

Saturday, 21.09.2019**CliniMARK Networking, WG and MC Meeting**

The presentations and posters will exhibit research activities by CliniMARK members in order to promote collaboration and joint projects.

Location: Amphitheater, Biomedical Research Foundation, Academy of Athens (BRFAA)
<http://www.bioacademy.gr/?lang=en> , Soranou Efessiou 4, 11527 Athens

8:30 Pick up of participants from President Hotel lobby <https://president.gr/> by Makis and transfer to BRFAA by Metro and walking

9:00-9:15	Welcome Luider, Vlahou, Zoidakis
9:15-9:45	Viviana Greco Institute of Biochemistry and Clinical Biochemistry, Catholic University of the Sacred Heart, Rome, Italy Direct analytical sample quality assessment for biomarker investigation: clinical perspective of high-resolution proteomics
9:45-10:15	Goran Gajski Institute for Medical Research and Occupational Health, Zagreb, Croatia Evaluation of multiple health-related biomarkers in vegetarians versus omnivores
10:15-10:45	Marei Sammar Department of Biotechnology Engineering, Braude College, Karmiel, Israel Extracellular vesicles from human placenta carry placental protein-13 (PP13) and levels are decreased in preeclampsia
10:45-11:15	Coffee Break and Poster Session
11:15-11:45	Viviana Roman Center of Immunology, Inst."Stefan S. Nicolau", Bucharest, Romania Personalized medicine in lung cancer
11:45-12:15	Saara Wittfooth Department of Biochemistry, University of Turku, Finland Antibody quality control in biomarker research
12:15-12:45	Peter Groenen Idorsia Pharmaceuticals Ltd, Basel, Switzerland Mass spectrometry-based biomarkers for rare diseases
12:45-14:00	Lunch and Poster Session
14:00-14:15	WG1 progress on deliverables Zanka Bojic-Trbojevic
14:15-14:30	WG2 progress on deliverables Peter Groenen
14:30-14:45	WG3 progress on deliverables Deborah Penque
14:45-15:00	WG4 progress on deliverables Andrea Wutte
15:00-15:15	Funding opportunities for research Jaroslav Katrlc
15:15-15:45	Coffee Break and Poster Session

15:45-16:15	STSM and ITC update Makis Zoidakis
16:15-17:15	MC meeting (budget topics, future meeting organization (Turkey/Spain), publications and dissemination activities, any other business)
17:15-17:30	Closing Remarks Luider, Vlahou, Zoidakis

17:30 Return to President Hotel

19:30 Pick up of participants President Hotel and transfer to Strofi Restaurant by Metro and walking together

20:00 Dinner at Strofi Restaurant <http://www.strofi.gr/en/>

Sunday, 22.09.2019

CliniMARK WG meeting focus on deliverables

Location: Biomedical Research Foundation, Academy of Athens (BRFAA)

<http://www.bioacademy.gr/?lang=en> , Soranou Efessiou 4, 11527 Athens

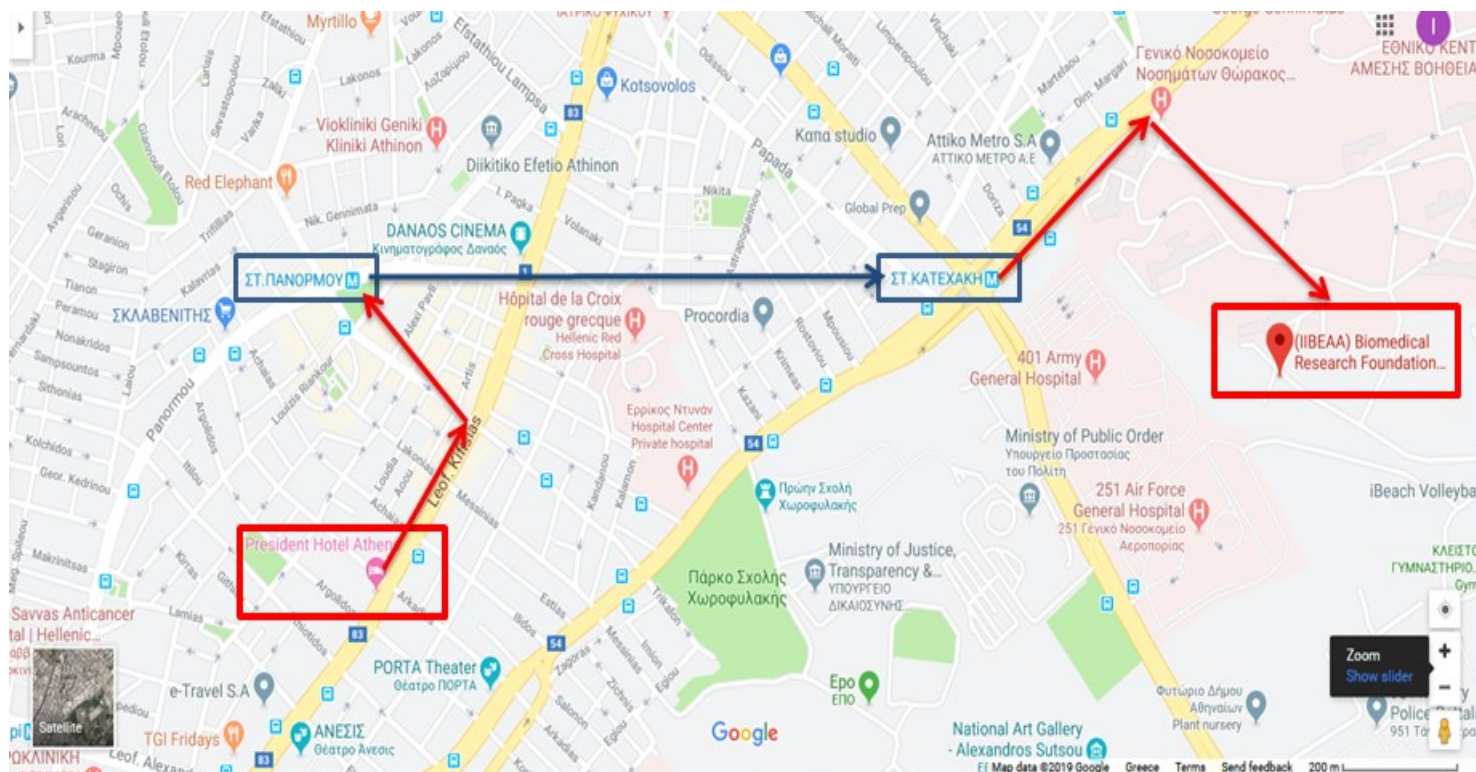
8:30 Pick up of participants by from President Hotel <https://president.gr/> by Makis and transfer to BRFAA by Metro and walking

