COST CLINIMARK TRAINING SCHOOL
Approaches for Biomarker Discovery and Validation

Spetses 23-27 September 2019

CA16113 - CliniMARK

Introduction

The quest for novel biomarkers is a popular research activity with high productivity. Thousands of studies are published claiming the discovery of biomarkers suitable for improving disease management. The stark reality though indicates that very few potential biomarkers are approved for clinical use. The application of omics approaches (Genomics, Transcriptomics, Proteomics, Metabolomics, etc.) in biomarker discovery has contributed significantly in increasing the number of publications reporting initial findings that are not validated. Major issues associated with this dire situation are the difficulty in analytical validation of robust biomarker assays, flawed study design, and the inability to exploit the full potential of high-throughput omics approaches. Thus, there is a waste of research resources without tangible benefits to society. Moreover, there are many unmet clinical needs that are not currently addressed by the available biomarkers in diseases of high prevalence and of high financial and social cost such as cancer, cardiovascular disease, chronic kidney disease, and chronic obstructive pulmonary disease. This situation is partly due to a lack of education resources dedicated to omics studies in biomarker research.

Teaching objectives and topics

The unique feature of the proposed workshop is that it will expose the problems associated with omics biomarker studies and train a new generation of scientists able to fix the flawed biomarker discovery and implementation paradigm. In order to achieve this ambitious goal the following specific teaching objectives are set so that students can:

1. obtain a global view of omics approaches and the biomarker life cycle from discovery to clinical implementation

2. acquire skills relevant to biomarker data analysis (analytical assay validation, clinical performance)

3. develop critical thinking by thorough evaluation of published biomarker studies, and improve writing and presentation skills

The following topics be covered during the workshop:

A) Introduction to the different biomarker types (diagnostic, prognostic, etc.)

B) Introduction to the different omics approaches and their application in the context of biomarker research

C) Emphasis on the importance of defining the biomarker context of use in the clinical setting before initiating a research protocol on biomarker discovery and validation

D) Presentation of good biomarker practice guidelines on:

1. study design (number and type of samples, proper statistical analysis, reporting of all findings, etc.)

2. analytical validation of assays (reproducibility, LOD, linearity, etc.)

3. clinical performance (sensitivity, specificity, etc.), validation in an independent large set of samples (ideally multi-center study) by different researchers (external independent validation)

4. comparison of the performance of the new biomarker with biomarkers already used in clinical practice (umbrella reviews)

5. tools for assessing if biomarkers are effective in improving concrete patient clinical outcomes (randomized trials, etc.)

6. implementation in sub-optimal conditions and different populations.

	Mon, Sep 23	Tue, Sep 24	Wed, Sep 25	Thu, Sep 26	Fri, Sep 27
09:00	Arrival and Registration	Introduction to omics and Biomarkers **Antonia Vlahou**	Antibody quality control in biomarker research **Saara Wittfooth**	Biomarkers in Screening for Obstructive Sleep Apnea **Deborah Penque**	Biomarkers for psychiatric disorders **Chris Turck**
09:30					
10:00		Biomarker panels by CE-MS **Harald Mischak**	Biomarker clinical implementation **Eva Martínez-Cáceres**	Proteomics for anxiety disorders: mind the mitochondria **Michaela Filiou**	Predictive biomarkers for CVD **Andreas Simm**
10:30					
11:00		Coffee break	Coffee break	Coffee break	Coffee break
11:30		Good Standardisation Practice in biomedical research **Andrea Wutte**	Targeted proteomics assays for biomarker evaluation **Virginie Brun**	Proteomics for biomarker discovery **Michalis Aivaliotis**	Biomarkers of healthy ageing **Niki Chondrogianni**
12:00					
12.30		**Student talks** 1-13	**Student talks** 18-30	Eureka: there is something rotten in the biomarker kingdom **Makis Zoidakis**	Genomics biomarkers **Lila Koumandou**
13:00					
13.30				Lunch Break Poster viewing	Lunch Break Poster viewing Discussions Free time
14:00		Lunch Break Poster viewing Discussions Free time	Lunch Break Poster viewing Discussions Free time		
14:30					
15:00					**Meet the expert** Biomarker assay validation Study design, MRM data analysis
15.30		High sensitivity immunoassays **Stanislav Kukla**	Validation of LC-MS/MS methods for the quantification of protein biomarkers: the example of soluble receptor for advanced glycation end products (sRAGE) **Rainer Bischoff**	Cultural excursion Free time	
16.00					
16:30		**Student talks** 14-17	**Student talks** 31-34		
17:00		Coffee break	Coffee break		Coffee break
17:30		Biomarkers in the pharmaceutical industry. Translating research into clinical benefits **Peter Groenen**	Biomarkers used in clinical practice for monitoring biological drugs **Begoña Oliver**		Biomarkers of Human Ageing derived from the MARK-AGE Study **Alexander Bürkle**
18:00					
18:30					Oxidative stress and biomarkers **Grune Tilman**
19:00	Welcome **Niki Chondrogianni Makis Zoidakis**	Molecular diagnostics: from bench to clinic **Daria Ler**	Liquid biopsy preparation **Chris Sutton**		
19:30	Biomarkers at the interphase of academia and industry **Alain van Gool**	**Poster session** Discussions	**Poster session** Discussions		**Summing-Up Round Table**
20:00					
20:30	Welcome reception	Dinner	Dinner	Dinner	**Farewell reception /awards**

Daily Program

Monday 23/09

9:00-19:00 Arrival and registration
19:00-19:30 Welcome **Niki Chondrogianni and Makis Zoidakis**
19:30-20:30 Biomarkers at the interphase of academia and industry **Alain van Gool**
20:30 Welcome reception

Tuesday 24/9

9:00-10:00 Introduction to omics and Biomarkers **Tonia Vlahou**
10:00-11:00 Biomarker panels by CE-MS **Harald Mischak**
11:00-11:30 Coffee break
11:30-12:30 Good Standardisation Practice in biomedical research **Andrea Wutte**
12:30-14:00 Student talks (1-13)
14:00-15:30 Lunch, Poster viewing, Free time, Discussions
15:30-16:30 High sensitivity immunoassays **Stanislav Kukla**
16:30-17:00 Student talks (14-17)
17:00-17:30 Coffee break
17:30-18:30 Biomarkers in the pharmaceutical industry. Translating research into clinical benefits **Peter Groenen**
18:30-19:30 Molecular diagnostics: from bench to clinic **Daria Ler**
19:30-20:30 Poster session, Discussions
20:30 Dinner

Wednesday 25/9

9:00-10:00 Antibody quality control in biomarker research **Saara Wittfooth**
10:00-11:00 Biomarker clinical implementation **Eva Martínez-Cáceres**
11:00-11:30 Coffee break
11:30-12:30 Targeted proteomics assays for biomarker evaluation **Virginie Brun**
12:30-14:00 Student talks (18-30)
14:00-15:30 Lunch, Poster viewing, Free time, Discussions
15:30-16:30 Validation of LC-MS/MS methods for the quantification of protein biomarkers: the example of soluble receptor for advanced glycation end products (sRAGE) **Rainer Bischoff**
16:30-17:00 Student talks (31-34)
17:00-17:30 Coffee break
17:30-18:30 Biomarkers used in clinical practice for monitoring biological drugs **Begoña Oliver**
18:30-19:30 Liquid biopsy preparation **Chris Sutton**
19:30-20:30 Poster session, Discussions
20:30 Dinner

