF EN ISO 15189. Facing the Lab medicine challenges and having in mind the medicalization of the profession, there is a strong need to have a coordinated strategy and to adopt a more proactive approach to respond to the changing of the international laboratory medicine landscape.

**Updates of Biomarkers in Prostate Cancer**

Qing H Meng

Department of Laboratory Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA

Prostate cancer (PCa) is the most commonly diagnosed cancer in the United States and the second most common form of cancer worldwide in men. The prostate-specific antigen (PSA) has been widely used to screen and assess treatment efficacy for prostate cancer. However, lack of specificity of PSA limits its use in accurately guiding therapy and biopsy. Development and introduction of new biomarkers with specific indications for diagnosis, prediction, prognosis, and therapeutic response become critical. Recent advances in research with new markers such as Prostate Health Index (PHI), prostate cancer antigen (PCA) 3, the kallikreins (4K score), and other emerging molecular markers show improvement over PSA alone in guiding biopsy and detection of PCa. Emerging genomic and molecular markers (TMPRSS2:ERG, PTEN, Annexin A3, GSTP1, and ctDNA etc.) may be a potential tool in risk stratification and prediction of metastasis, recurrence, and prognosis. Nevertheless, much work remains to be done before any of these biomarkers are fully validated both clinically and technically for clinical use. The combination of multiple biomarkers with various technical approaches (blood-based tests, urine-based tests, tissue-based tests, and liquid biopsy) will improve the sensitivity, specificity and accuracy of the early detection of prostate cancer.

**The human gut microbiome: its role in some pathological conditions**

Francesco Salvatore[1,2], Valeria D'Argenio[1,2], Lucia Sacchetti[1]

[1]CEINGE-BiotecnologieAvanzate, via G. Salvatore 486, 80145 Naples, Italy. [2]Department of Molecular Medicine and Medical Biotechnologies, University of Naples Federico II, via Pansini 5, 80131 Naples, Italy.

Different areas in the understanding of the relationship between the specific cells/tissues of the bacterial world and human beings have been explored by the metagenomic analysis of the environments in which the interaction between the two types of cells/tissues is particularly intense. Undoubtedly, the most widely investigated environment is the gut microbiome, where the studies have revealed the type and abundance of bacterial taxa (in some instances also of fungi, virus and other mixed microbial populations) by the high throughput nucleic acid sequencing methodology available today, particularly through the 16S ribosomal RNAs microbial characterization(1), that yields data concerning the different proportions of taxa present in various physiological or pathological conditions, that will also make it possible to foresee therapeutic approaches (2). Microbial colonization begins immediately at birth. Although influenced by a variety of factors, namely, diet, physical activity, travel, illness, hormonal cycles and therapies, the microbiome is practically stable in healthy adults. This suggests that the microbiome plays a role in the maintenance of a healthy state in adulthood. In addition, since microbiome composition can be evaluated and modified, it appears to be a promising target for the development of novel diagnostic, prognostic and, most important, therapeutic strategies based on its manipulation (2). However, most gut microbioma studies have investigated faecal material, not only in human beings but also in mice and mice animal models as surrogate of human situations/diseases. In the last few years our two groups have conducted a series of research projects that will be briefly described together with the results obtained and their possible interpretation and significance (3-5). These studies are mostly concerned with microbioma modifications in relation to Crohn's disease and celiac disease. More recently, my group also studied the microbioma content at mucosa level at two sites along the gut, the ileum and the sigma sections of patients at different stages of the transformation of chronic hepatitis to cirrhosis and then to hepatocarcinoma (unpublished data). Lastly, other instances of microbiota metagenomic approaches will be briefly described.

**The five rights of laboratory medicine**

Mario Plebani

Department of Laboratory medicine, University-Hospital of Padova, ITALY

A body of evidence collected in the last few decades demonstrates that the pre- and post-analytical phases of the testing cycle are more error-prone than the analytical phase. However, the paradigm of errors and quality in laboratory medicine has been questioned, analytical mistakes continuing to be a major cause of adverse clinical outcomes and patient harm. Although the brain-to-brain concept is widely recognized in the community of laboratory professionals, there is lack of clarity concerning the inter-relationship between the different phases of the cycle, interdependence between the pre-analytical phase and analytical quality, and the effect of the post-analytical steps on the quality of ultimate laboratory information. Analytical quality remains the "core business" of clinical laboratories, but laboratory professionals and clinicians a like should never lose sight of the fact that pre-analytical variables are often responsible for erroneous test results and that quality biospecimens are pre-requisites for a reliable analytical phase. In addition, the pressure for expert advice on test selection and interpretation of results has increased hand in hand with the ever-increasing complexity of tests and diagnostic fields. Finally, the data on diagnostic errors and inappropriate clinical decisions made due to delay or misinterpretation of laboratory data underscore the current need for greater collaboration at the clinical-laboratory interface. Laboratory medicine should therefore figure as the “5 R” discipline, the five rights being based on: (1) Appropriateness; (2) Personalized and patient-centered service; (3) Focus on outcomes, (4) Value-added, and (5) Diagnostic partnership.

**Creation of a biobank for type 2 diabetes, sequencing of 9.000 patients and controls and correlation to risk and outcome**

Ivan Brandslund

[1] Department of Clinical Immunology and Biochemistry, Vejle, Denmark

[2] Institute of Regional Health Research, University of Southern Denmark, Denmark

The Vejle Diabetes Biobank was established from 2007-2010 as a regional Biobank containing blood DNA, and urine samples from patients with diabetes and an age matched control population age 25-75 years.Antropometrics were obtained by physical examination, questionaires and interviews at the time of the inclusion into the biobank. The cohort was linked to the Danish civil registration system, the Danish national patient registry and the Danish national prescription registry.In total 4.255 non-diabetic individuals and 3.320 patients with diabetes were included. Type 2 diabetes patients had higher body mass index than type 1 diabetes and control subjects. Fasting levels of plasma triglyceride and blood pressure were higher in type 2 diabetes patients than type 1 diabetes patients, but low density lipoprotein, total cholesterol were lower in type 2 patients. Patients and controls were genotyped using the OmniPress array with 700.000 random DNA markers pr. genome. The genetic background for type 2 diabetes and it's complications has been published in 18 original papers of which 8 in Nature or Science.

