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Czech Annual Cancer Research Meeting

December 1–2 2022 | Olomouc, Czech Republic

17th Czech Annual Cancer Research Meeting
former Diagnostic, Predictive and Experimental Oncology Days

17th Molecular Pathology Days

105th Olomouc histopathology seminar

ABSTRACT BOOK

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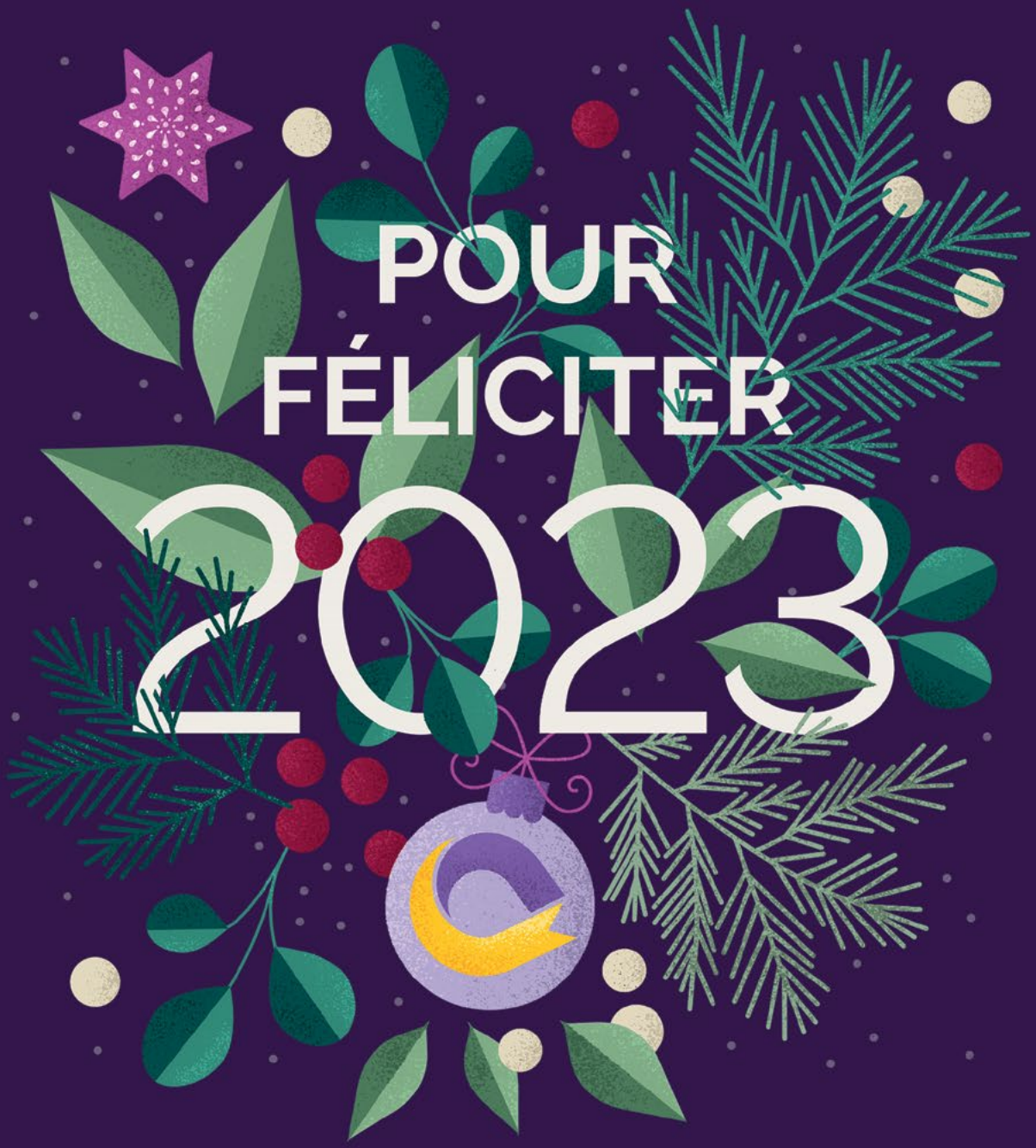

We wish you a Merry Christmas and a lot of personal and professional success in the new year 2023

CANCER RESEARCH
CZECH REPUBLIC

www.vyzkumrakoviny.cz

Czech Annual Cancer Research Meeting
December 1-2 2022 | Olomouc, CZ

Organizer

MedChemBio – cluster
Šlechtitelů 813/21
783 71 Olomouc-Holice

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Cluster of Medicinal Chemistry and Chemical Biology

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DEDICATION OF THE PROJECT

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PROGRAM

ČTVRTEK / THURSDAY 1. PROSINCE / 1ST DECEMBER, 2022

7:00 — 9:00 **REGISTRATION**

Cancer biomarkers and molecular targets / 9:00 – 10:30

Chairs: Petr Dzubak, Radoslav Goldman

9:00 – 9:30	Heparan 6-O-endosulfatases in HNSCC and other malignancies **Radoslav Goldman**
9:30 – 9:45	Characterization of cervical mucus: Definition of healthy proteome **Tomas Ozdian**
9:45 – 10:00	Proteomic biomarkers of lung cancer and COPD in exhaled breath condensate **Jana Vaclavkova**
10:00 – 10:15	Unlocking critical insights in immunotherapy with predictive single-cell functional proteomics **Dagmar Bezdekova**
10:15 – 10:30	Increase of cervical cancer prevention in the Czech Republic using self-sampling **Vladimira Koudelakova**

10:30 — 10:45 **COFFEE BREAK**

Anticancer drugs and therapies / 10:45 – 12:00

Chairs: Martin Mistrik, Lucia Kucerova

10:45 - 11:15	Novel approaches for circumventing cisplatin chemoresistance in human germ cell tumors **Lucia Kucerova**
11:15 - 11:30	Polymer therapeutics for photodynamic therapy and tumor imaging **Tomas Etrych**
11:30 - 11:45	Hyperthermia in cancer research and treatment **Zdenek Skrott**
11:45 - 12:00	The effect of morphine-based perioperative analgesia on circulating tumor cells dissemination in colorectal cancer patients **Pavel Stejskal**

12:00 — 13:00 **LUNCH / POSTER SECTION**

13:00 — 13:15 **OPENING CEREMONY**

Cancer immunotherapy / 13:15 – 14:45

Chairs: Marian Hajduch, Marek Kovar

13:15 – 13:45	Cancer immunotherapy through combination of immune checkpoint blockade and IL-2/anti-IL-2 mAb complexes selectively stimulating CD25+ T cells **Marek Kovar**
13:45 – 14:00	Chemical biology and cancer immunotherapy **Tereza Ormsby**
14:00 – 14:15	Forty (40) Color full spectrum flow cytometry and sorting panel for deep immunophenotyping of major cell subsets in human peripheral blood **Riccardo Pasculli**

ČTVRTEK / THURSDAY 1. PROSINCE / 1ST DECEMBER, 2022

14:15 – 14:30	Cytokine-antibody single-chain fusions for cancer immunotherapy **Jakub Tomala**
14:30 – 14:45	Molecular mechanisms in spontaneous regression of melanoma in pig model: analysis of cytokines **Helena Kupcova Skalnikova**
14:45 — 15:00	**COFFEE BREAK**
15:15 — 17:30	**NATIONAL INSTITUTE FOR CANCER RESEARCH FACULTY MEETING** Invited delegates only / Ventana Lounge

Cancer biomarkers / 15:00 – 16:00

Chairs: Ludmila Boublikova, Patrik Flodr

15:00 – 15:15	*In vitro* and *in vivo* characterization of miR-215-5p as a new tumor suppressor in colorectal cancer **Tana Machackova**
15:15 – 15:30	Molecular markers for disease monitoring and response prediction in solid tumors **Ludmila Boublikova**
15:30 – 15:45	Analysis of the expression of ALK/ROS1 proteins vz. rearrangement of ALK/ROS1 genes - comparison of methodological approaches **Anna Farkašová**
15:45 – 16:00	Systemic and localised amyloidosis associated with malignant neoplastic, uncertain neoplastic and non-neoplastic diseases **Patrik Flodr**
16:00 — 16:15	**COFFEE BREAK**