9:00-9:15	Update from the action Chair Theo Luider
9:15-11:15	WG1, WG2, WG3 satellite meetings for deliverable planning and presentation preparation for plenary session Focus on white papers/ deliverables
11:15-11:45	Coffee Break
11:45-13:00	WG1, WG2, WG3 satellite meetings for deliverable planning and presentation preparation for plenary session Focus on white papers/deliverables
13:00-14:00	Lunch
14:00-16:30	Plenary Session for presentation of WG 1,2,3 meetings results and decisions on deliverables and particularly on manuscripts
16:30-17:00	Concluding remarks Theo Luider

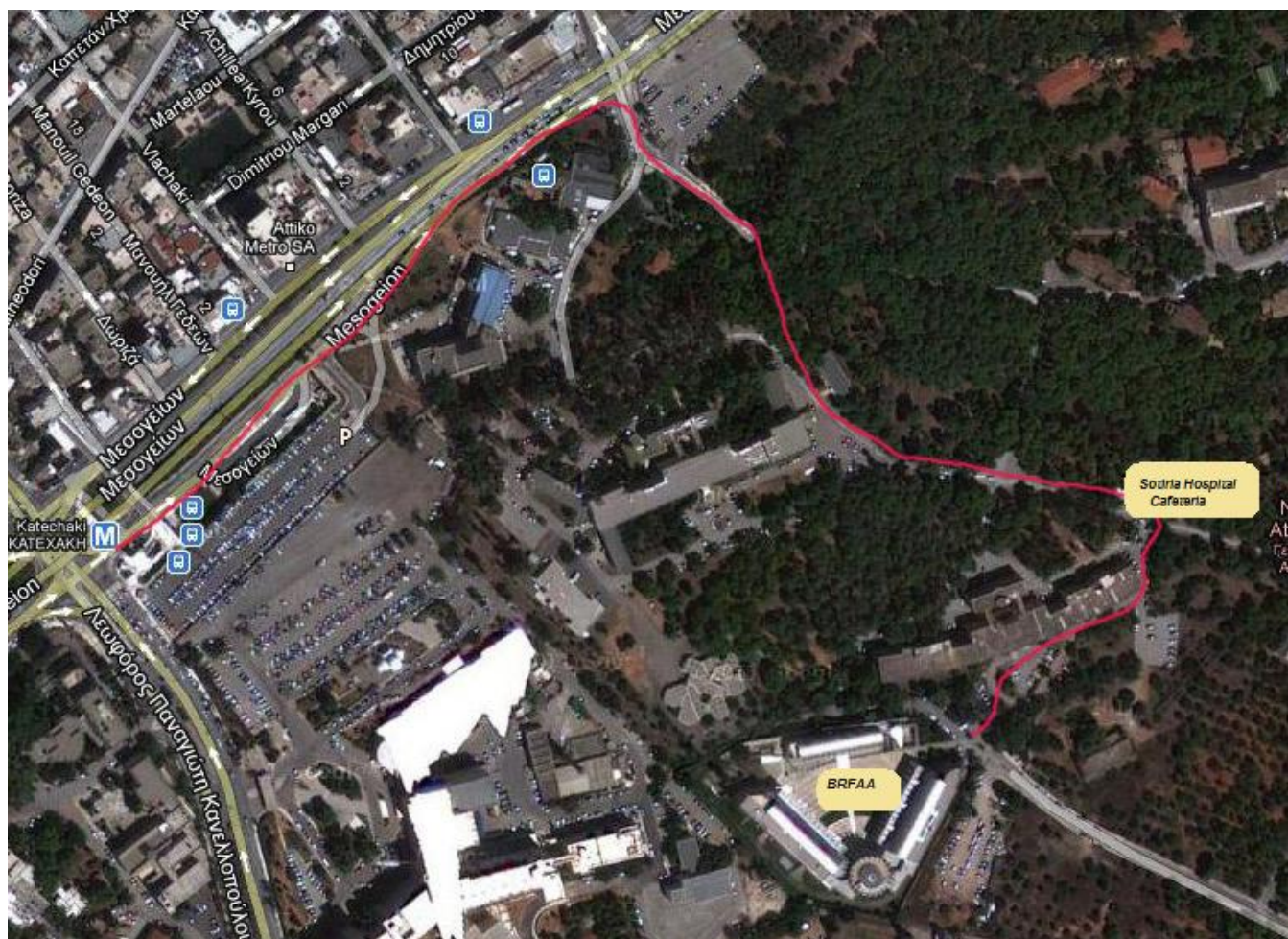
17:00 Return to President Hotel

Directions President Hotel to Biomedical Research Foundation:

- Walk from President Hotel to Metro Station Panormou
- Take the Metro from Panormou Station to Katehaki Station (Direction Airport/Doukissis Plakentias)
- Walk from Metro Station Katehaki to Biomedical Research Foundation Academy of Athens (IIBEAA/BRFAA)



Map for walking from Katehaki Metro Station to BRFAA:



POSTER ABSTRACTS

Poster 1

Proteomics alterations in colorectal cancer initiation and progression

Katarina Davalieva¹, Ivana Maleva Kostovska¹, Milcho Panovski², Marija Staninova Stojoska³, Aleksandar Dimovski^{1,3}

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³ Faculty of Pharmacy, University “St. Cyril and Methodius”, 1000 Skopje, R North Macedonia

Colorectal cancer (CRC) is the third most common and the second most deadly cancer worldwide with nearly 1.9 million new cases and 900 000 deaths each year. The clinical outcome of CRC treatment could likely be improved with a better understanding of the molecular alterations that impact CRC development. Our current research is focused on molecular characterization of proteins associated with progression of CRC aiming to identify the