Thursday 26/9

9:00-10:00 Biomarkers in Screening for Obstructive Sleep Apnea **Deborah Penque**
10:00-11:00 Proteomics for anxiety disorders: mind the mitochondria **Michaela Filiou**
11:00-11:30 Coffee break
11:30-12:30 Proteomics for biomarker discovery **Michalis Aivaliotis**
12:30-13:30 Eureka: there is something rotten in the biomarker kingdom **Makis Zoidakis**
13:30-14:30 Lunch, Poster viewing, Free time, Discussions
14:30-20:30 Cultural excursion, free time
20:30 Dinner

Friday 27/9

9:00-10:00 Biomarkers for psychiatric disorders **Chris Turck**
10:00-11:00 Predictive biomarkers for CVD **Andreas Simm**
11:00-11:30 Coffee break
11:30-12:30 Biomarkers of healthy ageing **Niki Chondrogianni**
12:30-13:30 Genomics biomarkers **Lila Koumandou**
13:30-15:00 Lunch, Poster viewing, Free time, Discussions
15:00-17:00 Meet the expert: Biomarker assay validation, Study design, MRM data analysis
17:00-17:30 Coffee break
17:00-17:30
17:30-18:30 Biomarkers of Human Ageing derived from the MARK-AGE Study **Alexander Bürkle**
18:30-19:30 Oxidative stress and biomarkers **Grune Tilman**
19:30-20:30 Summing-Up Round Table
20:30 Farewell reception / awards

Venue Information

The course will be held at Spetses Hotel in Greece (https://spetses-hotel.gr/en/)
The island of Spetses is easily accessible from and very well connected to Athens. Spetses is a well-established location for scientific training events and this hotel has successfully hosted many FEBS and IUBMB Advanced Courses in the past, with consistently positive experience and feedback. The hotel is easily accessible, but is in a quiet and secluded area of Spetses, allowing the participants to focus fully on the training course, and ensuring a perfect atmosphere for a relaxed but intensive interaction between the senior scientists and the trainees.
The hotel has full lecture facilities (the B. Clark lecture theatre with audio-visual aids, photocopiers, computers and free internet access) as well as ample space for poster sessions and informal meetings allowing direct interaction between participants, including the “meet the experts” session.
The meeting is on a full-board residential basis, so participants and lecturers will have all meals together, thus allowing additional informal discussions during these periods. The hotel has already confirmed reservation of the venue for the duration of the course and offers special group rates for the participants and lecturers of the course.

Course Organizers

Aivaliotis Michalis, Aristotle University of Thessaloniki, Greece
Chondrogianni Niki, National Hellenic Research Foundation, Greece
Filiou Michaela, University of Ioannina, Greece
Koumandou Lila, Agricultural University of Athens, Greece
Tilman Grune, German Institute of Human Nutrition Germany
Zoidakis Makis, Biomedical Research Foundation Academy of Athens, Greece

Speakers

Bischoff Rainer, University of Groningen, The Netherlands
Brun Virginie, Protein Dynamics Laboratory CEA, France
Bürkle Alexander, Department of Biology University of Konstanz, Germany
Martínez-Cáceres Eva, Immunology Division, Universitat Autònoma Barcelona, Spain
van Gool Alain, Radboud University Medical Center, The Netehrlands
Groenen Peter, Idorsia Pharmaceuticals, Switzerland
Kukla Stanislav, Merck Chemicals GmbH, Germany
Ler Daria, EUROFARM Centar Laboratory, Bosnia and Herzegovina
Mischak Harald, Mosaiques Diagnostics, Germany
Oliver Begona, Instituto de Investigacion Biomedica de Malaga, Spain
Penque Deborah, National Institute of Health Dr Ricardo Jorge, Portugal
Simm Andreas, Martin Luther University Halle-Wittenberg, Germany
Sutton Chris, University of Bradford, UK
Turck Chris, Max Planck Institute of Psychiatry Munich, Germany
Vlahou Tonia, Biomedical Research Foundation Academy of Athens, Greece
Wittfooth Saara, University of Turku, Finland
Wutte Andrea, Biobanking and BioMolecular Resources Research Infrastructure, Austria

POSTER ABSTRACTS

Poster 1

Expression of c-FOS and FosB proteins in association with MACC1 and its significance in human breast cancer progression

Daria Ler[1,2], Jasminka Mujic[1,2], Tea Bećirević[3], Karin Milde-Langosch[4]
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In previous report we have shown that higher MACC1 expression in breast cancer patients correlates with poor prognosis and shorter disease free survival but probably depends on different clinical futures such as metastases, tumor grade and stage, ER- and PR receptor. Up to date, there are a small number of articles about the promoter of MACC1 gene and its transcriptional regulation. Manisha J. *et al* has reported the possible promoter regulation of MACC1 by using promoter luciferase that directs the transcription of MACC1. They have identified binding sites for well know transcription factors involved in tumorigenesis and cell growth such as AP-1, Sp-1 and C/EBP expression and activity that are seen in many tumor types [1]. The AP-1 family consists of dimeric complexes of either homodimers of Jun family members (c-Jun, JunB and JunD) or heterodimers of Jun or Fos family members (c-Fos, FosB, Fra-1 and Fra-2). AP-1 proteins are involved in the regulation of a variety of cellular processes including proliferation, differentiation, growth, apoptosis, cell migration, and transformation [2]. The AP-1 is an oncogenic transcription factor found to be overexpressed in many cancer types demonstrating promising therapeutic targets. Additionally, AP-1 has been shown to promote proliferation of ER-positive breast cancer cells (i.e. MCF-7), where up-regulated AP-1 activity has been associated with tamoxifen resistance and increased invasiveness [3]. However, to our knowledge there is no information about the correlation of MACC1 expression and AP-1 proteins in breast cancer. In this study we report a strong correlation between c-FOS and MACC1 expression in ER+ receptor patients, which points to potential involvement of these markers in breast cancer genesis. Furthermore, a significant correlation was observed between FosB expression in group of breast cancer patients with lymph node negative (p=0.03) and progesterone- and estrogen positive status. These findings potentially indicate a significant role of Fos B in early stage of breast cancer. Finally, it is important to recognize a prognostic/predictive biomarker which could identify and stratify patients in subgroups that could most benefit from certain therapy and to avoid the use of chemotherapy for patients with estrogen receptor (ER)-positive breast cancer.

References

1. Manisha Juneja et al. Promoter identification and transcriptional regulation of metastasis gene MACC1 in colorectal cancer. Molecular oncology 2013, 929-943.
2. Smith LM, Wise SC, Hendricks DT, Sabichi AL, Bos T, Reddy P, et al. cJun overexpression in MCF-7 breast cancer cells produces a tumorigenic, invasive and hormone resistant phenotype. Oncogene.1999; 18:6063-70.
3. Belguise K, Kersual N, Galtier F, Chalbos D: FRA-1 expression level regulates proliferation and invasiveness of breast cancer cells. Oncogene. 2005, 24 (8): 1434-1444.

Poster 2

Metallothionein in serum of men with testicular germ cell tumors

Blanka Tariba Lovaković[1], Tanja Živković Semren[1], Vlatka Filipović Marijić[2], Marijana Erk[2], Marija Gamulin[3], Alica Pizent[1]

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Testicular germ cell tumors (TGCT) are uncommon but particularly important malignancies as they tend to affect children and young men, representing the most common tumor in men aged 15-35 years. Incidence rates have been increasing over the past 50 years in many industrialized populations. In clinical practice, there are three tumor markers commonly used in the diagnosis and prognosis of testicular cancer: alpha-fetoprotein (AFP), human chorionic gonadotropin (β-HCG) and lactate dehydrogenase (LDH). Although crucial in the management of testicular cancer, these markers are not specific for testicular cancer and their values are elevated in only 51% of testicular cancer cases. Metallothioneins (MTs) have been disclosed as a useful diagnostic factor for tumor progression and drug resistance in several malignancies. Their enhanced synthesis in rapidly proliferating tissues suggests its crucial role in normal and neoplastic cell growth. Increased levels of MT in blood serum have been found in patients with several types of cancer.