Molecular pathology / 16:15 – 17:45

Chairs: Jan Bouchal, Jozef Skarda

16:15 – 16:30	Restored biosynthetic pathways induced by MSCs serve as rescue mechanism in leukemia cells after L-asparaginase therapy **Julia Starkova**
16:30 – 16:45	Advances in plasmonic biosensors and their applications in oncohematology **Marketa Bockova**
16:45 – 17:00	Ultra high content imaging using MICS technology on the MACSima™ Imaging Platform **Bernd Müller-Zülow**
17:00 – 17:15	Immunohistochemical, histomorphological and electron microscopic evaluation of tissues around the implant material **Jozef Skarda**
17:15 – 17:30	Human-specific levels of endoplasmic reticulum stress markers in the pathological progression of Lewy body disease – a postmortem study **Dominik Hrabos**
17:30 – 17:45	Whats new in immunotherapy of malignant pleural mezotelioma from the perspective of a pathologist **Jozef Skarda**
19:00	**DINNER AND SOCIAL EVENING**

PROGRAM

PÁTEK / FRIDAY 2. PROSINCE 2022 / 2ND DECEMBER, 2022

Innovative cancer therapeutics / 9:00 – 10:45

Chairs: Marian Hajduch, Michal Hocek

9:00 – 9:30	Modified nucleosides, nucleotides and nucleic acids as potential cytostatics **Michal Hocek**
9:30 – 9:45	Novel insights into molecular mechanism of the anti-cancer drug emetine on DNA replication **David Lukac**
9:45 – 10:00	Beyond cancer: Repurposing of targeted therapeutics as an approach to advance clinical management of vascular anomalies **Petra Pokorna**
10:00 – 10:15	Targeting mitochondrial iron metabolism: selective killing of cancer cells via mitochondrially targeted deferasirox **Jaroslav Truksa**
10:15 – 10:30	Water-soluble polymer carriers for tumor treatment **Lenka Kotrchova**
10:30 – 10:45	Structure-assisted design of enzyme inhibitors **Pavlina Rezacova**
10:45 — 11:00	**COFFEE BREAK**

Prostate and ovarian cancers diagnostic and therapy / 11:00 – 12:15

Chairs: Jan Bouchal, Fred Santer

11:00 - 11:30	How to diagnose and induce HRD in prostate and ovarian cancer **Fred Santer**
11:30 - 11:45	The hidden potential of malignant ascites for ovarian cancer research **Vendula Hlavackova Pospichalova**
11:45 - 12:00	Ligands targeting prostate-specific membrane antigen (PSMA) for prostate cancer imaging and therapy **Cyril Barinka**
12:00 - 12:15	*In vivo* evaluation of enhanced blood retention and tumor uptake PSMA-targeting ^{225}Ac-labeled radioconjugates **Zbynek Novy**
12:15 — 13:15	**LUNCH / POSTER SECTION**

Inflammation and immunity in cancers / 13:15 – 14:30

Chairs: Karel Smetana, Zdenek Kolar

13:15 – 13:45	Cancer-associated fibroblasts **Karel Smetana, Jr.**
13:45 – 14:15	Smoldering inflammation and inflammatory threshold in tissue remodeling and cancer niche development **Luca Vannucci**
14:15 – 14:30	Mesenchymal cells associated with glioblastomas and brain metastases: Characterization and relationship to immune infiltrate **Magdalena Houdova Megova**
14:30 — 14:45	**COFFEE BREAK**

PÁTEK / FRIDAY 2. PROSINCE 2022 / 2ND DECEMBER, 2022

Lipid metabolism in cancer and non-communicable disorders 14:45 – 16:00

Chairs: Jan Bouchal, Juan Bautista de Sanctis

14:45 – 15:15	Perturbations of the sphingolipid and glycosphingolipid metabolism in colon cancer tissue **Miroslav Machala**
15:15 – 15:30	Clinical lipidomics: Applications in cancer and beyond **Ales Kvasnicka**
15:30 – 15:45	Molecular profiling of cardiovascular diseases applying an untargeted lipidomics approach **Lukas Najdekr**
15:45 – 16:00	Comparative effects of fatty acid supplementation on human NK cell cytotoxic activity of healthy and overweight young and old adults **Juan Bautista De Sanctis**
16:00 — 16:15	**COFFEE BREAK**

Personalized medicine and data analysis / 16:15 – 17:45

Chairs: Ondrej Slaby, Jozef Skarda

16:15 – 16:45	Impact of the comprehensive genomic profiling on the individual therapeutic planning in high-risk/refractory tumors: real-world precision medicine in pediatric oncology **Ondrej Slaby**
16:45 – 17:00	Automated NGS and its strong positive effect on an oncology patient survival rate and improvement on the healthcare service **Filip Drzik**
17:00 – 17:15	Applications and challenges of artificial intelligence in digital pathology **Mariam Gachechiladze**
17:15 – 17:30	The future of pathology **Agnieszka Ciesielska**
17:30 – 17:45	The drugs' mechanism of action identification based on digital-phase contrast images analyzed by AI **Jarmila Stankova**

17:45 — 18:00 **CLOSING CEREMONY**

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Ústav klinické a molekulární patologie

Čtvrtek / Thursday — 1. prosince 2022 / 1st December, 2022

Chairs: Petr Džubák, Radoslav Goldman

Heparan 6-O-endosulfatases in HNSCC and other malignancies

Radoslav Goldman

Georgetown University, Washington D.C., USA

Post synthetic editing of heparan 6-O-sulfation by the human endosulfatases SULF1 and SULF2 regulates extracellular matrix remodeling, growth factor signaling, or chemokine distribution. These pathways modulate outcomes of cancer diseases and patient responses to therapy. In this study, we carried out a translational study of multiple cancer diseases which confirms the impact of the 6-O-endosulfatases on the progression of head and neck squamous cell carcinoma (HNSCC). A pilot RNAscope study shows that SULF1 expression is predictive of oral cancer outcomes. We optimized the expression of the SULF enzymes in mammalian cell lines and began characterization of their structure, activities, and function in tumor biology. We expect that the advances will facilitate the detection and targeting of the enzymes in HNSCC and other malignancies.

Characterization of cervical mucus: Definition of healthy proteome

Tomáš Ožd'an[1], Jan Vodička[2], Jiří Dostál[2], Dušan Holub[1], Jana Václavková[1], Michal Ješeta[3], Pavla Kouřilová[1], Radovan Pilka[2], Petr Džubák[1], Marián Hajdúch[1]

[1]Institute of Molecular and Translational Medicine, Olomouc, Czech Republic
[2]Department of Gynecology and Obstetrics, University Hospital in Olomouc, Olomouc, Czech Republic
[3]Center of Assisted Reproduction CAR 01, Brno, Czech Republic

Cervical mucus is a viscous fluid functioning as a uterine cervix plug. It is formed and produced by cervical glands located in the cervix. During ovulation, cervical mucus becomes less viscous, which is a good window for non-invasive sampling. Our study focuses on the proteomic characterization of cervical mucus, which may thus act as a non-invasively acquired source of biomarkers for diseases and physiological conditions of the female genital tract. Our study aimed at two aims - the first is to optimize a proteomic workflow of cervical mucus processing. The second aim is to assess differences in the proteomic composition of cervical mucus in natural ovulatory cycles and IVF cycles with controlled ovarian hyperstimulation. The sampling was done in cooperation with women undergoing intrauterine insemination in a natural ovulatory cycle and women undergoing controlled ovarian hyperstimulation for IVF. The optimization of proteomic workflow, including an analysis by LC-MS/MS on a high-resolution Orbitrap Exploris 480 mass spectrometer. Data analysis revealed the protein composition and the differences in the cervical mucus between the natural and stimulated uterus.

This work was supported by Czech Health Research Council (NV-18-02-00291), by European Union – Programme EXCELES, ID Project No. LX22NPO5102, the Czech Ministry of Education, Youth and Sports (CZ-OPENSCREEN - LM2018130, EATRIS-CZ - LM2018133), and by the internal grant of Palacky University Olomouc (IGA_LF_2022_033) and European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0.

Proteomic biomarkers of lung cancer and COPD in exhaled breath condensate.