During this work it was concluded that type 2 diabetes seems not to be a genetic disease except for the MODY's. When comparing the Danish Greenland Inuit with the Danish caucasian population a large difference in fatty acid desaturases were found favoring energy production through fat consumption. Further, polymorphisms showed strong influence on weight and height in this population. These findings may be of importance for the increased prevalence of diabetes in the Inuit population now being fed on a western diet.

**Immune based diagnostics for cancer detection**

Karen Anderson

Biodesign Institute, Arizona State University and the Mayo Clinic Arizona, USA

While there is agreement that mammographic screening reduces breast cancer mortality, mammography has limitations, with reduced detection of cancers in women with dense breasts, over-diagnosis of benign breast lesions, and under-diagnosis of rapidly proliferative cancers. The performance of mammographic screening depends on various factors, including breast density and age. Both sensitivity and specificity decrease as breast density increases. Sensitivity and specificity are also lower in younger than older women, most likely because of the inverse association between age and breast density. Improvement of current screening strategies is thus most needed for younger women and women with dense breasts. In addition, for much of the world, systematic population screening by mammography is cost- and logistically-prohibitive. It has been suggested that circulating biomarkers could improve current screening strategies. These strategies include using circulating biomarkers as a complement to mammography in subsets of women for whom the sensitivity of mammography is the lowest, such as younger women and women with dense breasts, or between screening mammograms in women at high risk of breast cancer to improve the detection of interval cancers. Results of studies on the use of circulating biomarkers to detect breast cancer conducted to date, though, have been disappointing because of failure of biomarkers to be successfully validated and/or have low sensitivity. These results are not surprising given the heterogeneity of breast cancer which may limit the sensitivity of individual biomarkers. This talk will present data from multiple biomarker studies that support further evaluation of circulating biomarkers for screening for breast cancer, and emerging strategies for the design of biomarker validation studies.

**Clinical utility of small and long non-coding RNAs in bladder cancer**

Margaritis Avgeris and Andreas Scorilas

Department of Biochemistry and Molecular Biology, Faculty of Biology, University of Athens, Panepistimiopolis, 15701 Athens, Greece

Bladder cancer (BlCa) represents the fourth most common male malignancy and the cancer with the higher cost per patients for the health care systems of the Western world. Despite the reduced disease mortality of BlCa nowadays, which is mainly attributed to smoking reduction and to improvements of therapeutic management, BlCa remains a neglected disease, both in clinical and research area. Non-muscle-invasive bladder tumors recur frequently and can also progress to muscle-invasive stages, while muscle-invasive disease is highly aggressive and life-threatening. Therefore, the identification of the different molecular signatures of the disease could offer an alternative approach to improve disease prognosis and to support personalized treatment decisions. Non-coding RNAs (ncRNAs) are functional RNA molecules that are not translated into a protein. The family of ncRNAs includes several recently identified RNA, including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) implicated mostly in gene expression regulation at the transcriptional and post-transcriptional levels. The ability of ncRNAs to regulate gene expression has led to intense investigation of their functional role during cancer development and progression, as well as to elucidate their clinical value in patients' prognosis. In this regard, we have studied the clinical utility of miR-143/145 and miR-221/222 clusters, as well as miR-125b and miR-34a in urothelial bladder tumors. Deregulate expression levels were observed in bladder tumors for both clusters, while patients' survival analysis clearly highlighted their independent clinical ability to predict disease outcome following patients' treatment. Significantly reduced expression of GAS5, H19 and UCA1 lncRNAs was highlighted in bladder tumors, which were moreover correlated with unfavorable prognostic disease features, such as higher stage and grade tumors and high-EORTC patients' group. Finally, the loss of GAS5 and H19 was revealed to represent novel independent molecular markers for TaT1 patients short-term relapse and progression. In conclusion, small and long non-coding RNAs represent novel powerful predictors of bladder cancer prognosis.

*Acknowledgements*: This research was supported by the Hellenic Society of Medical Oncology and by the National and Kapodistrian University of Athens, Special Account for Research Grants.

**Diabetic Dyslipidemia: A Major Complication of Obesity and Diabetic States**

Khosrow Adeli

Senior Scientist, Molecular Medicine, Research Institute,
Head & Professor, Clinical Biochemistry, The Hospital for Sick Children, University of Toronto, Toronto, ON, Canada

Metabolic diseases particularly obesity, metabolic syndrome, and type 2 diabetes are increasing in prevalence and pose one of the major public health challenges worldwide. At least one quarter of the North American population has evidence of the metabolic syndrome and the prevalence is increasing. Metabolic syndrome describes a cluster of closely related risk factors for diabetes and cardiovascular disease (CVD), including abdominal obesity, hyperglycemia, hypertension, and dyslipidemia consisting primarily of hypertriglyceridemia and low HDL. The typical dyslipidemia (also referred to by some as 'the atherogenic dyslipidemia') observed in diabetic states is characterized by a cluster of quantitative and qualitative lipid and lipoprotein abnormalities. This includes increased plasma concentrations of fasting and postprandial apoB-containing, triglyceride-rich lipoproteins (TRL, including VLDL and chylomicrons), reduced HDL particle number and cholesterol content as assessed by plasma apoA-I and HDL-C respectively, and a predominance of small, dense LDL particles. Altered metabolism of TRL, both overproduction and impaired clearance, is central to the pathophysiology of the atherogenic dyslipidemia. Abnormalities can also occur in postprandial states due to overproduction of triglyceride-rich chylomicron particles. Thus lipid and lipoprotein abnormalities are commonly observed in diabetic states in both fasting and postprandial states. In this lecture, I will discuss the pathophysiology of diabetic dyslipidemia and its role in development of diabetic complications particularly atherosclerotic cardiovascular disease.