molecular mechanisms of initiation and progression and consequently finding biomarkers for improved clinical management of CRC. Colon tissue from patients with CRC (tumor and normal) was collected immediately after surgery, snap frozen in liquid nitrogen and stored at -80°C. The tumor samples were divided in 3 groups [(Localized (stage I/II), Advanced localized (stage III) and Metastatic (stage IV)] and were analyzed using LC-MS/MS on ACQUITY UPLC M-Class/ Synapt G2-Si (Waters Corp). The proteome profiling was done using UDMS^E label-free data independent acquisition with ion mobility. Raw data processing was done using Protein Lynx Global Server and Progenesis QIP (Waters Corp.) while statistical analysis included Shapiro-Wilk test, Mann Whitney and Spearman's rho correlation. We have identified 2621 proteins in all normal and tumor samples of which, 1934 were quantifiable (based on unique peptides) and 1896 were identified based on ≥ 2 peptides. Significant difference in abundance between control and cancer group (Mann Whithney $p \leq 0.05$) with fold change ≥ 1.5 showed 104 proteins. Among these, 74 have been found significantly correlated with cancer stage, (Spearman $p \leq 0.05$) of which 16 exhibited consistent regulation trend (up- or down-) across cancer stages. Majority of these proteins are closely related to progress, invasion and metastasis of malignant tumors and have been proposed as markers of biological aggressiveness in CRC by number of published studies. The identified biomarkers will be validated in a larger independent cohort as potential indicators of CRC progression towards aggressive forms.

Poster 2**Proteomics research on schizophrenia (choroid plexus proteome)**

Ivana Maleva Kostovska¹, Katarina Davalieva¹, Aleksandar Stankov³, Gorazd Rosoklija^{2,4,5}, and Andrew J. Dwork^{2,4,5,6}