Jana Václavková[1], Petr Džubák[1], Jana Vrbková[1], Pavla Kouřilová[1], Dušan Holub[1], Juraj Kultan[2], Petr Jakubec[2], Ondřej Fisher[2], František Kopřiva[3], Tatiana Gvozdiaková[3], Vendula Látalová[4], Marián Hajdúch[1]

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[2]Department of Respiratory Medicine, University Hospital and Faculty of Medicine and Dentistry, Palacky University, Olomouc, Czech Republic
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Lung cancer has always been associated with a bad prognosis and short survival of patients. However, when diagnosed in the early stages, it could be cured entirely, and the prognosis is much better. Lung cancer preventive screening recently started (2021) in the Czech Republic and is based on selecting the risk groups and screening algorithm with LDCT. However, intensive research is conducted to develop non-invasive methods suitable for more broad preventive screening. We have chosen an exhaled breath condensate (EBC) as a suitable biological matrix. It is collected non-invasively and considered a rich source of biomarkers from the respiratory tract. We have implemented our recently published mass spectrometry-based method, which we improved towards high resolution and reproducible in-depth protein identification.

Exhaled breath condensate was collected using Turbo 14 Turbo DECCS System (Medivac, Italy) from healthy and diseased adult individuals who breathed through a mouthpiece into the collection device for 10 minutes. Proteins in the sample are solubilized, denatured, reduced, trypsin digested, and purified using StageTip technology. Samples are measured by high-resolution mass spectrometry (HPLC-MS/MS-LTQ Orbitrap Elite) in 3 technical

replicates. A powerful protein search strategy was developed in Proteome Discoverer software version 2.5 (Thermo Scientific). Data are further statistically evaluated by Statistica and Bioconductor R – package. For biomarker validation, the SureQuant approach for targeted quantitation will be implemented.

In this work, we focused on non-small cell lung cancer (NSCLC) diagnostics. We have identified 4806 proteins, and of them, 4179 proteins were quantified at least in one replicate across 226 individuals' samples measured in triplicates. Combining univariate and multivariate statistical approaches and sensitivity analysis, we have suggested 72 potential biomarkers that could distinguish NSCLC patients from COPD and healthy individuals. Our models for NSCLC and COPD biomarker prediction worked well, seem promising, and will be further studied and validated. The validation is currently in process. The confirmed biomarkers will be used to develop an MS-independent kit for preventive screening of NSCLC, COPD, and possibly other lung cancer types.

This work was supported by European Union – Programme EXCELES, ID Project No. LX22NPO5102, the Czech Ministry of Education, Youth and Sports (CZ-OPENSCREEN - LM2018130, EATRIS-CZ - LM2018133), and by the internal grant of Palacky University Olomouc (IGA_LF_2022_033) and European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0 /0.0/16_019/0000868).

Unlocking critical insights in immunotherapy with predictive single-cell functional proteomics

Dagmar Bezděková

Accela, Prague, Czech Republic

Immunotherapies such as immune checkpoint inhibitors and cell therapies have made great advancements in fighting cancer, but challenges remain. Because many patients do not derive clinical benefit or long-term response, there is still a pressing need to better characterize the underlying mechanisms driving immune response and cancer resistance. In this presentation, we will show how highly multiplexed single-cell functional proteomics can help to deliver critical insights into biomarker discovery and accelerate the development of advanced, next-generation immunotherapies. Synthesis of fluorinated derivatives 2-phenyl-3-hydroxy-4(1H)-quinolinone and study of their anticancer activity

Increase of cervical cancer prevention in the Czech Republic using self-sampling

Vladimira Koudelakova[1,2], Hana Jaworek[1,2], Ondrej Majek[3,4], Marian Hajduch[1,5]

[1]Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacký University, Olomouc, Czech Republic
[2]Laboratory of Experimental Medicine, University Hospital Olomouc, Olomouc, Czech Republic
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[5]Cancer Research Czech Republic, Olomouc, Czech Republic

Introduction: The cervical cancer screening program is based on annual cytology with HPV triage in the Czech Republic. Screening HPV testing is covered by health insurance for all women aged 35 and 45 from 2021. The major challenge is the involvement of the women from a refractory population who do not attend the cervical cancer screening program for a long time. The objective of the study was to compare the different approaches to inviting women to the cervical cancer screening program.

Methods: The study was conducted in three arms. 6388 women from the database of dietary supplements company were included in the Arm A regardless of whether they participated in the cervical cancer screening program.Women who do not participate in the cervical cancer screening program for at least three years, and mostly had not previously reacted to several rounds of invitation, were selected from a database of a health insurance company (Arm B, 4813 women) and gynecologist database (Arm C, 653 women).EvalynBrush self-sampling devices (Rovers Medical Devices) were sent by Czech post to the women home address.All returned samples were analyzed using the Anyplex II HPV HR Detection kit (Seegene)/QIAscreen HPV PCR (Qiagen).

Results: The return rate was 7.6% (486/6388) in Arm A, 7.6 % (367/4813) in Arm B and 9.0% (59/653) in Arm C. HPV positivity was detected in 7.4% (36/486) of Arm A samples, 17.7% (59/334) of Arm B samples and 10.2% (6/59) of Arm C samples.

Conclusions: The return rate was highest in Arm C where the women were invited through their gynecologists. Based on these results, we are currently expanding Arm C to include additional 4000 invited women. The offering of self-sampling could significantly increase the attendance of Czech women in the cervical screening program.

This work was supported by grants: IGA_LF_2022_012, Programme EXCELES (LX22NPO5103), LM2018133 CZ.02.1.01/0.0/0.0/16_019/0000868 and charity Cancer Research Czech Republic.

Čtvrtek / Thursday — 1. prosince 2022 / 1st December, 2022

Chairs: Martin Mistrik, Lucia Kucerova

Novel approaches for circumventing cisplatin chemoresistance in human germ cell tumors

Lucia Kucerova[1,2], Katarina Kalavska[1,3,2], Silvia Schmidtova[2], Michal Mego[1,4,3]

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[2]Department of Molecular Oncology, Cancer Research Institute, Biomedical Research Center, Bratislava, Slovakia
[3]National Cancer Institute, Bratislava, Slovakia
[4]2nd Department of Oncology, Faculty of Medicine, Comenius University and National Cancer Institute, Bratislava, Slovakia

Germ cell tumors (GCTs) represent highly curable malignancies even in metastatic stage. Nonetheless, some patients do not have a durable complete remission with initial cisplatin (CDDP)-based chemotherapy. We established several in vitro and in vivo models for GCTs research including several clones of CDDP-resistant GCTs. We identified markers and potential therapeutic targets fibrilin-1, PD-L1 protein, PARP protein, DNA repair proteins, aldehyde dehydrogenase (ALDH), microRNA - mir-371a-3p, carbonic anhydrase IX and beta catenin. Even though, the PARP expression was confirmed in GCT cell lines and xenografts, veliparib failed to increase the CDDP cytotoxicity. Inhibition of aldehyde dehydrogenase using disulfiram resulted in reversal of cisplatin resistance in embryonic carcinoma cell line and led to initiation of the phase II clinical trial. Similarly, ALDH3 A1 inhibition by napabucasin overcame cisplatin resistance in ovarian germ cell tumor-derived cell line by inhibiting cancer stemness. We will discuss novel strategies of combinatorial treatment(s) that could circumvent CDDP resistance or target refractory germ cell tumors.

Grant support: VEGA 2/0124/21, APVV-20-0158, VEGA 1/0349/21, APVV-19-0411

Polymer therapeutics for photodynamic therapy and tumor imaging

Tomáš Etrych

Institute of macromolecular chemistry CAS, Prague, Czech Republic

In the past several decades, nanosized drug delivery systems with various targeting functions and controlled drug release capabilities inside targeted tissues or cells have been intensively studied. Understanding their pharmacokinetic properties is crucial for the successful transition of this research into clinical practice. Recently, photodynamic therapy (PDT) has arised as a smart approach which employs the light irradiation to activate photosensitizers in situ after the selective tumor accumulation. Here, the exogenous light stimulus induces the formation of cytotoxic oxygen free radicals, i.e., singlet oxygen species (1O2), resulting in the cell death.