**Molecular diagnostics of genetic cardiopathies by way of multigene panels**

Valeria D'Argenio[1,2], Maria Valeria Esposito[1,2], Giulia Frisso[1,2], Francesco Salvatore[1,2]

1 CEINGE-Biotecnologie Avanzate, via G. Salvatore 486, 80145 Naples, Italy.
2 Department of Molecular Medicine and Medical Biotechnologies, University of Naples Federico II, via Pansini 5, 80131 Naples, Italy.

Inherited cardiopathies are a group of heterogeneous genetic diseases usually classified according to functional and morphological abnormalities of the cardiac muscle and/or primary involvement of cardiac electric transmission. All these diseases are featured by a high clinical and genetic heterogeneity. Clinical presentation ranges from asymptomatic to severe rapidly worsening forms and the same symptoms can be the expression of different diseases. The age of onset is also extremely variable: sometimes an inherited cardiac disease is diagnosed before birth, while other subjects can show clinical signs later in the adulthood. Most relevant, all of these diseases can predispose to the development of malignant arrhythmias, heart failure and sudden cardiac death (SCD). It has been estimated that the majority of SCD in young individuals can be related to the presence of one of the above mentioned diseases. Therefore, the correct identification of the molecular alterations responsible for inherited cardiopathies is crucial for the correct patient's management and for the identification of all the at risk subjects within the affected families. The cardiopathies' clinical variability is reflected in the heterogeneity of their genetic basis. To date, tens of genes, showing different mechanisms of inheritance and incomplete penetrance, have been related to the onset of each inherited cardiopathy. However, they explain only a variable proportion of all cases suggesting the existence of other, still unknown disease-causative genes. In addition, the highly variable phenotipic expression, also in the presence of the same causative mutation and in the same family, have strongly suggested a role for additional inherited variants, in the same gene or in independently inherited genes, able to act as phenotype-modifiers. Finally, molecular variants in the same gene have been related to different cardiopathies. Taken together, all the above-mentioned issues explain the difficulties in the correct molecular diagnosis of these overlapping diseases. Within the last decade, the so called next generation sequencing (NGS) technologies, enabling the simultaneous analysis of large number of genes, have overcome these limitation, explaining the diffusion of NGS-based protocols for the molecular diagnosis of inherited cardiopathies. To assess the reliability of NGS for the study of the molecular basis of inherited cardiopathies, in proof-of-concept type of experiment, we designed and tested a DNA sequence capture procedure that was able to sequence simultaneously 202 cardiopathy-related genes. Our procedure allows the

simultaneous analysis of a large number of genes, thus obtaining a molecular diagnosis of alterations also in those patients for which traditional screening was not informative. In addition, it can identify mutations in other genes that, acting as phenotype modifiers, could be responsible for clinical variability. Furthermore, reducing time and costs and increasing the sensitivity of molecular testing, we could implement routine inherited cardiopathies molecular diagnostics and obtain a model easily applicable to other genetic diseases. Based on these findings, we have now designed three panels for the molecular diagnosis of inherited cardiopathies that are going to be introduced in routine practise improving the diagnostic flowchart of affected patients and of their families, also for preventative approach to monitoring and avoidance of the often fatal events like the SCD.

*Acknowledgements*: This work was supported by grants to F.S. from Regione Campania (Naples - Italy) and from MIUR (Rome - Italy).

**PT or EQA programs: A necessary evil or a guardian angel in Laboratory Medicine?**

Alexander Haliassos

Chair of the IFCC Task Force on Proficiency Testing (TF-PT)

The analytical quality in Laboratory Medicine is maintained using concurrently the Internal and External quality control programs. The role of Proficiency Testing schemes (ex. External Quality Control programs) is of primordial importance to the analytical quality, to the standardization of the methods and to the harmonization of the results, but there is a lack of interest from the commercial providers of such schemes either for the more new and complex tests and for the very old and simple measurands that involve a new calibration curve, because of the very subtle problems induced at the interpretation of the statistical results of their, already well-established and running, schemes. Also an involvement in this sector has small financial interest for them, in comparison to the scientific resources that they have to allocate to such a novel program. For this reason harmonization of the laboratory results is far to be achieved and in order to resolve these issues AACC introduced the project of the harmonization of clinical laboratory test results. Recently, a new IFCC Task Force has been created to be involved in the analysis and the exploration of the above mentioned of Proficiency Testing issues. This effort could lead to the establishment of specialized schemes under the hospices and recommendations of the IFCC and could greatly enhance and help to the prevalence of the methods derived from the work of the federation and to the harmonization of laboratory results.

**Long non-coding RNAs in cancer and their potential clinical applications**

Herbert Yu

University of Hawaii Cancer Center, University of Hawaii, Honolulu, HI, USA.

Long non-coding RNAs (lncRNAs) are a class of newly recognized DNA transcripts that have diverse biological activities. Dysregulation of lncRNAs may be involved in many pathogenic processes including cancer. Recently, we found an intergenic lncRNA, *LINC00472*, whose expression was correlated with breast cancer progression and patient survival. Our findings were consistent across multiple clinical datasets and supported by results from *in vitro* experiments. To evaluate further the role of *LINC00472* in breast cancer, we used various online databases to investigate possible mechanisms that might affect *LINC00472* expression in breast cancer. We also analyzed associations of *LINC00472* with estrogen receptor, tumor grade and molecular subtypes in additional online datasets generated by microarray platforms different from the one we used previously. We found that *LINC00472* expression in breast cancer was regulated more possibly by promoter methylation than by the alteration of gene copy number. Analysis of additional datasets confirmed our previous findings of high expression of *LINC00472* associated with ER-positive and low-grade tumors and favorable molecular subtypes. Finally, in nine datasets, we examined the association of *LINC00472* expression with disease-free survival in patients with grade 2 tumors. Meta-analysis of the datasets showed that *LINC00472* expression in breast tumors predicted the recurrence of breast cancer in patients with grade 2 tumors. In summary, our analyses confirm that *LINC00472* is a potential tumor suppressor, and that assessing its expression in breast tumors may have clinical implications in breast cancer management.