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Over the last decade, a biomarker for early detection of schizophrenia (SCH) has been an elusive goal of many research attempts. There are no validated laboratory tests or biomarkers for SCH diagnosis, prognosis or preferred treatment. Many proteomic studies have analyzed different brain regions and body fluids from individuals with schizophrenia; however, only a few have investigated the choroid plexus (CP) proteome. In addition to its main role of producing cerebrospinal fluid (CSF), which physically protects the brain and removes metabolites, recent studies suggest that CP plays an active role in the development, homeostasis, and repair of the central nervous system. The aim of our study was to identify proteins and biological processes that are dysregulated in CP with potential to be used as biomarkers in the CSF. Choroid plexus tissues from post-mortem brains from 7 clinical trials (SCH; major depressive disorder, [MDD] and without serious mental illness, [NoSMI] matched for age and sex were analyzed on ACQUITY UPLC M-Class/Synapt G2-Si mass spectrometer (Waters Corp.) using UDMSE data-independent scanning mode. Data processing was performed using Protein Lynx Global Server (PLGS) and Progenesis QIP software (Waters Corp.). Statistically significant difference ANOVA ($p \leq 0.05$) showed 204 proteins, of which 39 were altered in SCH compared to both groups (Mann Whitney test, $p \leq 0.05$). These 39 proteins were part of 15 biological processes. The main processes affected by those proteins include cell growth and maintenance, cell communication, signal transduction and energy metabolism. The present findings suggest that there are a number of proteins with altered abundances in CP of individuals with SCH. The next step will be the validation of some of candidate biomarkers in CSF and testing their clinical performance in larger and independent cohort.

ACKNOWLEDGEMENTS:

Financial support from NIMH-Fogarty Foundation, Grant Number: 4R01MH098786-05.

Poster 3**New approaches in the diagnostics of gestational diabetes mellitus (GDM).**

Izabela Burzynska-Pedziwiatr¹, Malgorzata Bukowiecka-Matusiak¹, Carla Ferreri², Anna Sansone², Monika Zurawska-Klis³, Katarzyna Cypryk³, Lucyna Alicja Wozniak¹

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Gestational diabetes mellitus (GDM) is glucose intolerance that begins or is first recognized during pregnancy. For several years, the Department of Structural Biology has been conducting research on understanding the molecular mechanisms underlying GDM development. The pathogenesis of GDM is not fully understood; however, it is known that the development of diabetes mellitus is, like in the development of type 2 diabetes (T2DM), increasing insulin resistance and secretory defect β -pancreatic cells. Currently, the relationship between insulin resistance and oxidative stress in women with GDM is suggested.

The result of previous studies conducted in our laboratory is to show the relationship between several genes, including SIRT1, PPARG and genes encoding adenosine A1, A2A, A2B and A3, and GDM receptors.

Continuation of research on the role of oxidative stress in GDM is a project aimed at determining the lipid profile in erythrocyte membrane and metabolic profile in plasma from patients diagnosed with GDM vs. normal glucose tolerant (NGT) pregnant women. The aim of our research is to identify potential biomarkers that could help discrimination NGT women from those with GDM. The second aim of our research is the identification of early molecular biomarkers for GDM and postpartum T2DM.

Our preliminary results indicate that the SFA-MUFA families may be involved in the pathophysiology of metabolic diseases such as GDM, but further studies are needed to confirm our hypothesis. In conclusion, the erythrocyte membranes of GDM women undergo remodeling resulting in abnormal fatty acid profiles, which are a reflection of the long-term status of an organism and can have significant impact on both the mother and her offspring.

ACKNOWLEDGMENTS:

Financial support from the Grant aimed at the development of young researchers and doctoral studies participants at the Medical University of Lodz 502-03/0-160-01/502-04-0036 is acknowledged.

Poster 4**Involvement of bacteria and silver nanoformulations for searching the COPD biomarkers and others.****Anna Kędziora¹, Mateusz Speruda¹, Maciej Wernecki¹, Gabriela Bugla-Płoskońska¹**¹ Department of Microbiology, Institute of Genetics and Microbiology, University of Wrocław, Wrocław, Poland

Silver, as antibacterial agent, has been known since ancient time, but the development of nanotechnology gave him second life in antibacterial therapy. Nanotechnological modification of silver is related with conversion of silver ions – Ag^+ (used to known) to silver nanoparticles (nanomaterials, nanoformulations) – Ag^0 , that have been described as 'material with any external dimensions in the nanoscale or having internal structure or surface structure in the nanoscale. The term 'nanoscale' is defined as size range from approximately 1 nm to 100 nm' with detailed physico-chemical properties such as surface area, charge, shape etc. (2011/696/EU). Silver nanoparticles are usually recommended as alternative way for killing difficult to eradication pathogens. Current medical uses of silver include the prevention and treatment of bacterial infection in wounds, with silver containing dressings for these purposes. In recent years the popularity of antimicrobial silver has grown outside of the clinic, silver nanoparticle formulations, and is routinely incorporated into a variety of domestic and personal products (e.g. food containers, sportswear, underwear, towels, carpets, assorted electronics, mobile phones, household goods, toilet seats). The antibacterial mode of action of silver ions and its mechanisms of bacterial resistance to silver ions are well known (especially in Gram-negative bacteria), but a mode of action and resistance mechanism of silver in nanoscale form remains unclear. Difficulties in explaining the phenomenon are added to the variety of available forms: powder, colloids etc. and their physico-chemical properties (such as size, shape, surface area, charge), therefore each nanoformulations should be consider as separate factor with different mode of action and mechanism of resistance. Applications of silver nanoparticles have a lot of positive aspects but overuse and ignorance about their behaviour in production, consumption and utilization may consequently cause damage to the environment, especially to human and animal health. Both, bacteria and silver nanoparticles, may be responsible for exacerbation of different disorders. Therefore, it would be a good idea to monitor the amount of silver nanoparticles in body fluid and cells together with microbial profile. Both may be the key to searching the biomarkers.