In this work, polymer platform suitable for efficient stimuli-sensitive therapeutics, diagnostics and even theranostics based on water-soluble and amphiphilic polymer conjugates is presented. Synthetic nanocarriers based on methacrylamide-based copolymers are highly attractive for in vivo application as they are fully biocompatible, water soluble and non-toxic biomaterials with tailored physico-chemical properties for application in medicine. Their favorable pharmacokinetics altogether with Enhanced Permeability and Retention effect-driven tumor accumulation enable a higher uptake in solid tumors with an enhanced therapeutic outcome. The set of polymer biomaterials differing in their inner structure, molecular weight and functionality was designed, synthesized and evaluated for their therapeutic, diagnostic and even theranostic properties. The presented work will comprise the synthetic strategy of polymer materials and their utilization as the carrier of the therapeutically or/and diagnostically active molecules. Within this aproach selected photosensitizers were used for the development of tumor-targeted theranostics for both efficient tumor imaging and PDT. Their physico-chemical, in vitro, and in vivo behavior were investigated and the results indicate that the attachment of the hydrophobic photosensitizer molecule results in the formation of micelles, which protects the active molecules during its transport. The cytotoxicity of developed polymer nanomedicines was remarkably increased when light was irradiated and they showed high tumor targeted accumulation based on the EPR effect, therefore these polymer systems are promising candidates for tumor diagnostics and treatment.

Hyperthermia in cancer research and treatment

Zdenek Skrott, Martin Mistrik

Institute of Molecular and Translational Medicine, Palacky University, Olomouc, Czech Republic

Due to malignant transformation, cancer cells are characterised by elevated levels of genotoxic, replication or proteotoxic stresses, which makes them vulnerable to various external conditions, including increased temperature. Thus, hyperthermia represents a very effective therapeutic modality usually combined with standard chemotherapy or radiation. Moreover, the elevated temperature is often used in experimental studies related to protein aggregation, cellular heat shock response and other proteostasis mechanisms relevant to cancer progression and treatment. Recently, we developed a single-cell method to inflict defined, subcellular thermal damage, adopting the plasmon resonance principle. Dose-

defined heat causes protein damage in subcellular compartments, rapid heat-shock chaperone recruitment, and ensuing engagement of the ubiquitin-proteasome system, providing unprecedented insights into spatiotemporal response to protein damage relevant to cancer. Using this versatile method, we discovered so-far unsuspected compensatory interplay of p97 translocase and HSP70 chaperone in the processing of heat-damaged proteins. As both, p97 and HPS70, represent potential cancer-relevant drug targets, these results can contribute to the development and better implementation of related therapies in cancer treatment.

The study was supported by a grant from the Ministry of School, Education, Youth and Sports of the Czech Republic: LM2018129 (Czech-BioImaging) and an Internal grant of the Palacky University (IGA_LF_2022_038).

The effect of morphine-based perioperative analgesia on circulating tumor cells dissemination in colorectal cancer patients

Pavel Stejskal[1], Josef Srovnal[1], Emil Berta[1,2], Alona Rehulkova[1], Lubomir Vecera[3], Tomas Gabrhelik[3], Filip Haiduk[4], Jan Maca[4], Jan Bruthans[5], Pavel Michalek[5], Pavla Kourilova[1], Marian Hajduch[1]

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[2]Ringerike Hospital, Honefoss, Honefoss, Norway
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[4]University Hospital Ostrava, Ostrava, Czech Republic
[5]General University Hospital, Prague, Prague, Czech Republic

Purpose: Circulating tumor cells (CTCs) are primary or metastatic tumor cells shed into the bloodstream and are considered precursors of distant metastatic spread and can act as an independent prognostic and predictive biomarker. Colorectal cancer (CRC) is the leading cause of cancer-related deaths worldwide and metastasis is the major cause of death. Previous studies showed poorer survival in CRC patients treated perioperatively with morphine in comparison to piritramide.

Patients and methods: In total, 150 CRC stage I-III patients undergoing either radical or laparoscopic surgery were enrolled in this prospective multicentric randomized study (NCT03700411). Patients were randomized into three arms using different perioperative analgesia – morphine, piritramide, and epidural analgesia. Three peripheral blood samples were collected preoperatively, one day and one month after surgery respectively. To preserve the cellular content of specimens, Cell-Free DNA BCT® (Streck, Inc.) blood collection tubes were used. The CTCs were identified using CytoTrack CT11TM (2/C, Denmark), a semi-automated immunofluorescence microscopy detecting the pan-cytokeratin and EpCAM signals. The method recovery rate was analyzed using SW-480 cancer cell line by flow cytometry.

Results: The CTCs recovery rate of the method was 72% and the method was certified by ISO 15189. The analyses of CTC presence revealed the highest positivity rate in the samples collected from patients treated with morphine on the first day after surgery (51.4 %). Also, a significant increase in positivity (from 23.5 % for the perioperatively collected samples) compared to samples from patients treated with epidural analgesia (31.1 % to 33.3 %) and piritramide (44.2% to 41.0%) was observed. The CTC presence was lower in control samples collected after one month irrespective of the analgesia type. The highest positivity among the control samples was in those associated with morphine analgesia (32.1 % versus 25.7% for epidural and 25.0% for piritramide analgesia).

Conclusion: A significant increase in CTC levels was found in postoperative blood samples of CRC patients treated with morphine compared to piritramide and epidural analgesia. Enrollment in the study is ongoing.

Čtvrtek / Thursday — 1. prosince 2022 / 1st December, 2022

Chairs: Marian Hajduch, Marek Kovar

Cancer immunotherapy through combination of immune checkpoint blockade and IL-2/anti-IL-2 mAb complexes selectively stimulating CD25+ T cells

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It has been shown previously that an elegant way how to dramatically increase in vivo biological activity of IL-2 is to use complexes of IL-2 and anti-IL-2 mAb (IL-2co)1. Moreover, it was demonstrated that IL-2co possess selective stimulatory activity for different subsets of IL-2 responsive cells depending on the clone of anti-IL-2 mAb used. Complexes of mouse IL-2 and anti-IL-2 mAb S4B6 (IL-2/S4B6) were found to be highly stimulatory for CD122high populations (memory CD8+ T and NK cells), while only moderate stimulatory activity for Treg cells was found. Conversely, complexes of IL-2 and anti-IL-2 mAb JES6-1A12 (IL-2/JES6) induced highly selective and potent expansion of CD25+ cell population (Treg cells)2. Importantly, administration of the same amount of IL-2 had negligible effect when compared to IL-2 complexes, confirming the enhancing effect of anti-IL-2 mAb on biological activity of IL-2 in vivo. Thus, it has been considered for more than a decade that IL-2/S4B6 are suitable for tumor immunotherapy while IL-2/JES6 are convenient for treatment of autoimmune diseases or to facilitate long-term allograft acceptance3-5.

We show here that IL-2/JES6 surprisingly possess anticancer activity per se and particularly in combination with blockage of CTLA-4 and PD-1 inhibitory molecules on T cells via respective mAbs (ICIs henceforth). However, the timing of ICIs and IL-2/JES6 administration is crucial since IL-2/JES6 administered before ICIs do not improve the antitumor activity of ICIs, however, IL-2/JES6 given after ICIs significantly potentiate it. The antitumor activity of IL-2/JES6 is highly counterintuitive due to the concomitant expansion of Treg cells. Nevertheless, we have an experimental evidence that Treg cell potential to inhibit CD8+ T cells, a crucial cell subset in tumor immunotherapy, is highly limited when strong sustained IL-2 signal is provided via IL-2 complexes.

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Chemical biology and cancer immunotherapy

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Our interdisciplinary team aims to develops new tools for the study, diagnosis and potentially treatment of cancer. We combine the expertise of medicinal chemistry with biochemistry, molecular biology and immunology to develop novel ligands targeting relevant receptors including cancer markers (PSMA, FAP) or receptors that are involved in cancer progression (PD-L1, CD73, CD64). We utilize not only the classical approach of structure-activity relationship studies but also mRNA and phage display that can be used for non-enzymatic targets. To test large number of compounds, we have established a very sensitive, high-throughput assay called DIANA. This method can be used as a diagnostic tool for detection of a particular target in blood serum as well. The best small molecules are used to generate probes for diagnostic and visualization of tumors. Selected small-molecule ligands will be also attached to the HPMA polymer to create synthetic antibody mimetics called iBodies. Previously, we have shown that iBodies can be used in all applications like monoclonal antibodies. Apart from antibodies, iBodies are versatile, easy to prepare and to modify with several types of ligands including fluorophore or a cytotoxic cargo. By attachment of multiple moieties, we are able to achieve the avidity effect that typically improves the activity of small molecules. We want to use iBodies to block specific cellular interactions that are advantageous for cancer growth, eliminate specific cellular populations or bring together an immune cells and cancer cell to trigger anti-tumor activity.