**High sensitivity and ultrasensitive cardiac troponin assays in the clinical laboratories**

Petr Jarolim

Brigham and Women's Hospital, Harvard Medical School, Boston, MA

Immunoassays measuring cardiac troponins I or T are critical tools for diagnosing acute myocardial infarction. Most contemporary cardiac troponin assays provide adequate diagnostic performance. However, the increased sensitivity and precision of the new, high sensitivity assays that have already been introduced into clinical practice, provide the potential to further shorten intervals between blood draws or the time needed to detect the first significant troponin elevation. In addition to the relatively modest benefits at the diagnostic end, the high sensitivity assays and the investigational ultrasensitive cardiac troponin assays offer improvements for predicting major adverse cardiovascular events, development of heart failure or transition to end-stage kidney disease. These novel high sensitivity assays can measure troponin concentrations in almost all healthy individuals and by enabling the measurement of patient's baseline troponin levels and their long-term monitoring, the may alert clinicians about deteriorating cardiorenal conditions. We will review the high sensitivity and ultrasensitive assays and their applications as tools for predicting individual risk of future adverse events and for guiding and monitoring corresponding adjustments of preventative therapeutic interventions.

**KLK6 proteolysis is implicated in the regulation of extracellular alpha-synuclein species and may represent a novel therapeutic approach**

Georgios Pampalakis[1], Vasia-Samantha Sykioti[2], Methodios Ximerakis[2], Kostas Vekrellis[2], Georgia Sotiropoulou[1,2*]

[1]Department of Pharmacy, School of Health Sciences, University of Patras, 26500 Rion-Patras; [2]Center for Neurosciences, Biomedical Research Foundation, Academy of Athens, Athens, Greece

Kallikrein-related peptidase 6 (KLK6) is a serine protease highly expressed in the nervous system. In synucleinopathies, including Parkinson disease (PD), the levels of KLK6 in CSF correlate inversely with those of α-synuclein. Recent evidences have implicated certain extracellular α-synuclein species (fibrils) in synucleinopathies through a prion-like propagation mechanism. Thus, normally regulated turnover of extracellular α-synuclein is of major importance yet the underlying mechanisms remain unexplored. We showed that KLK6 can degrade the naturally secreted α-synuclein directly and indirectly *via* a proteolytic cascade that involves downstream metalloproteases (Ximerakis et al. FASEB J 2014). Intergration of biochemical analyses and degradomics (TAILS, Terminal Amine Isotopic Labeling of Substrates) identified MMP2 and ADAMTS19 as members of this cascade that are activated by KLK6 in neuronal environment. Importantly, we found that KLK6 can readily cleave synthetic α-synuclein fibrils that are neurotoxic and have the ability for cell-to-cell propagation *in vivo* (Pampalakis et al. Oncotarget 2016). To demonstrate that our *in vitro* data are physiologically relevant, we have generated *Klk6*$^{-/-}$ mice (collaboration with Prof. Andras Nagy, University of Toronto, Canada) and assessed them by standard behavioral tests. *Klk6*$^{-/-}$ mice exhibited reduced grip strength, consistent with motor defects, which could be reminiscent of Parkinson-like symptoms. Intriguingly, at 3 months of age, *Klk6*$^{-/-}$ animals showed increased levels of high molecular weight α-synuclein species in their cortex. Further, KLK6-deficient cortical neurons showed increased uptake of fibrillar α-synuclein. We have constructed recombinant adenoviral vectors for KLK6 delivery and demonstrated that the levels of extracellular α-synuclein can be regulated by neuronally secreted KLK6. Our findings open up the possibility to exploit KLK6 as a putative pharmaceutical protein for synucleopathies and may represent a novel pharmacological approach for therapeutic intervention.

**Enhanced proteolytic activities in Acral Peeling Skin Syndrome: A role of transglutaminase 5 in epidermal homeostasis**

Dimitra Kiritsi[1*], Georgios Pampalakis[2*], Eleni Zingkou[2], Claus-Werner Franzke[1], Manthoula Valari[3], Georgia Sotiropoulou[1]

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Renewal of the stratum corneum is ensured by a finely tuned network of proteases and their endogenous inhibitors, in which kallikrein-related peptidases (KLKs) play key roles. De-regulated proteolysis seems to be a feature of skin pathologies of distinct genetic causes, which are nonetheless all characterized by defective epidermal barrier. Acral Peeling Skin Syndrome (APSS) is a rare autosomal recessive skin disorder characterized by painless, superficial blistering and peeling of the hands and feet. Very little is currently known on the pathogenetic mechanisms underlying APSS disease except that it is caused by mutations in the *TGM5* gene encoding transglutaminase 5. Since APSS is a desquamating disorder, we hypothesized that aberrant epidermal proteolysis could be the underlying cause of this pathognomonic feature. We analyzed the overall proteolytic activities in the epidermis of APSS patients and normal donors by *in situ* zymography using fluorescently-quenched gelatin, casein, and elastin substrates to map different protease specificities. We found that the APSS epidermis is characterized by aberrantly elevated proteolytic activities resulting in enhanced degradation of DSG1, thus, compromising skin barrier function. Whether the increased proteolysis is a secondary effect due to epidermal barrier dysfunction or the inability of inhibitors to crosslink into the stratum corneum remains to be elucidated.