ACKNOWLEDGEMENTS:

Financial support: STSM COST ACTION CA16113 for Anna Kedziora, Gabriela Bugla-Płoskońska and Maciej Wernecki.

Poster 5**Paper-Based Colorimetric Spot Test Utilizing Smartphone Sensing for Detection of Biomarkers****Eda Aydindogan†, Ayse Elcin Ceylan†, Suna Timurt, ‡, ****† Ege University, Institute of Natural Sciences, Department of Biochemistry, 35100, Bornova, Izmir, Turkey**‡ Central Research Testing and Analysis Laboratory Research and Application Center, Ege University, 35100, Bornova, Izmir, Turkey*

The need for a continuous, real-time monitoring of specific diseases represents an unmet scientific need. Evidently, cancer is one of the most important diseases where it is crucial to increase the rates of patient survival and monitor disease prognosis. Herein, a novel type of immunoassay was developed for detection of cancer biomarkers, using alpha-fetoprotein (AFP) and mucin-16 (MUC16) as model analytes. Using gold nanoparticle (AuNP) bioconjugates as a signal production tool, relevant antibody (Ab)-conjugated AuNPs were prepared on the nitrocellulose (NC) membrane and an affinity-based test platform was developed. To construct a spot-like point-of-care (POC) immunoassay, cysteamine conjugated AuNPs (AuNP-Cys) were immobilized on the NC membrane and relevant antibodies were conjugated to the nanoparticle on the detection pad, following a treatment with the samples that contains AFP or MUC16 which are well-known protein biomarkers for liver and ovarian cancer. By using the change in the colorimetric properties of AuNPs, detection of relevant tumor markers was achieved by using a smartphone image and color analysis software at the final stage. Image J application was used for the evaluation of color changes depending on the biomarker concentration in buffer or spiked synthetic serum samples. The linear range was found as 0.1 ng/mL-100 ng/mL with a correlation coefficient of and $R^2 = 0.988$ for AFP and 0.05-10 ng/mL with a correlation coefficient of and $R^2 = 0.981$ for MUC16. Limit-of-detection (LOD) was calculated as 2.123 ng/mL and 0.413 ng/mL for AFP and MUC16, respectively. Interferant molecules, Her2, Immunoglobulin G (IgG) and bovine serum albumin (BSA) were tested on the system. Furthermore, synthetic serum samples spiked with selected analyte molecule were applied on the system and measured successfully.

ACKNOWLEDGEMENTS:

This work is supported by TUBITAK COST Project ‘117Z609 Detection of Cancer Biomarkers by Low-cost Biochemical Assays for Early Diagnosis’.

Poster 6**Fluorescent Immunoassay Platform for Ethyl Glucuronide (EtG) as a Potential Biomarker of Acute Alcohol Consumption**

Ceren Durmus,^[a] Emine Guler Celik,^[a] Tulay Yilmaz Sengel,^[a] Z. Pinar Gumus,^[c] Hakan Coskunol,^[c] Shuhei Yamada,^[d] Takeshi Endo,^[d] Suna Timur*^[a,b] and Yusuf Yagci^[e,f]

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^[f] King Abdulaziz University, Faculty of Science, Chemistry Department, Jeddah/Saudi Arabia

Ethyl glucuronide (EtG), is a breakdown product of ethanol which can be detected in urine samples several days after consumption of alcohol. In this study, we constructed a practical fluorescence-based bioassay using quantum dots (QDs) as signal transducer for EtG analysis. In this platform, a polypeptide bearing polymer (EDOT-BTDA-Pala) was initially coated on the μ -well surfaces and EtG antibody was attached to the surface with glutaraldehyde. The analyte (EtG) was applied to the biofunctional surface for the selective capturing. At the final step, QD/Anti-EtG conjugate was added and the fluorescence intensity as a result of selective interaction with the EtG and QD-based probe was monitored. The linear range for the detection of EtG was found as 0.05–25 $\mu\text{g/mL}$ and defined by the equation of $y = 0.071x + 0.1642$ ($R^2 = 0.995$). The proposed platform was tested for the analysis in synthetic urine samples. Our findings showed that this immunoassay platform provides rapid, selective and sensitive results for the selected analyte.

Acknowledgements:

This work was supported by Scientific and Technological Research Council of Turkey (TUBITAK) under the "Industrial Focussed Undergraduate Thesis Support Program" numbered 2209-B.