Forty (40) color full spectrum flow cytometry and sorting panel for deep immunophenotyping of major cell subsets in human peripheral blood

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Until recently, developing fluorescence-based flow cytometry as-

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says with 40 colors has been merely aspirational, with many turning to competing technologies for high-parameter applications. No longer. With 64 fluorescence detectors and only 5 lasers, the Cytek Aurora now has the capability to resolve up to 40 colors in combination. Cytek has developed a 40-color human immunophenotyping panel acquired from a single tube, with outstanding resolution. This 40-color flow cytometry-based panel was developed for in-depth immunophenotyping of the major cell subsets present in human peripheral blood. Sample availability can often be limited, especially in cases of clinical trial material, when multiple types of testing are required from a single sample or timepoint. Maximizing the amount of information that can be obtained from a single sample not only provides more in-depth characterization of the immune system but also serves to address the issue of limited sample availability. The panel presented here identifies CD4 T cells, CD8 T cells, regulatory T cells, γδ T cells, NKT-like cells, B cells, NK cells, monocytes and dendritic cells.

Cytokine-antibody single-chain fusions for cancer immunotherapy

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Interleukin-2 (IL-2) is a multifunctional cytokine that is able to potently stimulate immune effector cells (e.g., CD8+ T and NK cells). Unfortunately, its concurrent promotion of regulatory T cells (Treg) and harmful off-target effects have limited its clinical efficacy. Boyman (1) reveal methods with which to mitigate these issues by complexing mouse IL-2 to anti-IL-2 mAb S4B6. These IL-2 complexes are superior to free IL-2, they manifest selective stimulatory activity for memory CD8+ T and NK cells and possess significant antitumor activity. However, the potential clinical use of these complexes is limited due to the mouse origin of IL-2 and the dissociation of the complexes at low concentrations. Based on our previous studies, we designed, engineered and produced translationally relevant protein chimera (immunocytokine, IC) consisting of hIL-2 linked through a flexible oligopeptide spacer to light chain of anti-hIL-2 mAb MAB602, either in unmodified or mutated version, functionally similar to scIL-2/S4B6 immunocytokine (2). This approach circumvents disadvantages of IL-2/S4B6 mAb complexes and exerts sufficient biological activity. We demonstrate that this IC we produced contained both IL-2 and mAb in a single molecule and IL-2 interacted with binding site of mAb. We also demonstrate its biophysical characteristics related to IL-2 receptor, its biological activity in vitro and in vivo and also therapeutic potential to eradicate experimental tumors.

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Molecular mechanisms in spontaneous regression of melanoma in pig model: analysis of cytokines

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Introduction: Spontaneous regression is defined as tumor disappearance without any therapeutic intervention. It occurs in approximately 30% of melanoma cases, mostly as a regression of minor part of the tumor lesion. Spontaneous regression is associated with tumor infiltration by immune cells and replacement of the tumor by fibrous tissue.

Melanoma-bearing Libechov minipig (MeLiM) is a porcine model of hereditary cutaneous melanoma with spontaneous regression occurring in approx. 70% of melanoma affected piglets. The spontaneous regression in MeLiM model is macroscopically detectable from the age 8 to 10 weeks as tumor flattering and depigmentation, and is also accompanied by changes in hematological profiles. In the remaining 30% of melanoma-bearing piglets, the melanoma growth continues with metastatic spreading to lymph nodes and inner organs, i.e. progression.

Cytokines and chemokines are major regulatory molecules of the immune system, however, they act also as regulators of cell survival, proliferation, differentiation and migration in general. In the tumor microenvironment, cytokines are frequently produced by malignant cells as well as stromal cells (e.g. fibroblasts).

Aims: The aim of the study was to quantify cytokine levels in tumors and blood plasma of MeLiM animals at early stages of spontaneous regression and compare them to progressive melanoma course and to melanoma-free control samples. The results are expected to elucidate regulatory processes in melanoma regression and to identify factors that distinguish progressive and regressive disease development.

Methods: The expression of selected cytokines was monitored in 6 to 12 week-old MeLiM piglets at the level of mRNA in tumor tissue and healthy skin (RT-PCR) and at the protein level in tumors and blood (xMAP assays). Melanoma-free crossbreds of MeLiM with white minipigs were used as controls.

Results: Presence of melanoma was characterized by elevated levels of pro-inflammatory cytokine interleukin 6 (IL-6) in both tumor and plasma. Spontaneous regression in comparison to progression was accompanied by changes in cytokines activating the IL-1 receptor (elevated IL-1α and IL-1β, while decreased IL-1ra levels) suggesting a pro-inflammatory milieu. In contrast, decreased IL-12 levels in both tumor and plasma were typical for melanoma progression, which may lead to an insufficient cytotoxic cell response in progression.

Conclusion: Our results suggest that the early phases of spontaneous regression of melanoma in MeLiM model are accompanied by pro-inflammatory environment supporting recruitment of immune cells. Further studies are required to specify immune cell infiltrates and function in the course of regression. Identified regulatory molecules might find future implications in the research of human disease and therapy.

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Čtvrtek / Thursday — 1. prosince 2022 / 1st December, 2022

Chairs: Ludmila Boublikova, Patrik Flodr

In vitro and *in vivo* characterization of miR-215-5p as a new tumor suppressor in colorectal cancer

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Decreased expression of miR-215-5-p was found in tumor tissue of patients with colorectal cancer (CRC) in comparison to healthy colon tissue. Moreover, expression levels of miR-215-5p were further decreased in metastatic lesions compared to primary tumor tissue. Overall, CRC patients with lower expression of miR-215-5p in tumors had significantly shorter overall survival and a higher chance of metastasis. We have shown that miR-215-5p significantly reduced proliferation, clonogenicity, and migration of CRC cells, lead to cell cycle arrest in G2/M phase and p53-dependent induction of apoptosis. The ability of miR-215-5p to inhibit tumor growth was confirmed in vivo. We proved epiregulin and HOXB9 to be the direct targets of miR-215-5p. Since epiregulin is an EGFR ligand and HOXB9 is its transcriptional inducer, we suggest that the primary molecular link between miR-215-5p and CRC cells phenotypes presents the EGFR signaling pathway, which is one of the canonical pathogenic pathways in CRC. In the follow-on study, we focused more on the role of miR-215-5p in the metastasis of CRC. MiR-215-5p was found to decrease invasivity, migratory capacity and tumorigenicity in vitro, and metastasis formation in the in vivo model of CRC. Transcriptome analysis identified signaling pathways involved in the process, and subsequent RT-qPCR validation indicates CTNNBIP1 to be a direct target of this miRNA. These results bring new insight into miR-215-5p biology and its tumor suppressive functioning in CRC.

Molecular markers for disease monitoring and response prediction in solid tumors

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While the advanced metastatic stages of adult solid tumors still remain incurable in the vast majority of cases, their current management can improve the survival significantly and control the disease for long periods of time. The consequent treatment failure is, however, inevitable due to changing genetic background and developing resistance to the therapy. Molecular profile monitoring in solid tumors during the disease course may be tricky but currently it has been made possible with the techniques of liquid biopsy.

The main aim of the project was to identify molecular aberrations associated with the pathogenesis and treatment resistance in specific types of solid tumors, assess their potential clinical impact for the disease monitoring, response prediction and prognostic estimates.

Patients with testicular germ cell tumors (TGCT) and metastatic colorectal cancer (mCRC) were enrolled. Primary tumor, metastatic samples and peripheral blood taken at diagnosis and defined time points were collected from each patient. DNA was extracted from primary and metastatic tumors and blood buffy coat (as a germline control), circulating free DNA (cfDNA) from blood plasma, their concentration was measured and quality checked. All samples were then sequenced on Illumina platform, TGCT with whole exome libraries, mCRC with targeted sequencing (cancer panel of 151 genes), the results were analyzed and correlated with clinical and laboratory data. cfDNA from healthy donors was used as another control.

cfDNA was detectable in all peripheral blood samples. Total cfDNA concentration was significantly higher ($p < 0.0001$) in TGCT patients (n=96 pts, 173 samples) than in normal controls (n=31) but without a clear threshold that may reliably distinguish between tumor and normal samples. Longer 360 bp cfDNA fragments were found in 58% of TGCT, including almost all samples from disease relapse or progression but in no normal controls ($p < 0.0001$). Contrary to TGCT, where no common specific molecular marker is available, a proportion of mCRC harbours typical mutations, most frequently in *RAS* genes, that are convenient markers for tumor detection. In a test cohort of mCRC with *RAS* mutation in primary tumors (n=9 pts), this mutation was found in 6/9 cfDNA samples. In 3 samples with undetectable *RASmut*, no other tumor-associated mutations or variants were identified. It suggests that the negative findings were due to the sensitivity limit of the method, and not due to *RASmut* loss under the selective pressure of prolonged systemic therapy and possible clonal evolution of the malignant cells.