**New Targeted Multimodal Therapeutic approaches for type 2 diabetes mellitus: the new kids on the block**

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Diabetes Mellitus is a common disorder whose incidence continues to rise due in part to genetic factors but advanced by the increasing prevalence of obesity and reduced physical activity. The disorder disturbs intermediary metabolism through a complicated set of pathophysiologic steps that results in accelerated microvascular and macrovascular complications and premature morbidity.

Until recently, only a few drugs were available to treat the disorder. In the last 5 years a new range of compounds have been introduced that target multiple steps in the pathophysiology of the disorder to normalise blood glucose control. These drugs include the DPP-IV inhibitors, the GLP-1 agonists and the SGLT-2 inhibitors, as well as new insulin analogues. These new kids on the block have shown dramatic clinical efficacy, an ability to promote weight loss and improved cardiac outcomes. This paper will review the evidence in brief.

**Therapeutic modulation of BDNF signaling in autism**

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Autism is a common neurodevelopmental disorder characterized by impairments in social communication and interaction and by repetitive behavior. Abnormalities in dendritic spines (sites of excitatory synapses) resulting in aberrant neuronal connections are thought to underlie autistic behavior. The molecular mechanisms responsible for altered connectivity and autistic behavior are unclear, impeding treatment options; identifying the molecular mechanisms underlying autistic behavior is necessary for the development of novel therapeutics. Reduced protein and signaling through the BDNF receptor (TrkB)-Akt pathway are found in postmortem brain tissue from subjects with idiopathic autism and in rodent brains following prenatal exposure to valproic acid (VPA), a known risk factor for autism in humans and an accepted model of environmentally-caused autism. Pre-adolescent mice prenatally exposed to valproic acid exhibit autistic-like behaviour in a sex-dependent manner in tests of sociability and repetitive behaviour. They also show decreased phosphorylated Akt and increased dendritic spine density. Treatment of these mice with the TrkB partial agonist LM22A-4 restores sociability, decreases repetitive behaviour and normalizes Akt phosphorylation and spine density. These findings confirm the feasibility of reversing autistic behavior postnatally and identify TrkB-Akt signaling as a potential therapeutic target for treating social deficits and repetitive behavior in idiopathic autism.

**Novel insight to the causal link between insulin signaling deregulation and the etiology of aggressive prostate cancer: Implications to the development of novel biomarkers and treatment targets**

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The systems interrogation of human derived clinical specimens has now become requisite to any comprehensive biomarker discovery and its functional validation research program. It relies heavily on the analysis of high throughput functional genomic features at multiple levels of molecular biology events, namely gene expression at the transcript, protein and metabolite levels. However, the clinical exploitation of such multi-omic observations, either in terms of gaining a mechanistic understanding of the molecules undergoing perturbations as their relate to specific disease processes, or how they effectively stratify disease down to the individual patient level (personalized medicine) regardless of their innate heterogeneity of presentation, has had limited success. To achieve this, we have developed mathematical models that extract the spatially (prostate tissue and matched serum), temporally (time dependent) and quantitatively robust information implicated in regulatory processes. It was found that the progression to aggressive prostate cancer implicated the disruption of DNA damage response elements, autophagy, apoptosis, insulin signalling and cell metabolism. Such a multi-parametric pipeline identified protein signatures that strongly correlated with aggressive prostate cancer relative to the benign state. Hallmark proteins to these signatures included pyruvate kinase M2 isoform, eEF isoforms 1-3, IGF BP isoforms 1-6, and MAP kinase isoforms 1 and 2 that could be used for the early prediction of aggressive prostate cancer and its treatment.

**GcfDNA as a liquid biopsy in transplantation**

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Biomarkers are needed to facilitate personalized immunosuppression. A particularly promising new approach for early detection of acute graft rejection is based on graft-derived cell-free DNA (GcfDNA), using droplet digital PCR. In two recent prospective studies, our group evaluated this new approach in liver and cardiac transplant recipients, followed over at least one year post-transplant. In a prospective multicenter cohort study in liver transplantation, it could be shown that GcfDNA values were about 9-fold elevated in patients (N=17) during biopsy proven acute rejection (BPAR) episodes, compared to median values observed in 88 patients during stable periods. GcfDNA values were already elevated 7-15 days prior to the diagnosis of BPAR. Conventional liver function tests (LFTs) showed low overall correlations with GcfDNA. GcfDNA provided additional LFT-independent information on graft integrity. The diagnostic sensitivity and specificity were 90.3% and 92.9% respectively for GcfDNA. In a further study cardiac recipients with BPAR (N=19) had about 6-fold higher GcfDNA test results compared to apparently stable patients (N=66) and about 3-fold higher values compared to biopsy negative patients (N=23). Fourteen patients already showed within 9 – 30 days prior to the diagnosis of acute rejection 5-fold higher GcfDNA results. There was only a low correlation of GcfDNA with hs-Troponin I (r=0.50). Plasma GcfDNA allowed for better discrimination of liver and cardiac transplant recipients with acute rejection, compared to conventional tests. GcfDNA provides actionable healthcare information, and may be helpful to personalize post-Tx immunosuppression.