Poster 7**Multiplexed MRM-based protein quantification of putative prognostic biomarkers for chronic kidney disease progression**

Georgia Kontostathi¹, Manousos Makridakis¹, Eleni Petra¹, Rafael Stroggilos¹, Szymon Filip¹, Flore Duranton², Harald Mischak³, Angel Argiles², Jerome Zoidakis¹, Antonia Vlahou¹

¹Biotechnology Division, Biomedical Research Foundation, Academy of Athens (BRFAA), Athens, Greece

²RD-Néphrologie, Montpellier, France

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Current diagnostic measures for Chronic Kidney Disease (CKD) include detection of reduced estimated glomerular filtration rate (eGFR) and albuminuria, which have suboptimal accuracies in predicting disease progression. The disease complexity and heterogeneity underscore the need for multiplex quantification of different markers. The goal of this study was to determine the association of six previously reported CKD-associated plasma proteins, [B2M (Beta-2-microglobulin), SERPINF1 (Pigment epithelium-derived factor), AMBP (Protein AMBP), LYZ (Lysozyme C), HBB (Hemoglobin subunit beta) and IGHA1 (Immunoglobulin heavy constant alpha 1)], as measured in a multiplex format, with CKD progression. Antibody-free, multiple reaction monitoring mass spectrometry (MRM) assays were developed, characterized for their analytical performance, and used for the analysis of 72 plasma samples from a longitudinal patient cohort. Five proteins [AMBP, B2M, LYZ, HBB and SERPINF1], were significantly associated with eGFR with the three formers also associating with unfavorable outcome. The combination of these markers provided stronger associations to outcome compared to the individual proteins. Collectively, our study describes a multiplex assay for the absolute quantification and verification analysis of previously described putative CKD markers, laying the groundwork for further assay use in prospective validation studies.

ACKNOWLEDGEMENTS:

This work is supported by the Greek GSRT (grant mELISA-CKD, T1EΔK-03551; EPAnEK Operational Programme)

Poster 8**Preliminary proteomic analysis of CD138+ cells for predicting the response of multiple myeloma patients to commonly used therapeutic regimens**

Vasiliki Lygiriou¹, Manousos Makridakis¹, Rafael Stroggilos¹, Ioannis V. Kostopoulos², Christine-Ivy Liacos³, Aikaterini Termentzi⁴, Jerome Zoidakis¹, Meletios A. Dimopoulos³, Ourania Tsitsilonis², Antonia Vlahou¹, Efstathios Kastritis³

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Multiple myeloma (MM) is a common hematologic malignancy accounting for 106,000 deaths in 2018, worldwide. Despite the considerable research efforts and established prognostic criteria, patient stratification and selection of therapeutic strategy require improvement. The aim of this study was to identify proteins and molecular mechanisms predictive of responsiveness to commonly used therapeutic regimens for MM. Nine MM patients of all stages were included in this study. CD138+ cells isolated from these patients prior to any MM-related treatment were analyzed with Liquid Chromatography coupled to tandem mass spectrometry. Patients were grouped in Deep-Responders (DR) and Non-Responders (NR) based on the IMWG criteria after treatment with either bortezomib- or lenalidomide/thalidomide- based regimens. Taking into consideration proteins that were present in at least 60% of samples in at least one group, a total of 944 proteins were identified. Differential expression analysis between DR and NR revealed 59 statistically significant proteomic changes (Mann Whitney p-value <0.05). Interestingly, functional annotation of the differentially expressed proteins showed that most of these proteins are associated with metabolism (25%), translation (17%), endoplasmic reticulum - protein folding (15%), cytoskeleton - motility (14%), immune response (7%) and ubiquitination (5%). Specifically, all proteins associated with translation and most of the proteins related to endoplasmic reticulum and protein folding were found upregulated in DR compared to NR, suggesting that response to treatment may rely on a phenotype characterized by increased protein production. This pilot proteomic analysis of CD138+ cells is the first comparison between DR and NR after treatment with different therapeutic regimens for MM. Our results suggest that increased protein production is a favorable phenotype for deep response to commonly used treatment. Proteomic analysis of a larger cohort and transcriptomics analysis of the same patients are ongoing and expected to validate our initial observations.

ACKNOWLEDGEMENTS:

The work presented here was funded by the Operational Programme Competitiveness, Entrepreneurship and Innovation 2014-2020 (EPAnEK) with code MIS 5032789, project title “Novel Biomarkers and Potential Therapeutic Targets for the Management of Patients with Multiple Myeloma” and acronym “My-BIOTag”.

Poster 9

LIST sensing technologies, opportunities for Point of Care and multiplexing Biomarkers

César Pascual García, Sivashankar Krishnamoorthy

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Nano-technologies bring new opportunities for bio-sensing that promise advances for Point of care devices and Personalised medicines. LIST strategy to contribute in the field is to improve sensitivity and reliability from design using smart nano-structures to improve the mass transport of analytes towards the sensors and develop fabrication of sensors with scalable industry compatible processes. These technologies improve the sensitivity and speed of current state of the art, providing sensors compatible with several multiplexing schemes for the detection of multiple biomarkers. In this poster we exhibit our main technologies using electrochemical and optical sensors including wafer scale plasmonic surfaces to implement protein assays, reliable field effect transistors and electrochemical manipulation of acid in microscale reactors.