NGS sequencing of TGCT

patients refractory to platinum-based chemotherapy (n=15 pts, 47 samples) revealed somatic molecular aberrations in several genes that are probably involved in TGCT development - e.g. *TPTE2* (testis-specific *PTEN* homologue) or *TSPAN16* (regulator of cell proliferation and signal transduction); and in genes that may be responsible for the acquired resistance to cisplatin – e.g. *CDC27* (tumor suppressor, cell cycle regulator), *RBMX* (*RBMY* homologue, spermatogenesis regulator), *PRDM1* (*TP53* regulator, involved in testis development), and others. Except for *CDC27*, these genes have not been associated with TGCT yet, although their function in testis development and/or other malignancies is well documented and thus the role in TGCT could be implied. In contrast, molecular aberrations detected in mCRC patients so far were mostly those previously well described – e.g. in *KRAS, NRAS, TP53, PIK3CA, BRAF, SMAD4, APC, ATM, PDGFRA, BRCA1,* etc.

An example of a mCRC patient followed up with a molecular marker, *TP53* mutation identified in the primary tumor, illustrates its potential clinical applications: (i) prediction of the treatment response – unusually high resistance to chemotherapy with multiple relapses and fast progression correlated with the known pathogenic effect of this mutation; (ii) proof of the tumor origin – presence of the mutation supported the metastatic character of a distant tumor lesion over a possible second primary, when histopathology examination including extensive immunohistochemistry was inconclusive; (iii) monitoring the treatment response and disease relapse in peripheral blood samples – here, the sensitivity limit of this approach should be validated, with single small metastatic lesions under 1 cm3 often being under the detection threshold of cfDNA analysis, in our hands.

In conclusion, the identification of specific molecular markers in solid tumors and peripheral blood cfDNA and their monitoring during the disease course is feasible, brings new insights into the tumor biology and, most importantly, has a clinical impact and potential to improve treatment strategies and patients´ outcome.

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Analysis of the expression of ALK/ROS1 proteins vz. rearrangement of ALK/ROS1 genes - comparison of methodological approaches

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Úvod: Analýza expresie ALK/ROS1 proteínov, resp. analýza prestavba ALK/ROS1 génov zohráva kľúčovú úlohu pri identifikácii pacientov s NSCLC, ktorý profitujú s cielenej liečby TKI inhibítormi. Za tzv. zlatý štandard v diagnostike ALK+/ROS1+ pacientov je stále považovaná in situ hybridizačná analýza, no pri využívaní imunohistochemickej detekcie expresie týchto proteínov sa objavujú tzv. diskordantné prípady, pri ktorých sú získavané rozdielne výsledky FISH vz. IHC analýz. Odpoveď na otázku, či v prípade prestavby génu bez imunohistochemicky potvrdenej expresie proteínov alebo naopak v prípadoch, kedy nie je dokázaná prestavba génu, no dochádza k expresii proteínu, skutočne vzniká aberantný proteín, ktorý je cieľom takejto terapie, by nám mohlo priniesť využitie analýz RNA.

Metódy: V období február 2022 – október 2022 sme analyzovali bioptické vzorky pacientov s NSCLC pomocou imunohistochemickej analýzy expresie ALK a ROS1 proteínov (s využitím klonom D5F3 (Roche Molecular Diagnostics), resp. D4D6 (Cell Signalling Technology), fluorescenčnej in situ hybridizačnej analýzy ALK a ROS1 génov (s použitím SPEC ALK Dual Color Break Apart Probe a SPEC ROS1 Dual Color Break Apart Probe (obe ZytoVision) a Idylla™ GeneFusion assay (Biocartis).

Výsledky: Počas 9-tich mesiacov sme analyzovali vzorky 252 pacientov s NSCLC. V tejto skupine sme identifikovali 5 prípadov s diskordantnými výsledkami imunohistochemickej analýzy expresie ALK proteínu a FISH analýzy prestavby ALK génu. Konkrétne v 2 prípadoch nebola detegovaná expresia ALK proteínu, no FISH metódou bola potvrdená prestavba ALK génu vo viac ako 15 % hodnotených nádorových buniek. Analýzou RNA sme nepotvrdili fúziu ALK génu ani v jednom z týchto prípadov. V 1 prípade nebola detegovaná expresia ALK proteínu, no FISH metódou bola potvrdená prestavba ALK génu vo viac ako 15 % hodnotených nádorových buniek. Analýzou RNA sme detegovali fúziu ALK génu. V 1 prípade nebola detegovaná expresia ALK proteínu, no FISH metódou bola potvrdená prestavba ALK génu vo viac ako 15 % hodnotených nádorových buniek. Analýzou RNA sme detegovali tzv. imbalanciu expresie ALK génu, ktorá indikuje prestavbu génu bez známeho fúzneho partnera. V 1 prípade nebola detegovaná expresia ALK proteínu, no FISH metódou bola potvrdená prestavba ALK génu vo viac ako 15 % hodnotených nádorových buniek. Analýzou RNA sme detegovali skipping mutáciu v exóne 14 MET génu. V ďalších 13-tich prípadoch s konkordantnými výsledkami IHC a FISH analýzy ALK proteínu/génu sme pri analýze RNA detegovali fúziu a imbalanciu expresie ALK génu. V 9 prípadov, v ktorých sme detegovali imbalanciu (8/9) alebo fúziu (1/9) ALK génu RNA analýzou, sme nepotvrdili

ani expresiu ALK proteínu IHC metódou, ani prestavbu ALK génu FISH metódou. V 4 prípadoch, v ktorých sme detegovali imbalanciu (3/4) alebo fúziu (1/4) ROS1 génu RNA analýzou, sme nepotvrdili ani expresiu ROS1 proteínu IHC metódou, ani prestavbu ROS1 génu FISH metódou. V 1 prípade bola FISH metódou detegovaná prestavba ROS1 génu, no tá nebola potvrdená ani imunohistochemickou metódou ani RNA analýzou.

Závery: Táto pilotná štúdia umožňuje dospieť k záveru, že využitie analýz RNA pomôže v identifikácii tých pacientov, u ktorých napriek nedetegovanej prestavbe týchto génov a/alebo nedetegovanej expresii proteínov doteraz používanými metódami pravdepodobne dochádza k vzniku aberantného proteínu. Na zhodnotenie, či aj takýto pacienti by profitovali z cielenej terapie, je nutné uskutočniť klinické skúšania, resp. sledovať ich terapeutickú odpoveď.

Poďakovanie: Táto práca je súčasťou projektu s názvom: Integratívna stratégia v rozvoji personalizovanej medicíny vybraných zhubných nádorových ochorení a jej vplyv na kvalitu života, kód projektu v ITMS2014+: 313011V446.