The aim of the study was to determine MT levels in serum of patients with TGCT, investigate the difference in serum MT levels among patients with different stages of TGCT and compare MT with commonly used markers. Serum samples were obtained prior to chemotherapy, after two cycles of chemotherapy and 1 year after chemotherapy. Concentration of total MT was determined in serum of 25 men with newly diagnosed TGCT (seminoma or non-seminoma) and in healthy volunteers.

Concentration of serum MT was significantly higher in TGCT patients than in healthy volunteers. A statistically significant difference in MT levels in patients with different stages of TGCT was observed in the serum of patients with non-seminoma obtained before chemotherapy. Although not significant, an increase in serum MT levels commensurate with the disease stage increase was also observed in patients with seminomatous TGCT.

Poster 3

Amino acids in urine of testicular cancer patients

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Testicular cancer is the most common malignancy in young men and in recent decades its occurrence has been growing rapidly. Tumour development induces changes in cell metabolism that can be monitored by analysing the metabolites in biological samples of the subject. This can be a useful tool for estimating the condition of an organism and further treatment of the disease. Amino acids are a source of energy and the building blocks for many specific molecules such as nucleotides and coenzymes. Recent studies have shown that alterations in amino acid concentrations in human biofluids can be related to cancer growth.

The aim of this study was to compare a quantitative profile of free amino acids in urine between men with testicular cancer and control subjects.

Concentrations of 30 amino acids were measured in the urine of 86 subjects with recently diagnosed testicular cancer and 68 control subjects by GC-MS using a Phenomenex EZ:faast kit for sample preparation. The results were corrected for urinary concentrations of creatinine. In statistical analyses of the difference between the groups, a Bonferroni correction was used to address the problem of multiple comparisons and reduce the possibility of false discovery. The results showed a significantly higher concentration of aspartic acid and significantly lower concentration of threonine, serine and histidine in urine of testicular cancer patients in comparison to the control group.

The results of this study suggest that the development of a tumour in the testicles possibly leads to a disorder in the citric acid cycle and the urea cycle. The obtained results can be used as a basis for further research on the role of the intermediates of the above-mentioned cycles in tumorigenesis, which could contribute to the possible discovery of new biomarkers for testicular tumours.

Poster 4

Correlation between early dynamics in circulating tumour DNA and outcome from FOLFIRI treatment in metastatic colorectal cancer

Amanda Frydendahl B. Johansen[1], Iben Lyskjær[1], Camilla S. Kronborg[2], Mads H. Rasmussen[1], Boe S. Sørensen[3], Karen-Lise G. Spindler[2] and Claus L. Andersen[1]

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Colorectal cancer (CRC) is one of the most frequently diagnosed cancers worldwide, and approximately 20% of patients present with metastatic disease. 5-Fluorouracil and leucovorin administered together with Irinotecan (FOLFIRI) is commonly used as a first- or second-line chemotherapy. However, resistance to chemotherapy in metastatic CRC (mCRC) is a major challenge and development of biomarkers for determining therapy response is vital to ensure optimal palliative treatment. We aim to provide evidence that ctDNA has the potential to be used as an early marker of therapeutic response of FOLFIRI.

Twenty-four patients diagnosed with mCRC, and with indication for first-line combination chemotherapy, were prospectively enrolled in this phase II study. Blood samples collected pre-treatment, at day 7, 14, 21, 60 and at progression were analysed for cell-free DNA (cfDNA) and ctDNA levels using digital droplet PCR. A subset of samples were additionally analysed by targeted next generation sequencing.

Patients with high levels of ctDNA or cfDNA levels (≥75th centile) before treatment had significantly shorter progression free survival (PFS) than patients with lower levels. Despite an overall decline in ctDNA levels from pre-treatment to first status CT-scan, serial analysis identified seven patients with temporary increases in ctDNA consistent with growth of resistant cells. These patients had shorter PFS and shorter overall survival. Targeted next generation sequencing analyses of cfDNA revealed dramatic changes in the clonal composition in response to treatment.

Our study suggests that increasing ctDNA levels during the first cycles of first-line FOLFIRI treatment is a predictor of incipient progressive disease and poorer survival. Thus, we demonstrate the potential of monitoring ctDNA levels as early as one week after treatment onset to enable early detection of treatment failure.

ACKNOWLEDGEMENTS

Financial support from The Danish Cancer Society, The Lundbeck Foundation and The Independent Research Fund Denmark is acknowledged.

Poster 5

Analysis of Plasma Cell-Free DNA by Ultradeep Sequencing in Patients With Stages I to III Colorectal Cancer

Thomas Reinert[1], Tenna V. Henriksen[1], Emil Christensen[1], Shruti Sharma[2], Raheleh Salari[2], Himanshu Sethi[2], Hsin-Ta Wu[2], Svetlana Shchegrova[2], Alexander Olson[2], Scott Dashner[2], Shruti Goel[2], Ryan Swenerton[2], Prashanthi Natarajan[2], Tony Tin[2], Hemant Pawar[2], Lene H. Iversen[1], Anders Husted Madsen [3], Cheng-Ho Jimmy Lin[2], Bernhard Zimmermann[2], Claus L. Andersen[1]

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Novel sensitive methods for detection and monitoring of residual disease can improve post-operative risk stratification with significant impact on patient selection for adjuvant chemotherapy (ACT), ACT duration, intensity of radiological surveillance, and ultimately outcome for patients with colorectal cancer (CRC). We investigated the prognostic and predictive impact of longitudinal ultra-deep sequencing of cell-free DNA in CRC patients. In this prospective, multicenter cohort study, ctDNA was quantified in the preoperative and postoperative settings of stages I to III CRC by personalized multiplex, PCR-based, next generation sequencing. The study enrolled 130 patients at the surgical departments of Aarhus University Hospital, Randers Hospital, and Herning Hospital. Plasma samples (n = 829) were collected before surgery, postoperatively at day 30, and every third month for up to 3 years. Pre-operatively, ctDNA was detectable in 89% of patients. Following definitive treatment, longitudinal ctDNA analysis identified 88% of the relapses. At the clinical decision points such as post-operative day 30 for ACT and post-ACT for additional therapy, ctDNA-positive patients were 7 and 18 times more likely to relapse than ctDNA-negative patients (day 30: HR=7.2, 95% CI 2.7-19,0 *P*<.001; post-ACT: HR=17.5, 95% CI 5.4-56.5, *P*<.001). In fact, 100% of the patients who were ctDNA-positive after ACT experienced relapse (n=7). Monitoring demonstrated that 30% of ctDNA-positive patients were cleared by ACT. During surveillance after definitive therapy, ctDNA-positive patients were over 40 times more likely to recur than negative patients were (HR, 43.5.0; 95% CI, 9.8-193.5; *P*<.001). In all multivariate analyses, ctDNA status remained an independent predictor of relapse. Thus, circulating tumor DNA analysis can potentially change the postoperative management of CRC by enabling risk stratification, ACT monitoring, and early relapse detection.

