Session 11: New trends in medicine – diagnostics and therapy

Lectures

L.11.1

The role of molecular diagnostics for targeted therapy in oncology

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Recent advances in the understanding of cancer biology have a strong impact on cancer detection, diagnosis and tailored targeted therapy, and fast development of new targeted molecular therapies Genetic/molecular diagnostics allows to identify the characteristics of genetic profile of patients or cancer cells to choose tailored treatment. Personalized selection of anticancer drugs based on the presence of applicable mutations has now become an integral part of cancer therapy. This has contributed to the fact that previously incurable cancers have become a chronic disease.

Targeted molecular therapy is usually based on knowledge of genetic alteration in cancer cells to use specific molecules to block those mechanisms for the development of cancer. Genetic tests are based on the evaluation of DNA or RNA sequences as well as on the evaluation of MSI or TMB. Depending on the molecular target, various molecular techniques are used, from the study of single changes in genes, through the evaluation of gene fusions, to the evaluation of several hundred gene panels or the complete genome.

There is also another important issue related to genetic testing, there are many questions regarding the choice of the method, quality standards that diagnostic laboratories should meet, and financing of these tests.

L.11.2

HIV transmission networks and variability after the onset of War in Ukraine

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Ukraine currently encounters one of the most significant HIV-1 epidemics in Europe, marked by an incidence rate of 37.5 cases per 100,000 individuals – fifteen times higher than the rates observed in Western European countries. Almost every region within the Former Soviet Union (FSU) is indicated by the dominance of the HIV-1 A6 lineage. Subtype B remains the prevailing variant across Western and Central Europe. Nevertheless, in recent years many countries have experienced an increase in the prevalence of subtype A6, including Poland and Germany. The ongoing conflict in eastern Ukraine could potentially reignite the dissemination of HIV-1 across Europe, leading to substantial shifts in infection patterns across the regions. Molecular sequences are increasingly being employed to investigate infectious disease transmission dynamics. Pathogens undergo genetic changes during transmission, leaving a genetic imprint of past transmission chains within their genomes. Through statistical analysis of these mutation patterns using phylogenetic models, we have identified connections between infections in both time and space, offering insights into the spatiotemporal spread among individuals and at-risk groups. The HIV-1 A6 lineage exhibits significant circulation dynamics in Poland. Migration from Ukraine has contributed to expanding the A6 variant in Poland. Continued surveillance and targeted prevention efforts are crucial for controlling the spread of HIV-1 A6.

L.11.3

Triglyceride-rich lipoproteins in atherogenesis – clinical implications

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Triglyceride-rich lipoproteins (TRL) are a group of particles at various stages of their metabolism, including newly secreted chylomicrons and very low density lipoproteins (VLDL) and their end products – remnants, in part belonging to intermediate density lipoproteins (IDL). TRL remnant particles, smaller than their precursors, depleted in TG and enriched in cholesterol and apoE, penetrate the arterial wall, accumulate in the subendothelial space and are susceptible to uptake by macrophages, forming foam cells. Free fatty acids released from accumulated remnants induce inflammatory response, also contributing to atherogenesis. Additionally, VLDL accumulation leads to accelerated lipid exchange between VLDL and HDL, causing pro-atherogenic changes in HDL and LDL particles.
Epidemiological studies have shown an association of remnant levels with cardiovascular events. TRL accumulation as a cardiovascular risk factor requires detection and treatment. High levels of atherogenic TRL in plasma are reflected by mixed hyperlipidemia, but a direct measure of TLR content is serum remnant cholesterol (Remn-C) concentration calculated as: Remn-C = total cholesterol – HDL-cholesterol – LDL-cholesterol. There are also analytical procedures for measuring Remn-C. The goal of treatment to reduce TRL accumulation is the level of triglycerides. Beyond the commonly used fibrates, new classes of drugs are being evaluated, including TRL production suppressors and lipoprotein lipase inhibitors.