Poster 10**The association of *GSTM1* genotype with the risk of renal cell carcinoma development and prognosis**

Vesna M. Coric^{a,c}, Tatjana P. Simic^{a,c}, Ana R. Savic Radojevic^{a,c}, Dejan P. Dragicevic^{b,c}, Tanja M. Radic^c, Zoran M. Dzamic^{b,c} and Marija S. Pljesa-Ercegovac^{a,c}

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Introduction: Glutathione S-transferases (GSTs) comprise a set of cellular proteins with various catalytic and non-catalytic functions, binding glutathione as cofactor. Some observations suggest that cytosolic GSTs may be implicated not only in the development, but also in the progression of renal cell carcinoma (RCC).

The aim: The aim of this study was to determine the significance of *GSTM1* polymorphism in RCC biology by investigating the influence of its deletion polymorphism on the risk of RCC development and postoperative prognosis as well.

Materials & Methods: *GSTM1* genotype was determined in 163 RCC patients and 240 matched controls by PCR method. The effect of *GSTM1* genotype on cancer specific survival was analyzed using Cox regression model and differences in survival were determined by Kaplan-Meier.

Results: Significant association between *GSTM1* genotype and RCC risk was found for the *GSTM1-null* genotype (OR=2.21, 95% IP=1.21–4.03, p=0.01). On the other hand, survival analysis indicated shorter overall survival for the patients with *GSTM1-active* genotype, compared to carriers of *GSTM1-null* genotype (p=0.022). Finally, *GSTM1-active* genotype was proved to be an independent predictor of higher risk for overall mortality in RCC patients. Namely, carriers of *GSTM1-active* genotype exhibited significantly higher HR (p<0.05), adjusted to recognized RCC risk factors and tumor clinical characteristics, analyzed in three Cox regression models compared to the carriers of *GSTM1-null* genotype.

Conclusion: Individuals with *GSTM1-null* genotype exhibited increased risk of RCC development while, on the other hand, favorable postoperative prognosis.

Poster 11**Quartz crystal microbalance coupled to assist the multilayer protein assembly by Langmuir Blodgett technique.****Claudio Larosa¹, Attilio Converti¹**¹Department of Civil, Chemical and Environmental Engineering, University of Genoa, Pole of Chemical Engineering, via Opera Pia 15, 16145 Genoa, Italy

Quartz crystal microbalance (QCM) is a valid scalable biolab to realize bio complex arrays in liquids or air streams. Enzymes, ligand folds¹, DNA fragments² often were anchored on quartz crystal and then anchored to bio-fragments in a dynamic liquid flow^{3,4}. Thus, as well known the physical principle of Nano gravimetry measurement is based on frequency shift of quartz crystal. The frequency is transmuted in mass respect to the empty quartz with a sensitivity limit in the nanogram scale. Frequencies shift values are utilized to monitoring the mass deposited on plate disks, coupled with the Langmuir Blodgett activities. Due to these reasons Nano gravimetry technique is an affordable utilized as surveillance to monitoring the uniformity of deposition grade in multi layers. Enzymes are validated examples for monitoring deposition on a quartz crystal with a significant reproducibility in Multi layers.

The monitoring of frequency respect time is a valid method to follow the correct depositions of proteins. It is also easy discovery alteration of bias deposition of protein in multi layers, whose are utilized as platform for laser beam sin croton to discovery new structure and discriminate new interaction between ligands. Discrete mass as frames step are utilized for multilayer deposition, whose give information on the reproducibility of method or anomalies during preparation. In summary Nano gravimetry is an easy approached to define multi-layer proteins coupled with Langmuir blodgget technique. The frequency change revel a periodic decrease of frequency in the order of 10. Hz fraction. Multi protein layers is a valid approach to define the structure of protein in alternative to crystalline protein⁵. Human biomarkers can be utilized and screen on quartz balance realized from human sieri using the complementary of chemical group or tasks.

References:

1. He, M.; Stoevesandt, O.; Taussig, M. J. In situ synthesis of protein arrays. *Curr. Opin. Biotechnol.* 2008, 19 (1), 4–9.
2. Ramachandran, N.; Larson, D. N.; Stark, P. R.; Hainsworth, E.; LaBaer, J. Emerging tools for real-time label-free detection of interactions on functional protein microarrays. *FEBS J.* 2005, 272 (21), 5412–25.
3. Nicolini, C.; Adami, M.; Sartore, M.; Bragazzi, N. L.; Bavastrello, V.; Spera, R.; Pechkova, E. Prototypes of newly conceived inorganic and biological sensors for health and environmental applications. *Sensors* 2012, 12 (12), 17112–27.
4. Spera, R.; Bezerra Correia, T. T.; Nicolini, C. NAPPa based nanogravimetric biosensor: preliminary characterization. *Sensor and Actuators, B*: 2013, 182, 682–8.
5. Nicolini, C.; Bragazzi, N.; Pechkova, E. Nanoproteomics enabling personalized nanomedicine. *Adv. Drug Delivery Rev.* 2012, 64 (13), 1522–31.