Acknowledgements

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Poster 6

Urinary collagen fragments: a potential prognostic tool in HF

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Fibrosis is associated with heart failure and may be highly relevant in relation to new-generation biomarkers. In the heart, fibrotic remodeling of the left ventricular wall leads to functional loss of systolic and diastolic function and is considered a forerunner of heart failure with reduced or preserved ejection fraction. Extracellular matrix turnover, a hallmark of fibrosis, might be reflected by changes in collagen fragments that make up the extracellular matrix. Studies on Type I collagen fragments in circulation and in urine showed primary evidence of their prognostic values for cardiovascular disease, suggesting the role of fibrosis in heart failure progression. In this study, we aim at investigating if type I collagen alpha 1 chain (COL1A1) fragments in urine or plasma can be correlated to severity of heart failure, and if they are predicative towards cardiovascular outcomes. The data derived from Hull (HOMAGE-Fibrosis) cohort consisted of 354 participants. The participants were followed at a median duration of 6.4 years. Multiple urinary and serum collagen-related peptides could be identified as significantly associated with outcome of heart failure, and other cardiac parameters including NYHA stage and left ventricular ejection fraction. They can potentially predict outcome of heart failure in a high-dimensional classifier. The correlation between serum and urinary peptides awaits more studies. We hypothesized that by monitoring the change in intensity in these collagen fragments, subclinical fibrotic changes that take place during heart failure may be detected, possibly also supporting our understanding of the pathophysiology of heart failure.

ACKNOWLEDGEMENTS

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Poster 7

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

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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 underscores 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 former 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

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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

ZnO nanostructure-based electrochemical biosensor for DNA detection.

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Portable DNA identification devices in condition of lack of complicating facilities are required in different areas. One of the type of the sensors that can be mentioned is an electrochemical detector based on zinc oxide nanostructures. Quite high value of the isoelectric point (approx. 9.5) makes ZnO suitable to use as an adsorbing surface for a working electrode. In this case, zinc oxide nanostructured devices can be used not just for DNA identification. Many types of biomolecules (enzymes, proteins, etc.) also have isoelectric points with a value less than six. In conditions of neutral pH the molecules are charged negatively and can be adsorbed by a developed zinc oxide surface.

The most widely used morphology of ZnO nanostructures are vertically aligned nanorod arrays. However sometimes other form of morphologies can be more effective. For example, nanotubes were more productive in the case of heavy metal ion determination. Therefore, one of the tasks of the study is to compare the efficiency of different ZnO morphologies developing a DNA electrochemical biosensor. The using of nanostructured materials in the production of biosensors makes it possible to detect the presence of traces of a particular protein at extremely low concentrations (up to several tens of nanogramms per milliliter), which makes these sensors particularly effective in the early diagnosis of cancer.

Poster 10

Proteomics alterations in colorectal cancer initiation and progression

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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 storred at -80^{o}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 ProteinLynx 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 agressivness 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 11

Proteomics research on schizophrenia (choroid plexus proteome)

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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 triads (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 ProteinLynx 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.

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Poster 12

Exosome-based biomarkers in urine for the diagnosis of prostate cancer

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Prostate cancer is the second most common cancer in men. Although significant advances in both diagnosis and treatment have increased the 10-year survival rate of prostate cancer to 98%, it is still the fifth leading cause of death from cancer in men. Early detection significantly increases patient survival, but prostate specific antigen (PSA) blood level, which has been used for early diagnosis for many years, produces a high number of false positives and has led to overtreatment. Prostate biopsy, which is the standard method for diagnosis and prognosis, can cause bleeding and infection, which may lead to dangerous complications in patients with compromised defenses of advanced age. Thus, there is a need for improved diagnostic methods that provide an early detection and can accurately estimate the risk of prostate cancer progression.

In the last years liquid biopsies have emerged as a promising alternative to traditional biopsies because of their several advantages. They are non-invasive, can be taken repeatedly and usually provide results faster than classical methods. Urine is a particularly interesting biofluid for prostate cancer due to its anatomical proximity to the prostate. Additionally, tumor cells have been detected in the urine sediment.

In our group we have several ongoing projects focused on the discovery of new prostate cancer biomarkers using different molecules present in the urine. Exosomes are small vesicles released by cells by fusion of multivesicular bodies with the cell membrane. It has been shown that exosomes derived from cancer cells contain tumor-related molecules. By isolating exosomes from urine and analyzing the differential expression of miRNA by next-generation sequencing and proteins by mass-spectrometry between patients with different stages of prostate cancer we expect to discover specific biomarkers correlated with tumor aggressiveness. Another of our projects focuses in developing a novel system to diagnose prostate cancer analyzing phosphopeptides in crude urine.

Poster 13

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

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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.

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Poster 14

Involvment of bacteria and silver nanoformulations for searching the COPD biomarkers and others.

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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 recommend 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 purpose. 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 it 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 still 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 behavior in production, consumption and utilization may consequently cause damage to the environment, especially to human and animal health. Both of them, bacteria and silver nanoparticles, may be responsible for exacerbation of different disorders. Therefore it would be a good idea to monitory the amount of silver nanoparticles in body fluid and cells together with microbial profile. Both of them may be the key to searching the biomarkers.

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Poster 15

Urinary volatile profiling of urological cancers: a bottom-up metabolomics approach

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Urine has emerged as a suitable biological matrix to search for biomarkers of urological cancers, benefiting from its proximity to the affected organs, non-invasive collection and large volume. In recent years, the analysis of volatile organic compounds (VOCs) present in urine has shown promising results for cancer diagnosis by detecting unique volatile odour signatures of several cancers (e.g., lung, breast). Recent advancements in the development of electronic nose devices offer the potential for new non-invasive tools to point-of-care clinical diagnosis based on the detection of volatile metabolites. Hence, one of the main challenges is to find a unique volatile signature of each type of cancer and then to develop a biomaterial tuned in specificity and selectivity for those signatures. A bottom-up metabolomics approach based on the volatile profiling of immortalized cell lines, tissue and urine (matrices of increasing complexity) will be used to determine specific signatures (multi-biomarker panels) characteristic of bladder, prostate and kidney cancers. Culture media, tissue extracts and urine from cancer and control cases will be analysed by gas chromatography coupled to mass spectrometry (GC-MS). The data generated will be pre-processed and analysed using bioinformatic tools. The potential multi-biomarker panels found for each type of urological cancer will be validated using an external set of urine samples of patients diagnosed with urological cancers and cancer-free individuals.

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Poster 16

Trinucleotide repeats in myotonic dystrophy type 1: from biomarker to drug target

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Myotonic dystrophy type 1 (DM1) is an autosomal dominantly inherited degenerative disease characterized by progressive loss of muscle mass, cataract, hypersomnia, fatigue, heart conduction abnormalities and respiratory failure.

The genetic mechanism of the disease is not completely elucidated, but most of the symptoms can be traced to a mutation present on a non-coding region of the DMPK (dystrophia myotonica protein kinase) gene. The mutation is characterized by a CTG expansion, which will be transcribed in $(CUG)_n$ RNA repeats. The CUG fragments are unstable and, in the cell, will form hairpin like structures, which are able to strongly bind several splicing factors such as, MBLN1 and CUGBP1. The depletion of these factors will in turn result in other RNA mis-splicing and protein synthesis defects.

The presence of the CTG expansion on the DMPK gene and the free CUG fragments in the cell are highly characteristic to myotonic dystrophy type 1 and can be considered a valuable biomarker for early stage diagnosis and prognosis. Moreover, based on the distribution of CTG expansion size, the occurrence and onset of the main symptoms, 5 clinical groups of DM1 have been defined: congenital, infantile, juvenile, adult and late on-set.

