Hungary-Serbia
IPA Cross-border Co-operation Programme

Opening conference of

Cross-Border biomarker research of ovarian cancer

(*CrossBiomark*)

(HUSRB/1203/214/091)

project by organization of

Department of Medical Chemistry, Faculty of Medicine, University of Szeged, Hungary;
Department of Oncotherapy, Faculty of Medicine, University of Szeged, Hungary
and
Oncology Institute of Vojvodina, Medical Faculty, University of Novi Sad, Serbia

within the Hungary-Serbia IPA Cross-border Co-operation Programme framework.

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10:30 - 11:00 **Registration**

11:00 - 11:05 **Opening speech**
Prof. Gábor Tóth,
Department of Medical Chemistry, Faculty of Medicine,
University of Szeged, Hungary

11:05 -11:20 *Title:* **Introduction of HU-SRB Co-operation**
Subtitle: **Description of "Cross-border biomarker research of ovarian cancer"**
Dr. Tamás Janáky,
Department of Medical Chemistry, Faculty of Medicine,
University of Szeged, Hungary

Abstract of lecture

The Hungary-Serbia IPA Cross-border Co-operation Programme belongs to the "new generation" of cross-border co-operation programmes in the budgetary period 2007-2013 of European Union financial framework under the Instrument for Pre-accession Assistance (IPA).
„CrossBiomark" is a supported project (HUSRB/1203/214/091) of the Hungary-Serbia IPA Cross-border Co-operation Programme. Lead beneficiary of the project is the University of Szeged (Department of Medical Chemistry and Department of Oncotherapy) and our Serbian partner is the University of Novi Sad (Oncology Institute of Vojvodina). The cooperation is based on high level scientific and technical background of partners.
The aim of our cooperation is to collect epidemiologic data on distribution of ovarian cancer in South Great Plain and Vojvodina regions. Our primary goal is to discover new biomarkers (panel) for early diagnosis of ovarian cancer having higher selectivity and specificity than that of the presently used protein biomarkers. Blood phospholipids are our candidates to find better biomarker(s). At the Department of Medical Chemistry we develop a new, state of the art 2D-LC/MS-MS method for qualitative and quantitative identification of more than 100 phospholipid species. Blood samples will be collected in both regions from healthy volunteers and patients with different stages of ovarian cancer and with non-malignant ovarian diseases. Phospholipid-profile of blood samples will be characterized in our laboratory and putative biomarkers will be selected through the comprehensive statistical evaluation of data. Data could also help us to better understand biochemical processes behind of ovarian cancer and could reveal new drug targets, too.

11:20-11:40 *Title:* **Why mass spectrometry?**
Subtitle: **An efficient tool in biomarker research**
Dr. Zoltán Kele,
Department of Medical Chemistry, Faculty of Medicine,
University of Szeged, Hungary

Abstract of lecture

Biomarker can be anything which indicates the actual biological state, but in the medical analytical chemistry the meaning of biomarker is limited to molecules that serve as indicators e.g. of disease state progression, or mechanism of action of drugs. Analysis of these usually low abundant molecules originated from complex matrices like different body fluids is challenge for analytical chemists. Due to the recent advances, the mass spectrometry and its coupled techniques play key role in biomarker discovery and evaluation owing to several important attributes, which include sensitive and selective detection, multi-analyte analysis, and the ability to provide structural information. Mass spectrometry is both powerful tool for analysis of small (metabolites) and large molecules (proteins), thus it is increasingly being used to support quantitative measurement to assist in the evaluation and validation of biomarker leads.
In this presentation mass spectrometric methods that are available for biomarker research will be presented.

11:40 - 12:00 *Title:* **Lipidmomics in Cancer Biomarker research**
Subtitle: **Identification of candidate tumor markers for ovarian cancer by phospholipid analysis**
Dr. Róbert Berkecz,
Department of Medical Chemistry, Faculty of Medicine,
University of Szeged, Hungary

Abstract of lecture

Lipidomics is a field involving analytical chemistry and molecular biology that focuses on the global composition and dynamic changes of lipids in biological systems such as cells and tissues. Blood serum and plasma are the most frequently used materials for clinical diagnostics as they constitute the most complete mixture of all kinds of metabolites and proteins. The choice of analytic technique used in lipidomics depends on the sample type (various biofluids or different tissues) and also the target lipids. Nowadays the most promising method used for lipid analyses of biofluids is mass spectrometry (MS). Besides the qualitative and quantitative results provided by mass spectrometry, main advantages of the coupled liquid chromatography-mass spectrometry (LC-MS) are the better sensitivity, selectivity, reproducibility and the requirement of small amount of sample.