Oral presentations

O.11.1

Roche – digital PCR – take the leap from research to producing clinically viable assays

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The unique characteristics of digital PCR give it significant potential to impact clinical research in oncology, infectious diseases, transplant and other areas. Recent and significant improvements in dPCR systems are leading labs to push the boundaries of clinical research. The Roche Digital LightCycler® System, is the digital PCR instrument of tomorrow. With a unique combination of 3 nanowell plate configurations, 6 advanced optical channels, and 5x concentrated DNA and RNA master mixes, it has the potential to help your lab to make the leap from publishing research to producing clinically viable assays. System that combines sensitivity, precision, flexibility, and integration in one powerful clinical research tool. It was designed to help laboratories push forward the boundaries of clinical research and has the potential to advance global medical knowledge.

O.11.2

Metagenomic approach in microbiomes analysis

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Development of next generation sequencing (NGS), have revolutionised the study and our understanding of microorganisms living in a different habitats, allowing for the comprehensive characterization of microbial communities without the need for cultivation. Using metagenomic approach we may analyse microbial composition and functional potential based on genomic data. Biotechnological companies offer ready-to-implement solutions to isolate total DNA from various samples, prepare libraries for NGS and sequence using different approaches that suit best to the project assumptions. This shortens the time required to perform the metagenomic analysis and leads to reduction in the costs. For that reason the interest in metagenomics arises rapidly in parallel with published data. However, the amount of data is growing disproportionately to the content of databases with known microorganisms, generating many unknowns. Moreover, the analysis of NGS data requires a diverse array of bioinformatics approaches to process, analyse and interpret the vast amounts of sequences. Here we utilized commercially available kits and mock community standards to prepare NGS libraries and sequence them using Illumina system, followed by computational analysis of the NGS data via different approaches for microbiome analysis. Our results highlighted the need to use optimised methods with certified standards that give the most reliable results to compare with published data and decrease the number of errors.

O.11.3

Correction of symptoms of Huntington disease in the mouse R6/1 model by genistein through the FOXO3-mediated autophagy stimulation

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Huntington disease (HD) is a neurodegenerative disorder caused by a mutation in the *HTT* gene. The expansion of CAG triplets leads to appearance of a misfolded huntingtin forming aggregates and leading to impairment of neuronal functions. In this communication, we demonstrate that stimulation of autophagy by genistein (4',5,7-trihydroxyisoflavone or 5,7-dihydroxy-3-(4-hydroxyphenyl)-4H-1-benzopyran-4-one) caused a reduction of levels of mutated huntingtin in brains of HD mice and correction of their behavior as assessed in a battery of cognitive, anxious and motoric tests, even if the compound was administered after development of symptoms in animals. Biochemical and immunological parameters were also improved in HD mice. Studies on molecular mechanisms of genistein-mediated stimulation of autophagy in HD cells indicated the involvement of the FOXO3-related pathway. In conclusion, treatment with genistein stimulates the autophagy process in brains of HD mice leading to correction of symptoms of HD which suggests that it might be considered as a potential drug for this disease. Together with a very recently published report, indicating that impaired autophagy may be a major cause of neurodegenerative changes, these results might indicate a way for development of effective therapeutic approaches for different neurodegenerative diseases by testing compounds (or possibly combinations of compounds) capable of stimulating autophagy and/or unblocking this process.