**Tumor cell-free DNA copy number instability (CNI) to early predict and monitor therapeutic response to anticancer therapy**

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High-quality genomic analysis is used for pharmacotherapy selection in patients with cancer. Tumor-specific genomic alterations can be identified in cell-free DNA (cfDNA) and can complement biopsies for real-time molecular monitoring of treatment, detection of recurrence, and tracking resistance. cfDNA is particularly useful when tumor tissue is unavailable or insufficient for testing. Next-generation sequencing, droplet digital PCR (ddPCR) or conventional PCR are currently used for this "liquid biopsy" testing, e.g. for *EGFR* mutations in patients with non-small cell lung cancer (NSCLC). Also allowing for the identification of resistance mutations selected by treatment, such as *EGFR T790M* under gefitinib. The evaluation of chromosomal aberration pattern in cfDNA seems a more universal approach. Such tumor derived changes can be quantified as genomic copy number instability (CNI) score of cfDNA. Changes in CNI scores were shown to be indicative of e.g. prostate cancer and can serve as early predictor of therapy failure to cytotoxic chemotherapy and for immunotherapy for various cancer types (e.g. NSCLC, colorectal, pancreatic ductal adenocarcinomas), by serial testing during therapy. Beside the positive impact on disease management, this has potential for cost savings by avoiding ineffective use of expensive new anticancer drugs. Nevertheless, larger validation studies on blood-based tumor genomic profiling are needed an in the way; nevertheless, cfDNA monitoring can provide clinically important actionable information for precision oncology approaches.

## *Mycobacterium brumae* cell wall fractions with potential immunotherapeutic activity for bladder cancer

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Bladder cancer is the fourth most common cancer among men, and the 14th most common type among women. Low mortality rate and high recurrence rate signifies its being a chronic disease and requirement for the development of new drugs. Intravesical Bacillus Calmette-Guerin application is a gold standard treatment for certain non- muscle invasive bladder cancer. However, it has several local and systemic side effects. We aimed to prepare less toxic and more potent therapeutic agents which can be used instead of a live strain for the treatment of bladder cancer and tried to find out the cell components which are effective in the treatment. Non-pathogenic strain *Mycobacterium brumae* which has immunostimulating and cytotoxic activity comparable to Bacillus Calmette-Guerin was used for this purpose. Cell wall fractions of the bacteria were subjected to HPLC. Tumour necrosis factor □ stimulating activities and the MALDI-TOF spectra of the samples were analysed. Two components, around 1800 Da and 3600 Da were detected as candidates for the immunostimulating activity. These components may have a potential for development of new drugs for the treatment of superficial bladder carcinoma.

**Demonstrating the Value of Laboratory Medicine**

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Rising costs and demands for improvement in the quality of healthcare are requiring increased services with reduced budgets and driving discussions on value in healthcare. The dominant model of funding for laboratory medicine is fee for service, which has focussed payers on costs and laboratory management on cost minimisation. The past period has seen an era where quality of laboratory testing has been under pressure with large scale automation and consolidation of laboratories being emphasised to achieve economies of scale to reduce costs. Opportunities for future stepwise reductions in clinical laboratory costs are likely to have diminished. The focus is now on delivering improved testing in a cost neutral or at least cost effective manner. This brings laboratory medicine into line with other health services that focus on value for money for payers and maximising health outcomes for patients. The IFCC advocates that laboratory medicine needs to 'add-value' to laboratory testing by improving patient outcomes on top of quality performance. This therefore raises the question as to how can this added value be demonstrated? One approach is to adopt the commercial concept of a Value Proposition which, when described for a particular test, will explicitly state a number of core attributes. These attributes include the suitability of patients for its use, the role the test result will play within the patient care pathway and the benefits and costs to all the relevant stakeholders. Application of the Value Proposition concept will extend existing principles of evidence-based medicine. It will importantly provide opportunities for the laboratory profession to provide leadership for both the adoption of new tests as well as modification of existing test usage rather than operating in the isolated and increasingly constrained laboratory medicine silo that typically describes current operations.

# POSTER ABSTRACTS

**Targeting epidermal KLK5 to rescue Peeling Skin Syndrome**

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The epidermis forms our protective barrier that is essential for body survival. Physiological skin desquamation depends on the tightly regulated activity of kallikrein-related peptidases (KLKs), while KLK5 is hypothesized to initiate a proteolytic cascade that results in corneocyte shedding and stratum corneum renewal. Our recent data suggest that KLK5 is the key enzyme in pathological overdesquamation associated with Netherton Syndrome (Furio et al. PLoS Genet 2015). Hyperactivation of KLK5 in epidermis has also been reported in other severe ichthyoses such as the Peeling Skin Syndrome-type B or Peeling Skin Disease (PSD) caused by mutations in the *Cdsn* gene that encodes corneodesmosin. *Cdsn-/-* mice recapitulate the disease although, as in many cases of invalidation of genes expressed in late differentiation of epidermis, they die soon after birth. Here, we have crossed *Klk5-/-*mice that we have generated with *Cdsn+/-* mice to generate *Klk5-/-Cdsn-/-* mice, in order to answer whether ablation of Klk5 is sufficient to reduce the unopposed proteolytic activities and to stabilize the desmosomes. We have characterized the epidermis of *Klk5-/-Cdsn-/-* for the normalization of epidermal differentiation, the levels of proteolysis and the function of the epidermal barrier. We applied proteomics/degradomics, NMR-based lipidomics and transcriptomics in mouse models to delineate alternative (yet unidentified) proteolytic pathways or other biomolecules, which could be involved in PSD and should be targeted in concert with KLK5. These o*mics* approaches may allow us to answer whether proteolysis represents a major pathogenesis mechanism in PSD. The findings of our study could also be beneficial to treat other ichthyoses or inflammatory skin diseases.