While at the present there is no available treatment for the disease, several groups focused their attention on finding small molecules that could disrupt the MBNL-1/CUG complex or bind to DNA $(CTG)_n$ repeat sequence thus preventing its transcription into harmful RNA.

In this study, we present our approach for the synthesis and purification of $(CTG)_n$ and $(CUG)_n$ for drug-target studies, along with an affinity capillary electrophoresis method for screening a broad range of small molecules as potential lead compounds for the treatment of DM1.

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Poster 17

Specific lipid fingerprint in women with high risk pregnancy and preeclampsia

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Although substantial progress in understanding pathophysiological mechanisms of preeclampsia (PEC) development has been made, this complication of pregnancy still remains one of the leading causes of maternal and perinatal morbidity and mortality, while premature delivery is the only effective treatment. Furthermore, despite a lot of research in this field, there is no single reliable predictive biomarker, as all biochemical parameters used in PEC prediction until now, have low prediction accuracy. It is thought that women prone to PEC development have an irregular remodeling of the spiral artery, resulting in placental hypoperfusion. In hypoxic environment, placenta is a source of various inflammatory and oxidative modified markers responsible for generalized endothelial dysfunction and inflammation found in these women. Today, we are aware that lipids are decisive factors in cell signaling pathways involved in regulating significant cellular functions, while lipotoxicity is recognized as an important mediator of maternal endothelial dysfunction and incomplete trophoblast invasion. The introduction of the lipidomics has allowed characterization, identification and quantification of different lipid species and represents a powerful approach in understanding lipid biology. Pregnant women with moderate or high risk factors for PEC development will be monitored throughout the pregnancy, from their enrollment into the study until the delivery. Maternal blood samples will be taken at the end of each trimester. Afterwards, circulating profiles of sterols and sphingolipids in women with high-risk pregnancy will be determined by high performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS) at the Department of Medical Biochemistry. The data obtained will be analyzed in order to define a specific lipid fingerprint of women with PEC. We will also investigate the correlation of such a fingerprint with conventional biochemical parameters to highlight specific lipid species that could be relevant in assessing the outcome of high-risk pregnancy and identification of women prone to PEC development.

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Poster 18

Nephrotoxicity biomarkers of novel potential antidiabetic agents

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The actual therapies of diabetes mellitus are effective, but often restricted because of their ways of application, side effects, loss of the efficacy after long-term use, cost and unavailability in rural areas where they are mostly needed. The development of new potent and comfortable classes of blood glucose-lowering medications with minimal side effects to supplement actual therapeutics is urgently needed. Polyoxometalate compounds (POMs) were reported as a novel class of inorganic compounds with normoglycemic properties in diabetic animal models, thus considering them as promising antidiabetic drugs. However, there is a relative lack of relevant data related to toxic properties of POMs. As the kidney constitutes the major excretory organ for most substances, during the development of a new drug it is necessary to examine the nephrotoxic properties of potential pharmaceutical agents. Additionally, some studies reported the highest accumulation of some polyoxotungstates in the kidneys after the oral application. Therefore, our research was designed to monitor nephrotoxicity biomarkers in *Wistar* rats treated with POMs exhibiting hypoglycemiant action. The obtained results of our recent study showed that the two-week oral administration of some POMs (20 mg/kg/day) induced increase of serum urea and creatinine levels. Also, dark and oedematous mitochondria in the tubules of the kidney samples from the treated animals were observed using transmission electron microscopy. Accordingly, the changes in the tested parameters after the POM treatment suggest the need for monitoring the panel of renal function biomarkers such as specific functional biomarkers (cystatin C and urine albumin), up-regulated proteins (kidney injury marker-1, neutrophil gelatinase-associated lipocalin, and interleukin 18), and enzymes (lactate dehydrogenase, glutathione-S-transferase, and alanine aminopeptidase).

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Poster 19

Cardiac oxidative stress parameters and cardiometabolic markers in monocrotaline-induced heart failure in Wistar albino rats: influence of subchronic vitamin B6 application

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Monocrotaline (MCT) induces pulmonary arterial hypertension (PAH), characterized by remodeling mechanisms of the pulmonary arterial vessels with increased pulmonary vascular resistance, and with the occurrence of consequent right ventricular hypertrophy, right-sided heart failure, decompensation and ultimately to fatal outcome. The aim of this study is to test the hypothesis that subchronic application of vitamin B6 could affect heart failure (HF) induced by MCT and with the modulation of oxidative stress parameters and cardiometabolic biomarkers. Biochemical and inflammatory parameters together with histomorphometric analysis will be assessed in blank solution-exposed controls (C1 physiological saline 1ml/kg one day n=8; C2 physiological saline 1ml/kg 28 days n=8)**,** MCT-induced HF **(**MCT 50mg/kg, n=8**),** B6 (vitamin B6 7mg/kg/day, n=8) and MCT+B6 (MCT 50mg/kg, vitamin B6 7mg/kg/day, n=8) male Wistar albino rats (b.w. 160 g, at start)**.** Superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities together with parameters of oxidative damage of proteins, thiol- and carbonyl groups, will be determined in cardiac tissue. Echocardiography will also be performed in order to confirm MCT-induced rat heart failure. The obtained results will be correlated with each other, in order to investigate if vitamin B6 attenuates hypertrophy of RV wall and how it will modulate oxidative stress which is involved in PAH pathogenesis and subsequent HF.

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Poster 20

Advantages of multimarker approach for diagnosis and prognosis of colorectal cancer

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Colorectal cancer (CRC) is complex, multifactorial disease that is induced by both genetic and environmental factors. Whilst CRC incidence is increasing in modern world, adequate laboratory diagnostic and prognostic tools are still lacking. Namely, diagnosis of CRC is based on highly invasive procedure of colonoscopy, whilst currently available serum markers for monitoring and prediction of risk for CRC do not have sufficient analytical and clinical performances. Therefore, since single markers lack in sensitivity and specificity in CRC diagnostics, a multimarker approach is proposed. Such approach comprises the use of transcriptomics and proteomics data for development of novel and easily obtainable markers, which should have sufficient discriminative power to detect CRC in early phases of development and to improve disease prevention and prognosis. Development of CRC is associated with obesity, lipid profile disorders and inflammation. Bearing this in mind, biomarkers which reflect specific interactions between obesity, dyslipidemia and inflammation during the onset and progression of CRC might be useful in clinical practice. Simultaneous analyses of advanced lipid status markers, circulating concentrations of cytokines and their mRNA levels in peripheral blood mononuclear cells (PBMCs), as well as the assessment of their interrelations, are performed in plasma samples of patients with CRC and healthy controls at the Department of Medical Biochemistry, Faculty of Pharmacy, University of Belgrade. The obtained data will be used for detection and evaluation of suitable biomarkers which might be used for prediction, diagnosis and prognosis of CRC. Additionally specific single nucleotide polymorphisms (SNPs) will be analyzed in order to get an insight into their causal relationship with mRNA instability and changes in protein levels that are associated with CRC.