Posters

P.11.1

The role of heat shock protein 90 in atopic dermatitis

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Atopic dermatitis (AD) is one of the most common chronic non-infectious inflammatory skin diseases characterized by intense itching and recurrent skin lesions. While the primary events and key drivers of AD are topics of ongoing debate, cutaneous inflammation due to inappropriate IgE (auto)antibody-related immune reactions is frequently considered. Heat shock proteins (Hsps), including Hsp90 and Hsp70, are intra- and extracellular molecular chaperones implicated in cellular homeostasis and immune processes and are induced by cell stress such as inflammation. We aimed to investigate the role of extracellular Hsp90 and Hsp70 in patients with AD. Here, serum samples derived from AD patients and age- and gender-matched healthy controls were screened for presence of circulating Hsp90 or Hsp70, as well as anti-Hsp90 or -Hsp70 IgE by enzyme-linked immunosorbent assays. We found that serum levels of Hsp90 were significantly elevated in AD patients when compared to healthy controls and positively correlated with the disease's activity. In addition, circulating levels of anti-Hsp90 IgE autoantibodies were significantly elevated in AD patients when compared to healthy controls. In contrast, levels of Hsp70 or anti-Hsp70 IgE were similar between both groups. Our results suggest that extracellular Hsp90 or anti-Hsp90 IgE autoantibodies deserve attention in the study of the mechanisms that promote the development or maintenance of AD, as well as provide potential novel disease biomarkers.

P.11.2

L-asparaginases in cancer cell proliferation and apoptosis

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L-Asparaginases are amidohydrolytic enzymes used in the treatment of acute lymphoblastic leukemia. Recombinant proteins are generally produced in *Escherichia coli*, although their use may introduce the risk of bacterial endotoxin contamination (LPS), which can stimulate pyrogenic responses. This research aimed to obtain specific endotoxin-free L-asparaginases and evaluate their *in vitro* anticancer potential through cell proliferation and apoptosis assays. Commercially available cell lines (MOLT-4, THP-1, HL-60, Raji) were utilized to investigate the anticancer properties of selected enzymes. Prior to their application in cell cultures, different enzyme preparations underwent assessment of thermal stability and L-asparaginase activity in cell growth media. The proliferation assay showed a growth inhibition effect of the EcAIII protein on the MOLT-4 cell line. Apoptosis assays were also conducted using flow cytometry and annexin V /7-AAD staining, which revealed the ability of the tested L-asparaginases to induce apoptosis in the examined human cancer cells. These findings indicate the potential utilization of this class of L-asparaginases as future biopharmaceuticals.

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P.11.3

Cellular degradome in the multiple myeloma biology research and new drug design

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Multiple myeloma (MM) is a hematologic neoplasm arising from plasma cells that begin to grow out of control and accumulate in the bone marrow. MM cells outnumber healthy blood-forming cells, cause the destruction of bones and anemia. By producing large amounts of abnormal monoclonal paraprotein (M protein) cause kidney dysfunction. Most MM patients treated with currently used therapies experience relapse resulting from the outgrowth of resistant cell clones. Therefore, an urgent need is to understand MM's biological basis and seek new therapeutic approaches and molecular targets for drug design. The PROTAC strategy enables protein targeting through protein degradation's cellular machinery. It is based on the use of complex compounds consisting of a ligand that binds the protein of interest and a ligand that recruits E3 ligase, both linked and stabilized by a linker. We verified the validity of the use of the PROTAC strategy in MM cells by targeting bromodomain-containing protein 4 (BRD4), which is essential for sustaining the growth and survival of MM cells. The prospective outcome of obtained results directed us toward testing PROTAC compounds that induce degradation of other molecular targets of MM, such as anti-apoptotic proteins apoptosis regulator BCL-2 (BCL-2) and induced myeloid leukemia cell differentiation protein MCL-1 (MCL-1). Further research includes the comparative studies of selected PROTACs with inhibitors of BCL-2 and MCL-1 used to date in MM studies.