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**Molecular cloning of novel alternatively spliced transcripts of human kallikrein-related peptidase 10 (*KLK10*) using next-generation sequencing**

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The newly introduced next-generation sequencing (NGS) technology has enabled genome-wide studies, providing massively parallel DNA sequencing. NGS applications constitute a revolution in molecular biology and genetics and have already paved new ways in cancer research. Tissue kallikrein and kallikrein-related peptidases (KLKs) form the largest group of serine proteases in the human genome, sharing many structural and functional characteristics. Multiple alternative transcripts have been reported for the most human *KLK* genes, while many of them are aberrantly expressed in various malignancies, thus possessing significant prognostic and/or diagnostic value. Alternative splicing of cancer-related genes is a common cellular mechanism accounting for cancer cell transcriptome complexity, as it affects cell cycle control, proliferation, apoptosis, invasion, and metastasis. In this study, we describe the identification and molecular cloning of eight novel transcripts of the human *KLK10* gene using 3' rapid amplification of cDNA ends (3' RACE) and next-generation sequencing (NGS), as well as their expression analysis in a wide panel of cell lines, originating from several distinct cancerous and normal tissues. For this purpose, total RNA was extracted from 55 human cell lines, followed by first-strand cDNA synthesis using an oligo-dT-adaptor sequence as primer. Next, nested 3′-RACE PCR was applied for the molecular cloning of novel *KLK10* transcripts. PCR products were then cleaned-up and used for library construction. The quality and concentration of the constructed library was determined with quantitative real-time PCR. In the next step, NGS was carried out on an Ion PGM™ system. Bioinformatics analysis revealed a total of eight novel *KLK10* transcripts, which contain new alternative splicing events between already annotated exons as well as novel exons. In addition, investigation of their expression profile in a wide panel of cell lines was performed with nested RT-PCR using variant-specific primers. Since many *KLK* mRNA transcripts possess clinical value, these newly discovered *KLK10* transcripts appear as new potential biomarkers for diagnostic and/or prognostic purposes or as targets for therapeutic strategies.

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## *BCL2L12*: A multiple spliced gene with a significant potential as biomarker in breast cancer

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Introduction: Apoptosis is a highly orchestrated, genetically regulated form of cell death, the impairment of which is crucial in breast cancer (BC) development and progression. *BCL2L12*, a member of the BCL2 family of apoptosis-related genes, has been studied in various malignancies, revealing its potential role as a tumor biomarker. It has been recently found that *BCL2L12* is subjected to alternative splicing, resulting in the generation of 13 alternatively spliced variants. The aim of this study was the quantification of *BCL2L12* splice variants 1 and 4 (v.1 and v.4) expression at the mRNA level and the assessment of their biomarker potential in BC. Methods: Total RNA was extracted from 80 pairs of BC and normal tissues. Thereafter, RNA was reverse transcribed into first strand cDNA, which in turn was used as template in a SYBR Green based Real-Time PCR assay. Relative quantification analysis was conducted using the comparative CT ($2^{-ddCT}$) method, and the associations of *BCL2L12* variants expression with various clinopathological parameters, were evaluated by statistical analysis. Results: *BCL2L12* v.1 mRNA levels were found to be significantly ($p \leq 0.001$) higher in malignant compared to their matched non-cancerous breast tissues. Moreover, *BCL2L12* v.1 demonstrated increased expression in women with PR negative ($p=0.036$) and Grade III tumors ($p= 0.002$). Interestingly, significant *BCL2L12* v.1 upregulation ($p = 0.024$) was observed in triple negative BC. Regarding *BCL2L12* v.4, a significant upregulation was observed in malignant compared to their matched non-cancerous breast tissues ($p = 0.004$). Moreover increased *BCL2L12* v.4 expression levels were associated with ER- and PR negativity ($p = 0.015$ and $p\leq 0.001$ respectively ). Finally, similar to *BCL2L12* v.1, *BCL2L12* v.4 upregulation ($p\leq 0.001$) was observed in triple negative BC. Conclusion: Our preliminary results indicate a possible involvement of *BCL2L12* v.1 and v.4 in BC progression and suggest their potential as biomarker in this malignancy.

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**Expressional status of the tumor-suppressor miR-195 in lobular, ductal and benign breast tumors: a new potential biomarker in the molecular subtyping and the prognosis of breast adenocarcinomas**

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The potential of microRNAs (miRNAs) as novel tumor markers has been the focus of recent scrutiny because of their tissue specificity, stability, and association with clinicopathological parameters. MicroRNA-195 (miR-195) is a tumor suppressor, since one of its direct targets that has been identified is the antiapoptotic *Bcl-2*, playing an important role in tumorigenesis. The aim of the present study was to analyze the expression levels of miR-195 in 68 benign and 137 malignant breast tumors so as to explore its clinical value in breast cancer. Total RNA was extracted, polyadenylated, and reversely transcribed to cDNA from tissue specimens. Subsequently, a highly sensitive quantitative real-time PCR protocol was developed and miR-195 levels were then estimated by applying the $2^{-\Delta\Delta C_T}$ method by using *RNU48* as a reference gene. The relative quantification units measured for miR-195 were finally subjected to comprehensive statistical analysis. The analysis indicated that miR-195 significantly downregulated in malignant compared to benign tumors, highlighting its value in discriminating these breast lesions (AUC: 0.633; 95% CI: 0.552 – 0.713; $P = 0.002$). Moreover, the comparison of invasive ductal and lobular adenocarcinomas revealed a significant ($P < 0.001$) increase in miR-195 levels of lobular tissues. Regarding the correlation of miR-195 expression with molecular subtypes, HER2 positive tissues displayed significantly more elevated expression of miR-195 ($P = 0.001$) than those of luminal and even more than those of basal-like ones. A positive correlation was observed between miR-195 levels and primary tumor staging ($P = 0.001$). In addition, miR-195 expression levels were increased in the samples with positive hormonal ($P < 0.001$) and node status ($P = 0.031$). Overall these results recommended that miR-195 expression constitutes a promising molecular marker for the diagnosis, prognosis and classification of breast carcinomas.