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Poster 21

DNA methylation and telomere length in diabetes mellitus

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Diabetes mellitus (DM) is one of the most common chronic disorders and represents a major public health issue. Development of microvascular and macrovascular complications due to late diagnosis or inefficient treatment is thought to be the main cause of the high rate of mortality and morbidity. Although it is well recognized that persistent hyperglycemia leads to the development of diabetes complications, the molecular mechanisms responsible for their onset have not been fully elucidated. The investigation of the role of epigenetic modifications, including DNA methylation, on the onset and progression of the disease, is particularly important for multifactorial diseases, such is DM. Indeed, analysis of global DNA methylation provides comprehensive insight into the interaction of traditional risk factors and environmental factors on gene expression, but this epigenetic modification has not been sufficiently investigated in diabetes. So far, the differences in DNA methylation are shown in patients with DM compared to healthy subjects, but also in patients with and without diabetic complications, suggesting that DNA methylation could be a valuable prognostic biomarker. Available data indicate that diabetes may also affect the telomere length (TL), a biomarker of cellular aging. Specifically, TL in patients with diabetes is reduced compared with healthy individuals. Furthermore, DM patients were characterized by dyslipidemia and oxidative stress, highlighting the potential for the interactive role of all above-mentioned mechanisms in the development of DM complications. Therefore, our study will focus on the establishment of the multimarker approach for the prediction of diabetic complications development. Traditional risk factors, global DNA methylation and TL and biomarkers of oxidative stress, dyslipidemia and inflammation will be determined in DM patients with and without complications and analyzed to identify new clinically relevant biomarkers or set of biomarkers which could improve prevention, early diagnosis and monitoring of the disease.

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Poster 22

New approaches in the diagnostics of disorders of glycoconjugate metabolism

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Rare disorders are still underestimated group of inherited diseases, although the number of patients suffering from this disorders are greater than the numbers of patients with cancer and AIDS together. The wide variety of molecular basis and clinical signs classify rare diseases as difficult to diagnose. Disorders of glycoconjugate metabolism are a rapidly expanding group of rare diseases that include congenital disorders of glycosylation (CDG) and lysosomal disorders (LSD). It is estimated that 40% of CDG patients are still waiting for their diagnosis. Current conventional diagnostic methods used in health care have many disadvantages, but the cooperation between the scientific institutions and physicians has the potential to eliminate them. The innovation of methods for diagnostics of disorders of glycoconjugate metabolism in the form of personalized "OMICS" approach using modern analytical approaches can thus lead to rapid, reliable and precise diagnosis. Samples of suspected patients, where traditional methods for diagnosis and treatment are no longer sufficient, or in the aim of the determination of the efficacy of the administered therapy will be sent from the clinical center directly to the Institute of Chemistry, where they will be analyzed through a personalized approach including glycomic, glycoproteomic, metabolomic and genetic analyses. The obtained complex data will be correlated with the clinical phenotype and biochemical parameters of the patients and together with the biological samples stored in the biobank for the needs of other workplaces dealing with the diagnosis of metabolic disorders. Circulating microvesicles will be isolated from the patient's blood and characterized by established "OMICS" methods to discover a new, clinically relevant biomarker, or a set of biomarkers of glycoconjugate metabolism disorders.

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Poster 23

Experimental study about the expression of blood plasma biomarkers expressed during the transformation of colon adenoma to adenocarcinoma in a murine animal model.

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The 5-year survival rate for the CRC that is located at the time of diagnosis is 90%, but it can decrease to 68% if it is regionally compromised and to 10% if they are discovered with metastasis. This indicates the need for developing early diagnosis systems. Currently, techniques such as NGS or PCRq serve as support for new research related to the early diagnosis of CRC. This work can be summarized by adding a Conceptual Hypothesis and an Operating Hypothesis.

CONCEPTUAL HYPOTHESIS: The combination of a panel of serum biomarkers that occur before the transformation of adenomatous lesions into adenocarcinomas in colorectal cancer and detect the presence of miRNAs can predict early the conversion of an adenomatous lesion of the colon into a neoplastic lesion.

OPERATIONAL HYPOTHESIS: By comparing the presence and concentration of certain MicroRNAs with other diagnostic systems such as endoscopy and histopathology, a correlation can be established between them in order to develop an early diagnosis through the use of MicroRNAs.

For this study animals of type Rattus norvegicus, strain Pirc F344 / NTac-Apcam^{1137} were used, all with 2 months of age. The procedure under inhalation anesthesia with Sevoflurane consisted of: (1) Extraction of blood by venipuncture of the lateral coxigeal veins using a 25G needle collected in EDTA tubes for subsequent plasma extraction once centrifuged to 3G for 5 minutes at 4° C, (2) endoscopic examination using a 2.9mm optical rigid cystourethroscopy with a total thickness of 5 mm, and a fiberscope of 7.5 Charr, and (3) extraction of the different findings found during the colonoscopy and a biopsy of healthy tissue, using a biopsy forceps through the working channel of the cystourethroscope, fixed in 4% formaldehyde.

ACKNOWLEDGEMENTS

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Poster 24

Evaluation of soluble IFNß receptor (sIFNAR2) as IFNß response biomarker in multiple sclerosis patients.

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Background: There is a need to identify response biomarkers that help the choice of treatment that will be most effective for MS patients. The soluble IFNß receptor (sIFNAR2) is an isoform generated by alternative splicing, that can be detected in serum and is able to modulate the activity of both endogenous and systemically administered IFNß. We previously described that untreated-MS patients showed significantly lower serum levels of sIFNAR2 than healthy controls, in two independent cohorts, so it was proposed as diagnostic biomarker. Now, our aim is to assess sIFNAR2 levels as IFNß response biomarker as well as to evaluate its relationship with other clinical variables.

Methods: The longitudinal study included 51 MS patients (basal, 6 and 12 months after IFNß treatment onset) classified as responders (R) and non responders (NR) according to the Rio Score. Additionally, 12 MS patients were analyzed during the relapse and in the remission period. For the clinical form, 143 relapsing remitting, 43 secondary progressive and 12 primary progressive were analyzed. Quantification of sIFNAR2 was performed by ELISA developed and validated in our laboratory. Each assay included a standard curve, 2 quality controls and a negative control. Non parametric tests were used.

Results: Before the onset of treatment, NR patients had significantly lower levels of sIFNAR2 compare to R patients (p=0.026). Then, a logistic regression analysis showed that patients with basal sIFNAR2 levels lower than 43.2ug/ml have OR of 5.1 (p=0.012 CI [1.42-18.25]) of being a non-responders to IFNß treatment. The model was adjusted for possible confounding variables such as sex, age and evolution time. NR patients increased sIFNAR2 levels after 6 months of treatment (p= 0.010), while sIFNAR2 levels while levels remain stable in R patients.

On the other hand, elevated levels of sIFNAR2 were observed in patients during the relapse compared with the same patient in remission (p=0.002). Regarding the clinical form, PP had elevated sIFNAR2 compared to RR and SP (p= 0.042, p= 0.010).

Conclusions: Basal levels of sIFNAR2 is a predictive value of response to IFNß treatment and could have clinical applicability at the time of choosing the treatment. **The lower basal levels of** sIFNAR2 in non responders patients are restored **six months after** IFNß treatment and reach similar values to responders patients. sIFNAR2 could be involved in the pathogenesis of MS and IFNß treatment could modulate its levels.

Poster 25

Immunomonitoring of HLA-DR expression and ratio nCD64/mHLA-DR as predictive biomarkers of infection in critical patients at the Intensive Care Unit

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Introduction

Sepsis is characterized by a simultaneous imbalance of hyperinflammation and immunosuppression.

The expression of HLA-DR in monocytes (mHLA-DR) and CD64 expression in neutrophils (nCD64) are considered, respectively, predictive and diagnostic biomarkers of infection. The ratio nCD64/mHLA-DR has been described as a prognostic biomarker of sepsis.

Objective

To evaluate mHLA-DR expression and ratio nCD64/mHLA-DR in patients admitted to the Intensive Care Unit (ICU) and their relationship with the development of infection.