Poster 12**Evaluation of urinary volatile metabolites as potential biomarkers for prostate cancer diagnosis**

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Prostate cancer (PCa) is the second most common malignancy in men. Prostate specific antigen (PSA) is the most frequently used biomarker for PCa screening but, due to its recognized limitations, U.S. Preventive Services Task Force now recommends against PSA screening. The analysis of volatile compounds emanating from biological samples is a major promising approach for finding new effective diagnostic markers for PCa. The purpose of this work was to study the urinary volatile metabolic profile of patients with PCa ($n=40$) and non-cancer controls ($n=42$) with the aim of identifying a potential urinary volatile pattern as a non-invasive strategy to detect PCa. A metabolomics approach based on headspace solid-phase microextraction gas chromatography–mass spectrometry was performed to investigate volatile organic compounds (VOCs) in general and, more specifically, volatile carbonyl compounds (VCCs) present in urine samples. Considering both approaches, a panel of 6 volatile compounds descriptive of PCa was defined, capable of discriminating PCa patients from controls. Furthermore, an external validation set ($n=18$ PCa and $n=18$ non-cancer controls) was used to calculate the sensitivity, specificity and accuracy of the defined panel. Our results revealed that this 6-volatile panel was able to predict PCa, with a sensitivity of 89%, a specificity of 83% and an accuracy of 86%. Overall, our results disclose a biomarker panel that has the potential to be used in clinic for PCa diagnosis.

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Poster 13**The secretome cargo of MSCs: from players in intercellular communication to relevant molecular biomarkers in the wound healing process**

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The success of wound healing is impaired in several medical conditions resulting in increased morbidity. The current available therapeutic options fail to promote full tissue regeneration, though MSCs represent promising alternatives to the repair and regeneration of damaged tissues. Moreover, diagnostic molecular biomarkers may help to identify and stratify subsets of non-healing patients for whom biomarker-guided approaches may aid in wound healing. Recently, the secretion of exosomes by mesenchymal stem cells (MSCs) has been suggested as a dominant mechanism by which these cells exert their healing function. As such, our study aimed at evaluating the role of exosomes, derived from umbilical cord matrix MSCs primed by 3D culturing, on wound healing using *in vivo* methodologies paired with integrative proteomics. Specifically, the objective was to characterize the content of the exosomes and unveil the mechanistic pathways behind their therapeutic effects. Namely, to identify and validate specific markers to understand the response to therapy. Exosomes from 3D (Exo3D) and 2D (Exo2D) MSC cultures were isolated by size exclusion chromatography. Size distribution of the isolated exosomes (135.9 ± 54.0 nm and 265.0 ± 37.2 nm for Exo2D and Exo3D, respectively) pointed out the influence of the culture system in its morphology, however without compromising the presence of CD9 and CD81 exosomal surface markers. Moreover, proteomic analysis of the isolated exosomes revealed that 3D conditions led to higher protein diversity than the 2D environment. Indeed, Exo3D show 18 specific proteins, some of which involved in cell chemotaxis, division and proliferation. The therapeutic potential of exosomes on skin regeneration was further evaluated *in vivo* in a rat wound-splinting model. Macroscopic observations show that Exo-treated wounds exhibited accelerated wound closure when compared to control wounds. As a result, histological examination revealed that Exo3D-treated wounds show an improvement in the healing profile, by promoting wound margin closure and complete tissue regeneration with hair re-growth. Overall, the results indicate that 3D MSC-derived exosomes promoted wound healing, thus granting them a potential new role as active players in cell-free-based therapies. Accordingly, -omics approaches may help on the identification of new diagnostic biomarkers for improved therapeutic outcomes.

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Poster 14

Personalized medicine in lung cancer

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Poster 15

Enzyme activity modulation and oxidative stress responses as toxicity biomarkers

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Poster 16

Determination of protein glycosylation in cancer and other diseases by lectin-based protein microarray

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Changes in glycan structures of proteins and cell surfaces are in relationship with many biological functions (pathogen-host interactions, immune system, stem cells, fertilization, etc.), with various diseases (e.g. cancer, inflammatory diseases, neurological diseases, psychiatric diseases, congenital diseases of glycosylation) and with structure and function of pathogens. We are focused on development and application of affinity bioanalytical techniques based on biochips analysing glycans for applications in biomedicine, biology and biotechnology. As biorecognition elements are used lectins, special proteins recognizing particular glycan structures enabling glycoprofiling of proteins, cells and tissues. Lectin-based glycoprotein microarrays enable effective high-throughput glyco-profiling of samples and screening/analysis of glyco-biomarkers. Here we report developed microarray platforms used for the study of glyco-biomarkers of colorectal cancer (CRC) and congenital disorder of glycosylation (CDG). Exact identification of glycan structures by MS techniques complements and confirms data obtained by lectin-based protein microarray. We use lectin-based microarray system also for glycoprofiling of samples related to other diseases as e.g. autoimmune diseases, psychiatric diseases or peritoneal dialysis.