**Elevated tissue levels of miRNA-28-5p predict short-term relapse and poor overall survival of patients with colorectal adenocarcinoma**

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Many small non-coding RNAs, including microRNAs (miRNAs) are aberrantly expressed in cancer and leukemia. MicroRNA-28-5p (miR-28-5p) is involved in cancer-related cellular processes, including cell growth and cycle control, proliferation, and apoptosis. miR-28-5p targets several cancer-related genes and is hence involved in cell proliferation, migration, invasion, and epithelial-mesenchymal transition. In this study, we investigated the potential diagnostic and prognostic significance of miR-28-5p expression in colorectal adenocarcinoma, the most frequent type of colorectal cancer. Therefore, total RNA was extracted from 182 colorectal adenocarcinoma specimens and 86 non-cancerous colorectal mucosae. After polyadenylation of 2 μg total RNA by poly(A) polymerase and subsequent reverse transcription with an oligo-dT adapter primer, we quantified miR-28-5p levels using an in-house–developed reverse-transcription real-time quantitative PCR method, based on the SYBR Green chemistry using *SNORD43* (*RNU43*) as endogenous control. Extensive biostatistical analysis revealed the comparison of miR-28-5p levels among 86 pairs of colorectal tumors and their adjacent non-cancerous mucosae uncovered the downregulation of miR-28-5p expression in the majority of malignant colorectal tumors. More importantly, high miR-28-5p expression predicts poor disease-free survival (DFS) and overall survival (OS) of colorectal adenocarcinoma patients. Multivariate Cox regression analysis revealed that miR-28-5p overexpression is a significant predictor of poor prognosis in colorectal adenocarcinoma, independent of tumor size, histological grade, TNM staging, radiotherapy and chemotherapy. Interestingly, strong miR-28-5p expression retains its predictive potential regarding relapse among patients with negative regional lymph nodes, and predicts poor OS in patients diagnosed with non-metastatic colorectal adenocarcinoma. High miR-28-5p expression predicts poor poor disease-free survival (DFS) and overall survival (OS) of colorectal adenocarcinoma patients, independently of clinicopathological prognosticators and standard patient treatment currently used for prognosis., including chemotherapy and radiotherapy. *Acknowledgements:* This work was financially supported by the Hellenic Society of Medical Oncology.

**Discovery of novel transcripts of the SCAF1 gene in human cancer cells, using next-generation sequencing technology**

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The human SR-related CTD associated factor 1 (SCAF1) gene is a new member of the human SR (Ser/Arg-rich) superfamily of pre-mRNA splicing factors, that has been discovered and cloned by members of our group. SCAF1 interacts with the CTD domain of the RNA polymerase II polypeptide A and is firmly involved in pre-mRNA splicing. Although it was found to be expressed widely in multiple human tissues, its mRNA levels vary a lot. Additionally, many studies have clarified that SCAF1 mRNA transcript was found to be overexpressed in a series of human tumors, confirming its significant prognostic value as a cancer biomarker. In this study, we describe the identification and molecular cloning of fifteen novel transcripts of the human SCAF1 gene, using nested PCR and NGS technology. For this purpose, total RNA extraction from 55 human cancer cell lines was performed, followed by first-strand cDNA synthesis using an oligo-dT-adaptor as primer. Following, nested PCR was carried out for the molecular cloning of novel SCAF1 transcripts. PCR products were then purified and used for library construction. The concentration of the created library was assessed using quantitative real-time PCR. In the next step, NGS was performed on an Ion PGM™ system. Computational and bioinformatics analysis of the obtained NGS data revealed the existence of nine novel alternative splicing events between annotated exons of SCAF1. As a result, a total of fifteen novel SCAF1 alternative transcripts were discovered and their expression profile was investigated with PCR experiments using variant-specific primers, followed by agarose gel electrophoresis. From all the fifteen newly discovered transcripts, seven have an open reading frame (ORF) and are hence predicted to encode for novel SCAF1 protein isoforms, whereas the rest eight are nonsense-mediated mRNA decay (NMD) candidates as they contain a premature stop codon. Since SCAF1 is implicated in many human malignancies, qualifying as a potential biomarker, the quantification of the presented novel transcripts in human samples may have clinical applications in different types of cancer.

## Changes in angiogenesis biomarkers in the early phase of knee osteoarthritis

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Major changes in osteoarthritic joint tissues were long treated as merely the consequences of degeneration (arthrosis), m.p. incurable. Angiogenesis in the case of osteoarthritis (OA) has been relatively little explored, although it is likely that namely angiogenesis contributes to persistence of inflammation. Today dozens of pro- and anti-aniogenic factors are described that are believed to have a role in OA, but there is no clarity regarding how to guide the process *in vivo*.

*Aim:* To identify the main biomarkers, resp. possible regulators, of angiogenesis in the early phase of knee osteoarthritis (kOA).

*Material and methods:* Sixty subjects (mean age 50 +/- 7.3 years, 25 female and 35 male) from an Estonian population-based symptomatic cohort were investigated. Radiographic diagnosis of knee OA based on the Nagosa-Doherty system (Kumm et al. 2013). Luminex technology was used to determine plasma values of 26 angiogenesis-related cytokines.

*Results.* Already in grade 1 sumOA, significant changes were noted: upregulation of PDGF-BB, ANG-1 and CCL5/RANTES concurrent with an increase of neovascularization-inhibiting TIMP-4 and simultaneous downregulation of angiogenesis inhibiting plasma CLCL10/IP-10. Futher, the level of CLCL10 / IP-10 in grade 2 sumOA did not differ any more from that in sumOA 0. When osteophyte formation is regarded as an early separate feature of OA, then it is associated with an increase of plasma TGF-β1 and sICAM1.

*Comments:* The attempt to find the main biomarkers of angiogenesis in early KOA was successful. Already in OA grade 1, simultaneous activation of both angiogenesis promoting and inhibitory factors was revealed. Gender differences in inflammatory regulation of kOA may prove useful in personalized management of knee OA patients in the future.

*Perspectives for therapie:* Activation of CLCL10/IP-10 or chemokine CXC receptor 3 may provide a novel therapeutic agent to inhibit neoangiogenesis (Yates-Bender et al. 2012). Inhibition of certain cytokines or angiogenic growth factors could become an important method for treatment of knee OA for the subgroup with extensive synovitis (Hamilton et al. 2016).

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**NOTES**