Methods

Prospective study of 77 patients admitted to the ICU from our hospital (HGTiP) due to stroke or severe traumatic brain injury. The mHLA-DR and nCD64 expression were analyzed in whole blood samples at baseline, +3, +6, +9, +12 and +15 days after admission, using a standardized flow cytometry protocol.

Results

During the follow-up, 71% of patients became infected (infection without sepsis, sepsis or septic shock).

Infected patients showed – already after three days of admission- a lower percentage of mHLA-DR+ (85.8 ± 16.22% *vs*. 92.5 ± 12.13%, $p < 0.001$). Interestingly, on day +3, infected patients also had a higher ratio nCD64/mHLA-DR (0.12 ± 0.19 vs. 0.04 ± 0.08, $p < 0.001$) than the non-infected ones.

Conclusion

The immumonitoring of mHLA-DR expression and ratio nCD64/mHLA-DR may help to evaluate those patients with susceptibility to develop infection and sepsis at the ICU.

Poster 26

Non-invasive sensitive method for oxidative stress quantification in exhaled air

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Inhalation of particulate matter (PM) tends to induce inflammation and endogenous reactive oxygen species (ROS) production. ROS overproduction, in turn, lead to oxidative stress and increase the risk of respiratory diseases, including chronic obstructive pulmonary disease (COPD). The diagnosis and assessment of COPD currently relies on respiratory functional exploration test using spirometry. However, this technique does not allow early diagnosis nor early detection of exacerbation episodes. Therefore, in addition to spirometry, alternative methods focusing on exhaled air as non-invasive matrix are being developed in the Respiratory disease Occupational Biomonitoring Collaborative Project in the framework of CliniMARK EU-COST Action (CA16113) in order to access the biochemical information related to lung oxidative stress.

One studied approach focuses on the simultaneous quantification of a panel of tree oxidative stress biomarkers in exhaled breath condensate (EBC) samples by LC-MS/MS technique: 8-hydroxy-deoxyguanosine, 8-isoprostaglandin F2α and malondialdehyde. Providing that EBC is a strongly diluted sample, the main challenge consists of reaching low limit-of-quantification as well as robust sample pre-concentration step.

In parallel, we aim at optimizing a portable photonic system, the OPEA analyzer, able to determine lung oxidative potential via direct analysis of exhaled air (1L). The detection principle relies on multiscattering-enhanced absorbance strategy that enables fast and sensitive determination of the homeostatic redox balance if lining fluid droplets contained in the exhaled breath aerosol. Optimization aspects related to instrument design and sample storage will be addressed.

Both biomarker quantification approaches will be discussed in terms of analytical validation and expected context-of-use in the COPD paradigm.

Poster 27

Identification and Targeting Breast Cancer Stem Cells

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Breast cancer is the most common cause of deaths due to cancer in women due to complications with its diagnosis and resistance to therapy. Till to date several studies have shown that, similar to other solid tumors, breast tumors contain a subpopulation which is responsible for resistance to therapies and relapse of cancer and is termed as Cancer stem cells (CSC). Additionally apart from CSC's natural existence in tumors, prolonged treatment of cancer cells with commercial chemotherapeutics can also turn these cells into CSCs through epithelial to mesenchymal transition (EMT). So there is a dire need to discover such biomarkers which can identify CSCs and drugs which can target this subpopulation specifically. Moreover similar to tumors, established breast cancer cell lines also contain CSCs. So in this project using commercial cell lines gene expression data and cytotoxicity data (from CCLE and CGP datasets), we identified such biomarkers (15 genes) which can identify CSCs and drugs to target these. Midostaurin was identified as the drug which can target CSCs and another drug, Lapatinib can target non-CSCs. We validated our biomarkers and drugs using *in silico* and *in vitro* approaches in several datasets and three different biological settings respectively. Moreover identified genes also has prognostic importance in Paclitaxel treated patient cohorts identified through *in silico* analysis. Additionally in literature, it has been reported that treating mesenchymal cells (CSCs) with several natural compounds like forskolin and curcumin, can convert these to epithelial phenotype (non-CSCs) and then these can be targeted with conventional chemotherapeutics. This approach has an advantage as natural compounds like forskolin and curcumin do not pose any side effects to normal cells. So we are screening CSC like cell lines to convert them to epithelial phenotype and targeting these with non-CSC drugs. This approach currently is being validated.

To conclude our findings, we have identified CSCs biomarkers and the drugs which can target both CSC and Non CSC.

Poster 28

Paper-Based Colorimetric Spot Test Utilizing Smartphone Sensing for Detection of Biomarkers

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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.

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Poster 29

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

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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 µg/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.

Poster 30

Non-invasive profiling of the breast microenvironment to monitor breast health

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Nipple aspirate fluid (NAF) is a breast-specific proximal fluid, secreted naturally by the epithelial cells lining the breast ductal system from which 85% of breast cancer cases arise. NAF is, therefore, an attractive resource for breast cancer biomarker discovery as it can be attained simply and non-invasively through breast massage and screened for biomarkers indicative of disease development. For biomarkers to progress from laboratory to clinic, they must be identified using a reproducible, high-throughput method, allowing disease indicators to be identified rapidly.

In our preliminary study, 10 matched pairs from both healthy volunteers and breast cancer patients were reduced, alkylated, and trypsin digested prior to 1D-LC/MS analysis on Orbitrap Fusion, to accelerate our understanding of the protein composition and consistency of NAF. One invasive carcinoma NAF pair was selected as an inter-experiment control, analysed in triplicate within each experiment for quantitative proteomic profiling. MS/MS fragment mass lists were searched against Swiss-Prot database (Homo sapiens) through Proteome Discoverer 2.2, using Mascot software for protein identification.

In the triplicate analysis, a total 799 proteins with 766 quantifiable were identified in the invasive carcinoma patient samples. A minimum Pearson correlation coefficient of $R^2 = 0.93$, ($p<0.001$) highlighted a significantly strong correlation between all 3 data sets, indicating high repeatability and reproducibility of the method. Comparison of proteomes highlighted signature proteins expressed at significantly distinct levels in non-cancer and cancer groups. Out of which, 174 proteins were significantly upregulated in cancerous samples compared with those non-cancerous ($p<0.05$), playing key roles in cell adhesion ($p= 0.0273$), cell migration ($p=0.0272$), tissue morphogenesis ($p= 0.0273$) and angiogenesis ($p= 0.0273$).

Overall, the results highlight the great potential NAF carries as a liquid-biopsy for monitoring breast health. Analysis in 1D-LC/MS provides relatively rapid insight into the breast microenvironment.

Poster 31

Quantitative profiling of Cytochrome P450 2S1 in Colorectal cancer by PRM assay

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Cytochromes P450s (CYPs) constitute a superfamily of xenobiotic metabolising enzymes responsible for metabolism of many pharmaceuticals in the liver. Elevated mRNA levels of specific isoforms, such as CYP2S1, are associated with poor prognosis in colorectal cancer (CRC), and represent novel therapeutic targets for biotransformation of prodrugs to potent cytotoxics at the cancer site. In order to understand the expression of CYP2S1 protein, we have developed a parallel reaction monitoring mass spectrometry (PRM MS) assay to screen CRC samples. Peptides (n=3) uniquely associated with CYP2S1 were synthesized and used as standards to optimise analytical performance (fragmentation conditions, LC retention time, LOQ, LOD, dynamic range) in an Orbitrap Fusion-based PRM MS assay. Levels of CYP2S1 were then determined in protein extracts of CRC, relative to the standards. The PRM MS assay yielded quantitative data over 3 orders of magnitude. CYP2S1 was detected in C106, CaCO2, HCC2998, HT55 and DLD1 cell lines (0.05-1.08pg) and HT55 and DLD1 xenografts (0.29-0.41pg). CYP2S1 was also detected in patient-specific CRC tissues, with elevated levels in Stage III tumours. The PRM MS assay provides a specific, sensitive, high throughput method for identifying CYP2S1 compared to established enzyme and immunoassays. CYP2S1 levels vary considerably across CRC sources highlighting the importance of screening to identify the correct models for new drug development and the right patients for subsequent treatment.