Systemic and localised amyloidosis associated with malignant neoplastic, uncertain neoplastic and non-neoplastic diseases

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Amyloidosis is a heterogeneous acquired or hereditary, systemic or localised disease that results from the abnormal and insoluble deposition of beta-sheet fibrillar protein aggregates in various tissues with variable distribution in extracellular space, mainly oligomers and protofibrils produce tissue damage. Current nomenclature classification distinguishes 36 amyloidogenic proteins, 18 proteins in systemic amyloidosis and 22 proteins as a part of localised forms (ISA 2020). Our file contains 339 positive specimens (in total 749 FFPE and native samples analysed) with amyloid deposits diagnosed in a period of 15 years 2007-2022 in variable tissues and organs stained with Congo red and/or Saturn red as a diagnostic step with consequent immunohistochemical analysis (IHC) and/or proteomic analysis (laser captured microdissection-liquid chromatography/tandem mass spectrometry - LMD-LC/MS/MS) as a typing steps. Our results are shown in enclosed tab. The most frequent neoplasms related to an amyloidosis (AL amyloidosis, 100 cases) are multiple myeloma (MM) and monoclonal gammopathy of undetermined significancy (MGUS). Another B-cell non-Hodgkin lymphomas (B-NHLs) may be associated with an amyloid deposition of AL type, in our cohort namely extranodal marginal zone lymphoma of MALT-type (ENMZL MALT-type, 10 cases), lymphoplasmacytic lymphoma (LPL, 4 cases) and 7 cases fall in a category provisionally named “Highly suspected B-NHL with plasmacytic differentiation” which is currently classified as „AL amyloidosis with a localised B-cell neoplasia of undetermined significance“ or as „AL amyloidosis with an associated predominant kappa or lambda light chain expressing plasma cell population without evidence for clonality” according to a detection of clonal rearrangement of heavy or light chains (sub)genes. The senescent plasmacytic evolution in B-NHL related to a recent therapy may also bring local AL deposits (1 case in our cohort). Detected hybrid amyloid types are highly important in differential diagnosis of coincidental diseases both producing amyloid deposits (commonly AL amyloidosis + another non-AL one). Such a situation is challenging the correct interpretation of amyloid protein deposition process e.g. a/ deposits from different specimens or organs, b/ deposits from the same specimen and organ in diverse microspaces, c/ deposits from the same specimen and organ in the same microspace. These microanatomical variabilities reveal distinct subtypes of amyloid fibrilogenesis. The analysis of amyloid deposits irrespective of the origin and localization is appealing for diagnostic and experimental precising including also amyloid signature proteins (SAP, HSPG, SAP, apo-AI, apo-AIV, apo-E and others). Presented algorithm shows highly valid method with crucial impact on a novel therapeutic decisions (siRNA, antisense mRNA, anti-human-SAP antibodies, monoclonal antibody biding amyloid protofilaments, accelerators of fibrillation, etc.) which are different in particular amyloidosis and concurrent diseases (e.g. ATTRwt amyloidosis in elderly with MGUS/MM without AL amyloidosis, and more combinations exist). Polymorphisms and mutation burden is another horizon in amyloid deposits survey. Supported by AZV-16-31156A and LF_2021_005 from Palacky University Olomouc.

Čtvrtek / Thursday — 1. prosince 2022 / 1st December, 2022

Chairs: Jan Bouchal, Jozef Skarda

Restored biosynthetic pathways induced by MSCs serve as rescue mechanism in leukemia cells after L-asparaginase therapy

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Current treatment of childhood acute lymphoblastic leukemia (ALL) significantly improves the survival of patients, but still, about 15% undergo relapse. Relapses are associated with resistance to the chemotherapeutic agents. The primary goal of this project was to elucidate the resistance mechanism of L-asparaginase (ASNase), one of the crucial drugs used in the ALL therapy. We previously showed that ASNase caused metabolic reprogramming by which leukemic cells escaped cytostatic effect of the treatment. Herein, we investigated the role of main aspects of the in vivo environment on the resistance mechanism of leukemic cells. By co-culturing them with mesenchymal stromal cells (MSCs) and treating them with ASNase-pretreated culture media, we mimicked the bone marrow matrix and the in vivo half-life of the drug. Our results showed that leukemic cell survival was increased compared to the "classical" in vitro treatment. While ASNase-mediated metabolic rewiring of leukemic cells persisted in both, mono and coculture; reduced glycolysis and increased fatty acid oxidation; activity of mTOR-regulated biosynthetic pathways differed. In both cultures, the phosphorylated forms of S6 (mediator of protein synthesis) and CAD (mediator of nucleotide synthesis) were inhibited after ASNase treatment. However, the effect was significantly less profound in the coculture model. Similar changes in phospo-S6 were observed in primary BCP-ALL cells isolated from pediatric patients treated with ASNase. As shown by stable isotope tracing asparagine synthetized de novo and released from MSCs compensated for asparagine depletion (after ASNase administration) and induced resistance of leukemic cells. Asparagine was sufficient to restore protein and nucleotide synthesis and partially rescued the viability of leukemic cells. In conclusion, presence of MSCs sustains biosynthetic pathways, making leukemic cells more accessible to bioenergetic rewiring, which may counteract ASNase cytotoxicity. These findings present a potential therapeutical target for resistant patients.

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Advances in plasmonic biosensors and their applications in oncohematology

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Label-free optical biosensors present a promising technology that can address a broad range of medical applications – from unraveling molecular foundations of diseases to their diagnosis and treatment monitoring. Biosensors based on optically excited surface plasmons present the most advanced optical affinity label-free biosensor technology. Over the past two decades, plasmonic affinity biosensors have become a key method for real-time investigation of biomolecular interactions. However, their penetration to clinical applications has been much slower [1, 2].

Here, we present recent advances in developing plasmonic biosensors for medical diagnostics. Specifically, we report new approaches in plasmonic sensor instrumentation for parallelized and sensitive detection of biomolecular analytes, discuss advanced functional coatings and methodologies suppressing the adverse effects of non-specific adsorption, and present new assays allowing for the detection of low levels of biomolecules in complex biological media. Finally, we describe medical applications of plasmonic biosensors related to the diagnosis of Myelodysplastic syndromes (MDS) which is a group of hematological malignancies with a high risk of progression into acute myeloid leukemia (AML). Two specific projects are discussed. First, we present the use of a plasmonic

biosensor to quantify interactions between selected MDS-related proteins immobilized on the surface of the plasmonic imaging sensor and blood plasma of MDS patients and healthy donors. We demonstrate that this interactomic approach is able to discriminate among different MDS subgroups as well as between MDS patients and healthy donors [3]. Second, we use a plasmonic biosensor combined with a novel assay based on the oligonucleotide-triggered nanoparticle release from a sensor surface for ultrasensitive detection of MDS-related miRNAs (miR-125b, miR-16) in blood plasma at concentrations as low as 349 aM [4].

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Immunohistochemical, histomorphological and electron microscopic evaluation of tissues around the implant material

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Introduction: Fracture healing is a multistep complex of physiological processes in which, in addition to bone cells, the surrounding soft tissue also participates. Fractures may heal spontaneously, but in some cases, the treatment of fractures requires the use of supportive instruments – such as implants – to stabilize the bone. Orthopaedic implants can be made of various materials such as metal (titanium, stainless steel), ceramic or polymers. Implant materials do generate wear particles, which may lead to foreign body reaction, chronic inflammation, local bone and soft tissue destruction and other complications. Difficulties associated with this condition can lead to implant destruction and treatment failure.

Material and methods: In our study, we examined tissue from 106 patients who were treated for complicated fractures of the upper and lower limbs. We evaluated tissues from three locations surrounding the original fracture; soft tissue, bone tissue and implant-adhering tissue of multiple origins. The sample tissues were formalin-fixed, decalcified and paraffin-embedded, and 3µm sections were stained by the haematoxylin and eosin method. The sections were analysed using light microscopy, and representative paraffin blocks retrieved from each sampling site were selected for subsequent immunohistochemistry analysis (CD11b, CD15, CD34, CD44, CD68, Cathepsin K, TRAcP). Deparaffinized and unstained tissue sections on glass slides were coated with a thin layer of Au/Pd and analysed by scanning electron microscopy at the accelerating voltage of 15 kV (SEM-EDS, ThermoFisher Scientific).

Results: In general, histological evaluation of the samples revealed similar changes in the assessed tissues. Typical histopathological reactions were: the presence of inflammatory infiltrate (polymorphonuclear cells, diffuse macrophage infiltrates, round-cell inflammatory infiltrate), formation of non-specific granulation tissue, foreign body reaction, reactive fibrosis, angiogenesis, necrosis and calcification. Furthermore, metal debris was present mainly intracellularly as small black pigment particles. This pigmentation, as

well as necrosis and calcification, were observed more in the implant-adhering tissue than at other locations. However, the incidence of fibrosis was the same in all localities. In the immunohistochemical study of the soft tissue, higher expressions of CD68, CD34, CD15 and CD11b markers were observed than in other locations: bone tissue and implant-adhering tissue. TRAcP and Cathepsin K markers were expressed more in the bone tissue than in the implant-adhering tissue. On the other hand, there was no significant difference in expression for the CD44 marker observed. Performed statistical analysis support these findings. A number of elements were detected by the SEM-EDS method, such as Ti, Fe, Ni, Cr, Nb, Al and other.