P.11.4

Gankyrin protein as a modulator of response to bortezomib in multiple myeloma

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High levels of gankyrin protein, encoded by *PSMD10*, are a particularly unfavorable prognostic factor in multiple myeloma (MM).
The aim of this study is to experimentally validate the effect of gankyrin on cell sensitivity to proteasome inhibitors, using human myeloma cell lines in which we deleted *PSMD10* expression by CRISPR/Cas9 method.
The cytotoxic effect of proteasome inhibitors was evaluated on the single cell-derived clones, both wild-type (wt) and *PSMD10*-KO. Activities of proteasome subunits were evaluated by Cell-Based Proteasome-Glo™ Assays and expression of genes encoding them by RT-PCR. RNA-seq analysis was performed.
We observed statistically significant differences in IC50 values in response to proteasome inhibitor, bortezomib but not to carfilzomib. Cell lines were divided into two groups of response differing in NRAS status. *PSMD10*-KO from one group showed lower IC50 values than wild-type while *PSMD10*-KO from the other group showed the reverse effect. A comparison of results from RNA-seq analysis with patient data showed changes in the serine-glycine biosynthetic pathway. We also observed differences in proteasome activity.
Our results indicate that gankyrin could be a potential modulator of response to bortezomib in NRAS-mutated multiple myeloma.

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P.11.5

Streptavidin-based FGF1 oligomer as HSPGs targeting agent – toward novel drug carrier for treatment of pancreatic cancer

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Heparan sulfate proteoglycans (HSPGs) are cell surface glycoproteins involved in the regulation of cell-to-cell communication and modulation of cell signaling. HSPGs are overexpressed in pancreatic cancer, thus, HSPGs are considered as an attractive molecular target in precision medicine approaches. The natural ligand for HSPGs are extracellular fibroblast growth factors (FGFs) that together with the fibroblast growth factor receptors (FGFRs) transmit signals across the plasma membrane. HSPGs, FGFs and FGFRs form signaling complexes adjusting important cellular processes.
In our previous findings we showed that oligomerization of FGF1 strongly increases affinity for heparans. Here we constructed FGF1-based tetrameric ligand through its uncoupling from FGFRs and re-directing it fully towards HSPGs. As a scaffold for oligomerization of FGF1 we employed engineered streptavidin (SA), which can simultaneously bind four biotinylated ligands.
We assessed the oligomeric state of purified proteins using DLS and SEC calibrated column. Increased affinity for heparans was confirmed by BLI and FPLC elution profile analyses. Using fluorescent microscopy, we assessed the increased efficiency of heparan-dependent endocytosis. We attached a cytotoxic drug to the tetramer and the cytotoxic potential of the conjugate will be assessed on cancer cell lines of pancreatic cancer with overexpression of HSPGs.

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P.11.6

Coiled coil and GFPp-based assemblies of FGF1 – precise targeting of HSPGs and promising drug delivery agents for pancreatic cancer cells

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Heparan sulfate proteoglycans (HSPGs) are cell surface glycoproteins playing crucial role in cell physiology. HSPGs forms ternary signaling complexes with fibroblast growth factors (FGFs) and their receptors (FGFRs) leading to transmission of diverse signals and regulation of essential cellular processes e.g., differentiation, proliferation. Additionally, it has been proved that HSPGs are overexpressed in several cancer types, mainly in pancreatic cancer, making these proteins an interesting and promising molecular target for anti-cancer drugs in precision medicine approaches. Here, we designed and tested multimeric ligand assemblies (MLAs) based on FGF1 protein, natural HSPGs ligand, containing point mutations preventing its binding to the FRFRs, targeting only HSPGs. We used two different oligomerization scaffolds – coiled coil motifs and GFP polygons for controlled oligomerization of FGF1. Firstly, we confirmed the oligomeric state of MLAs using calibrated SEC column and DLS. Next, using BLI and FPLC elution profile analyses we proved that oligomerization increases MLAs affinity for heparans in comparison to its monomeric forms. Finally, fluorescent microscopy showed that oligomerization of FGF1 increased its endocytosis via HSPGs.
For now, we attached cytotoxic drug to designed MLAs for further investigation of its cytotoxicity for different panels of pancreatic cell lines.