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Poster 32

Proteomic Profiling of Matched Normal and Tumour Tongue Biopsies from Smokers and Non-Smokers

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Head and neck squamous cell carcinoma (HNSCC) has a yearly incidence of 600,000 cases worldwide, with ≥40% mortality rate. Due to late detection, survivors often have significant debilitating physical impairment to their day-to-day life. Hence, there is an unmet clinical need to identify biomarkers for early detection and intervention that can lead to less detrimental impact on lifestyle. An iTRAQ proteomic approach was used to profile protein changes in matched normal and tumour tissues for clinical applications. Matched normal and tumour tissue biopsies from non-smoking and smoking patients with tongue carcinomas were subject to cryo-pulversiation and protein determination. Extracts were pooled, trypsin digested and iTRAQ 4-plex labelled. Data generated by 2D-LC/MS on an Orbitrap Fusion, searched using the Mascot search engine to identify proteins and relative difference between disease and healthy, and between smoking and non-smoking quantified by LAMMA statistics. Significantly changed (± SD) proteins associated with cause were evaluated using bioinformatics tools (STRING, DAVID, PANTHER). A total of 3426 proteins were identified and quantified. Comparison of non-smoker tumour with smoker tumour identified 64 proteins upregulated and 62 downregulated, smoker tumour vs smoker normal identified 349 proteins upregulated and 395 downregulated, non-smoker tumour vs non-smoker normal identified 469 proteins upregulated and 431 downregulated. Collagen PTM enzymes and apoptotic proteins were differentially expressed between non-smokers and smokers. The biological significance of the results indicated the importance of using biopsies with good clinicopathological data for experimental design. The approach provides an important step towards comprehensive stratification of HNSCCs based on cause and region-specific pathology and identification biomarkers suitable for further validation.

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Poster 33

Potential of AGE-modified peptides as an early diagnostic marker for Alzheimer's disease – A pilot study

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The research community strives for early diagnostic markers in Alzheimer's disease (AD) to prevent disease-driven neuronal damage. A major known risk factor for AD, diabetes, is characterized by the carbohydrate-induced glycation of proteins, forming long-lasting, non-reversible and often cross-linking advanced glycation end products (AGEs) which are also implicated in the development of AD.

Thus, the question arose whether such protein-alterations could serve as biomarkers for AD-development in a sub-set of patients.

CSF of 5 control and 5 patients with assured AD diagnosis was tryptically digested and analyzed by an LC-MS/MS-workflow. The results were examined for AGE-containing peptides, which were unambiguously identified and could be quantified by their MS-signals. Of those, potential biomarker-candidates were determined by statistical analyses.

We quantified 164 glycated peptides in this study of which 139 are possible biomarker-candidates. AUC-analysis of those indicated that the data-set might include two interesting peptides, containing sugar- and glyoxal-induced modifications, that discriminate between AD and controls. These two candidates will be investigated in more detail. Additionally, sample size estimation based on this pilot study indicated, that with a reasonable number of samples, statistical significance could be reached for the top-candidates.

In conclusion, AGE-modified peptides show the potential to be used as biomarkers in AD.

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Poster 34

Serum proteome-wide changes in end-stage renal disease-related protein-energy wasting patients and correlation with clinical and nutritional state

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PEW is a malnutrition state with depleted body reserves of protein and energy fuel in advanced stage of chronic kidney disease (CKD stage 5/end-stage renal disease, ESRD) patients. It is a known risk factor for morbidity and mortality, especially in patients undergoing renal replacement therapy. Currently, there is a paucity of specific biomarkers for the early prognosis/diagnosis of ESRD-related PEW patients (ESRD/PEW patients). The application of proteomics can potentially uncover stage-specific biomarkers, offering a tailored disease intervention. This study aims to demonstrate that directly integrating serum proteomic ESDR/PEW patients profiles and nutritional status adds a highly informative level to the disease evaluation and management. Using a comparative high-throughput MS-based serum proteome profiling of 20 patients receiving hemodialysis, differentially expressed and modified blood proteins were screened and correlated with their dietary information. Quantitative serum proteomics analysis involved in-solution protein tryptic digestion coupled on line with high resolution, accuracy and sensitivity nLC-MS/MS analysis for protein identification and relative quantitation. >2000 proteins were identified and relatively quantified in all patients, highlighting unique patient-proteomic signatures. Most of the identified proteins participated mainly in biological processes related to blood regulation (e.g. plasma lipoprotein particle remodeling, complement activation and blood coagulation). Among the known/unknown proteins possibly implicated in the manifestation of ESRD/PEW patients were apolipoproteins (9), complement proteins (21), fibrinogen proteins (3), serum amyloid proteins (4), coagulation factor proteins (5) and serum albumin. Moreover, significant differences among the patients pertained to protein modification differences, e.g. proteolysis, phosphorylation ubiquitylation, etc. Concluding, integrating MS-based quantitative proteomics data with assessment of protein and energy nutritional status of ESRD/PEW patients using standard clinical methods may advance our understanding of the disease. Such a joint, multidisciplinary approach provide new view of proteomics, linking risk factor protein derangements, nutritional care and personalized therapeutic strategies in ESRD/PEW patients.

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Poster 35

A novel bioinspired proteasome activator: Potential anti-ageing strategies offered by Mother Nature (but not only)

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Proteasomes are constituents of the cellular proteolytic networks that maintain protein homeostasis through regulated proteolysis of normal and abnormal (in any way) proteins. Proteasome activation in human primary fibroblasts has been shown to result to cellular lifespan extension. Using *Caenorhabditis elegans* as a model, we were also able to promote proteasome activation in the organismal level. More specifically, proteasome activation in *C. elegans* either through genetic means or through compounds resulted in enhanced levels of proteasome activities that led to a SKN-1- dependent lifespan extension. The elevated proteasome function conferred lower paralysis rates in various Alzheimer's disease (AD) nematode models accompanied by decreased Aβ deposits thus ultimately decelerating the progression of AD phenotype. Similar positive results were also delivered in human neuroblastoma cells and in murine cortical neurons.
Based on these results we have searched for natural and synthetic compounds with proteasome activating properties. In the context of the present study, novel hybrid compounds, combining the structural features of the natural antioxidant vitamin E and of hydroxytyrosol (the main polyphenolic constituent of olive oil with a variety of biological properties), in one scaffold, were designed and synthesized. The new analogues were evaluated for their ability to activate the proteasome in human primary fibroblasts *in cellulo* as well in the test tube using highly purified 20S proteasome. The identified activators were administered to the cells throughout their replicative lifespan and exhibited an extending effect. Their anti-ageing properties were further tested and verified in the multicellular level, using the nematode *Caenorhabditis elegans*.
In total, our results suggest that proteasome activation exerts downstream positive outcomes on ageing and AD and they unveil the need for identification of anti-ageing and anti-amyloidogenic compounds with proteasome activating properties.

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