Glycerophospholipids (PLs) as a major class of lipids are classified by their polar head group. According to chemical composition the main classes of PLs are the following, phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylglycerol (PG) and phosphatidic acid (PA). In this project the research focuses on phospholipids (PLs) which are major components of cell membrane, signaling molecules and involved in the progression and metastasis of cancer when tumor cells undergo major morphological and molcecular changes. Previous researches highlighted the importance of

(lyso)phopshatidic acids (PA) as potential biomarkers for ovarian cancer (OC). Elevated plasma PA levels were detected in patients with OC compared with healthy control. However beside PAs additional PLs might be promising biomarkers in the early detection of ovarian cancer before symptoms appear. Therefore we aimed to develop sensitive, accurate and automated two-dimensional liquid chromatography-mass spectrometry (2D-LC-MS) method which is suitable to identify and quantify large number of PL species from human plasma. The separation of all PL classes will be performed in the first dimension with hydrophilic interaction chromatography (HILIC), and as many species within classes as possible will be separated in the second dimension with reversed phase chromatography (RP).

Analysis of membrane PLs are challenging both in identification and quantification point of view for analytical chemist because of wide range distribution of PLs in tissues and the diverse behavior of different PL classes during the separation and also ionization process. In a previous study we developed a RP LC-MS technique to investigate the effect of different treatment on the changing the PLs levels in the mouse brain. This kind of separation is based on the hydrophobic character of PLs. However the RP-separation has limitation due to the large number of lipid species and the limited capacity of reversed phase column. In many cases overlapping of isobaric inter- and intra-class PL species were observed decreasing the accuracy of measurement. Generally, one dimensional chromatographic techniques are unable of providing resolution of complex samples (brain and plasma extract could contain several hundreds PL species) consisting of many components due to the lack of separation space of solid phase. Therefore with 2D-LC techniques we can overcome the limited resolving power of common 1-dimensional LC techniques. Combination of two chromatographic steps with different separation mechanism results in better separation of species. Knowing mechanisms of RP and HILIC separations the combination of both separation as two dimensional LC separation using an ESI-MS(MS) detection can be one of the best solution for the purpose to achieve accurate qualitative and quantitative analysis of PL species from human plasma and determination of candidate OC biomarkers.

12:00 - 12:20 *Title:* **Ovarian cancer - place of tumor markers in clinical practice**

Dr. Aljosa Mandic,
Oncology Institute of Vojvodina, Medical Faculty,
University of Novi Sad, Serbia

Abstract of lecture

The ovarian cancer is the sixth major cause of death, with an incidence of 3.4 per 100 000 females. 68.6% of patients are younger than 60, with a loss of up to 19.7 years of life per person. According to Cancer register of Vojvodina ovarian cancer is the third most diagnosed female cancer in Vojvodina, with incidence rate 11,2/100.000 and mortality rate 5,9/100.000. About 1 in every 83 women in Vojvodina have ovarian cancer in a lifetime.The sensitivity and specificity of diagnostic tools used in early ovarian cancer are very different from study to study. The biomarkers used in ovarian cancer are for diagnosis and/ or monitoring disease progress. CA 125 and HE4 are the most used in clinical practice nowdays.

CA-125. (Mucin 16); CA-125 is a high molecular weight protein, and most commonly used biomarker for ovarian cancers. CA-125 is produced by coelomic epithelium which includes mesothelial cells and Mullerian tissues. This marker is elevated in 80% of epithelial ovarian cancer. The inadequacy of CA-125 alone as a tumor marker for screening is related to the high rate of false

positive values with a low sensitivity of 62% for patients with early stage disease and 90% for patients with advanced stage ovarian cancer, and a specificity of 94% –98.5%. CA125 may be elevated in many benign processes, including endometriosis, pelvic inflammatory disease, pregnancy, and diverticulitis.

HE4 (human epididymis protein 4) is a low molecular weight glycoprotein whose exact function has not been characterized. Its expression in cortical ovarian cysts suggests that formation of Mullerian epithelium is a prerequisite step in development of some types of epithelial ovarian cancer. It is overexpressed in 93% of serous, 100% of endometrioid, and 50% of clear cell (not mucinous) ovarian carcinomas. Several studies have reported that HE4 is a better marker compared with CA125 for diagnosing ovarian cancer. Higher sensitivity of HE4 compared with CA125 specifically in premenopausal women results in better detection of early-stage ovarian malignancies and borderline tumors. In a 2012 study of 1218 patients including 252 with ovarian cancer and 79 borderline ovarian tumors, specificity was 62.2% for CA125 and 63.4% for HE4 with a sensitivity of 94.4%.

Nowadays, these tumors markers are used in combinations with other diagnostic tools such as combinations of CA125, menopausal status and ultrasonography, the risk malignant index (RMI), combination of CA 125 and HE4 called risk of ovarian malignancy algorithm (ROMA), and combines imaging, CA125, and four other biomarkers, prealbumin, apolipoprotein A-1 , β2-microglobulin, and transferrin (OVA1).

The use of biomarkers to triage adnexal masses continues to be an active area of research and recently osteopontin, mesothelin, lysophosphatic acid, haptoglobin, and transthyretin demonstrated some potential.

12:20 - 13:00 **Sandwich lunch and consultation**