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P.11.7

Ramipril may overcome platinum resistance in ovarian cancer – bioinformatic analysis of TCGA datasets

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Introduction: Ovarian cancer causes significant mortality and is diagnosed at advanced stages. Treatment strategy includes surgery and adjuvant chemotherapy of platinum plus taxanes recognized as gold standard. Other available chemotherapeutics are usually used after failure of platinum-based treatment. Due to the acquired resistance to therapy, a new personalized approach therapy is needed and molecular biomarkers may help for it.
Material and Methods: Expression levels of genes identified as being responsive to RAM were analyzed based on the TCGA and included: identification of RAM-responsive genes between platinum-sensitive and resistant, finding positively and negatively correlated genes.
Results: We identified ACE, MT1H and FIGF genes as significantly different expressed between platinum-sensitive and platinum-resistant patients. In the case of ACE and MT1H association with patients' survival time was observed. Moreover, the genes co-expressed with MTIH and ACE and their coupled molecular pathways influencing PT-sensitivity and their are associated with sequestration of platinum, with genes associated with promotion of transcription, with base excision repair process, TP53 regulation of transcription of DNA Repair Genes, ALK signaling in cancer, with BCL2L11 (BIM) transcription and YAP1-mediated transcription.
Conclusions: ACE, MT1H and FIGF genes are potential candidates as biomarkers for assessment of platinum-based treatment in ovarian cancer.

P.11.8

Serum metabolites as a potential biomarkers in children with type 1 diabetes

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Recent studies suggest connection between specific metabolic profile and islet autoimmunity. Early metabolic changes are attributed to many biochemical pathways which may have important implications for the understanding of type 1 diabetes (T1D) pathophysiology and early disease detection and prevention. The aim of our study was a pilot assessment of the metabolic profile of children with T1D.
Study included 30 patients with T1D and 11 healthy children as a control group. Metabolic profile (188 analytes) was analyzed with the AbsoluteIDQ® p180 kit (Biocrates Life Sciences) using LC-MS method.
Seven of amino acids were decreased in T1D serum samples. 5 biogenic amines were statistically different ($p<0.05$) between T1D and control samples. 36 significant changes ($p<0.05$) in glycerophospholipids were mainly phosphatidylcholines. 15 metabolites showed good discriminatory power in ROC analyzes. The highest diagnostic power to discriminate T1D patients was shown for acylcarnitine C18:1 and phosphatidylcholine PC aa 42:5, with AUC: 0.923 and 0.915, respectively.
Certainly, metabolites showing altered concentrations can be suggested as a potential serum biomarker for the diagnosis of T1D, but confirmation of the results in a larger cohort is required. Considering the changes in the phosphatidylcholines group, one can speculate about the relationship between barrier damage and the development of type 1 diabetes.

P.11.9

RNA methylation signatures as potential regulators of HNSC pathogenesis

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The diversity of head and neck cancer, its molecular pathogenesis, and progression are driven not only by the accumulation of genetic alterations but also by the epigenetic landscape: histone modifications, non-coding RNA activity, DNA, and poorly described RNA methylation. Since most epigenetic alterations promote cancer formation and progression, they can be used as biomarkers for the clinical outcome and will reveal new therapeutic opportunities for cancer patients. Here, we have focused on signs of RNA methylation in 45 HNSC patients (tumor and normal tissues) and we determined the methyltransferase 3 (METTL3) knock-out impact on HNSC cells *in vitro*.
LC-ESI-MS/MS analysis was used to calculate the percentage ratio of m^6A to A in total RNA samples. We have determined the differences in mRNA level of selected RNA methylation machinery genes in HNSC patients' cancerous tissues compared to normal tissues. We have correlated these results with patients' clinical data and suggest probable essential factors in HNSC tumorigenesis. Moreover, we found that METTL3 knock-out (using the CRISPR-Cas9 system) slowed down divisions and induced apoptosis of cancer cells, supporting cell arrest in the G2 phase, increased apoptosis activity in response to cisplatin treatment, and increased the stemness markers expression.
Thus, we speculate that the RNA methylation process may significantly impact HNSC progression