Conclusion: Many materials used in implants are considered biocompatible and inert; however, the release of small particles of material into the surrounding tissue – confirmed by many previous studies – can still have some harmful effects. Therefore, it is necessary to develop new surface treatments and materials for use in bone implants to modulate the surrounding biological environment to eliminate adverse effects affecting the healing process.

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This study was supported by the Doctoral grant competition VSB – Technical University of Ostrava, reg. no. CZ.02.2.69/0.0./0.0/19_073/0016945 within the Operational Programme Research, Development and Education, under project DGS/TEAM/2020-029 „Submicron particles in human tissues and microplastics in drinking water".

Human-specific levels of endoplasmic reticulum stress markers in the pathological progression of Lewy body disease – a postmortem study

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Objectives: The concept of Lewy body disease as neurodegenerative diseases characterized by the presence of alpha-synuclein pathology in neurons includes Parkinson's disease, Parkinson's disease dementia, and dementia with Lewy bodies. One of the potential culprits responsible for neurodegeneration in these diseases is chronic endoplasmic reticulum stress-induced unfolded protein response. The role of this cellular mechanism in the pathogenesis of Lewy body disease, and its extent and anatomical distribution in the human brain during disease progression have not yet been fully understood. In recent study, we systematically analyzed its potential activation, most importantly through changes in levels of Grp78 and eIF2alpha in human brain affected by the disease. Analyzing a variety of brain areas in different stages of the disease, our objective has been to determine the areas most affected by endoplasmic reticulum stress.

Methods: In the study, we have used postmortem brain tissue from 45 subjects in total. Subjects were selected as symptomatic Parkinson's disease/Parkinson's disease dementia/dementia with Lewy bodies patients with Braak stage 5-6 pathology (n=14), subjects with incidental Lewy body disease with Braak stage 1-4 pathology (n=19) and healthy controls (n=12). Frozen tissue was analysed by western blot, formalin-fixed paraffin-embedded sections were used for IHC and IF analysis.

Results: Levels of total eIF2α were increased in the striatum of the patients ($P<0.01$), whereas levels of phospho-eIF2α did not change with accumulation of alpha-synuclein pathology. Importantly, total eIF2α levels positively correlated with alpha-synuclein levels in the striatum ($r=0.721$, $P<0.0001$). Grp78 levels increased in the amygdala of patients ($P<0.05$) and were correlated with alpha-synuclein levels ($r= 0.866$, $P=0.0005$). Additionally, Grp78 was upregulated in substantia nigra pars compacta in immunohistochemical analysis. Interestingly, we also observed that Grp78 co-localise with alpha-synuclein only rarely, and exclusively around the outer layer of the fully developed Lewy bodies.

Conclusion: The levels in the proteins of unfolded protein response increase with the progression of the alpha-synuclein pathology in selected areas of the human brain and positively correlate with alpha-synuclein levels. The anatomical aspect of the study suggested uneven distribution of endoplasmic reticulum stress and helped to focus future studies towards analysing specific areas of the brain.

Whats new in immunotherapy of malignant pleural mezotelioma from the perspective of a pathologist

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Introduction: Expression patterns and prognostic significance of PD-L1 and PD-1 are still not clear in human malignant pleural mesothelioma (MPM).

Methods: Formalin-fixed paraffin-embedded (FFPE) tumor samples from 203 MPM patients with treatment without immunotherapy were collected from 5 European centers.The expression patterns of PD-L1 and PD-1 of tumor cells (TCs) and tumor-infiltrating lymphocytes (TILs) were detected by immunohistochemistry and correlated with clinical parameters.

Results: High (>10%) PD-L1 TC and PD-1 TILs expressions were found in 18 (8%) and 39 (24%) patients, respectively. PD-L1 was rarely expressed by TILs [≥1%, n=13 (8%); >10%, n=1]. No significant associations were found between the PD-L1 or PD-1 expression of TCs or TILs and clinicopathological parameters such as stage or histological subtype. Notably, patients with high (>10%) TC-specific PD-L1 expression exhibited significantly worse median overall survival (OS) (6.3 vs. 15.1 months of those with low TC PD-L1 expression; HR: 2.51, P<0.001). In multivariate cox regression analysis high TC PD-L1 expression (>10%) proved to be an independent negative prognostic factor for OS (HR: 2.486, P=0.005). But there was no significant correlation between PD-L1 or PD-1 expression of TILs and OS.

Conclusions:

High (>10%) PD-L1 expression of TCs independently predicts worse OS in MPM. Further studies are warranted to investigate the value of PD-L1/PD-1 expression as a marker for treatment response in MPM patients receiving immunotherapy.

Pátek / Friday — 2. prosince 2022 / 2nd December, 2022

Chairs: Marian Hajduch, Michal Hocek

Modified nucleosides, nucleotides and nucleic acids as potential cytostatics

Michal Hocek

Institute of Organic Chemistry and Biochemistry o the CAS, Prague 6, Czech Republic

The lecture will summarize the research in the Hocek group on the design and synthesis of modified nucleic acids components as cytostatic or cytotoxic agents. Main focus will be on base-modified nucleosides (in particular substituted or fused 7-deazapurine ribonucleosides) that were discovered and studied in collaborartion with the Hajduch group at IMTM UPOL. Also diverse nucleotides and prodrugs will be discussed. The last part will cover our on-going research on hypermodified DNA aptamers against targets relevant to oncology.

Novel inights into molecular mechanism of the anti-cancer drug emetine on DNA replication

Dávid Lukáč, Zuzana Machačová, Pavel Moudrý[1]

Institute of Molecular and Translational Medicine, Olomouc, Czech Republic

DNA synthesis of the leading and lagging strands works independently and cells tolerate single-stranded DNA generated during strand uncoupling if it is protected by RPA molecules. Natural alkaloid emetine is used as a specific inhibitor of lagging strand synthesis, uncoupling leading and lagging strand replication. Here, by analysis of lagging strand synthesis inhibitors, we show that despite emetine completely inhibiting DNA replication: it does not induce the generation of single-stranded DNA and chromatin-bound RPA32 (CB-RPA32). In line with this, emetine does not activate the replication checkpoint nor DNA damage response. Emetine is also an inhibitor of proteosynthesis and ongoing proteosynthesis is essential for the accurate replication of DNA. Mechanistically, we demonstrate that the acute block of proteosynthesis by emetine temporally precedes its effects on DNA replication. Thus, our results are consistent with the hypothesis that emetine affects DNA replication by proteosynthesis inhibition. Emetine and mild POLA1 inhibition prevent S-phase poly(ADP-ribosyl)ation. Collectively, our study reveals that emetine is not a specific lagging strand synthesis inhibitor with implications for its use in molecular biology.

Beyond cancer: Repurposing of targeted therapeutics as an approach to advance clinical management of vascular anomalies

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Vascular anomalies are rare defects in vasculature development, whose clinical manifestation can range from birthmarks to large debilitating lesions. Especially in managing large-scale anomalies, standard therapeutic modalities consisting mainly of surgical approaches exhibit limited efficacy, and patients often undergo lengthy therapeutic procedures that bring only minimal benefit. In addition to chronic pain, esthetic issues, and the overall decrease in quality of life, patients may develop systemic complications, such as chronic coagulopathy. Recent research focused on clarifying their genetic background showed that alterations in genes involved in two significant intracellular signaling pathways, RAS/MAPK and PI3K/AKT, are causative in most vascular anomalies. Both pathways are frequently altered in various cancer types, and multiple drugs that target these alterations are already used in cancer treatment. This opens new therapeutic opportunities for vascular anomalies following the precision medicine paradigm.

Thirty-eight patients with vascular anomalies underwent molecular-genetic testing using different targeted DNA sequencing approaches. The analysis was carried out using FFPE tissue specimens from lesion biopsies. For 82% of patients (n = 31), causal alterations of either germline or somatic origin were found. As the cohort mainly consisted of patients with venous malformations, *TEK* and *PIK3CA* gene alterations accounted for 27/31 findings. *TEK* gene mutations were all previously described alterations leading to constitutive receptor signaling, with *TEK* L914F being found in 12/19 cases. *PIK3CA* gene variants were hotspot mutations, which are also established drivers of carcinogenesis. Other findings included alterations in *KRAS, GLMN, PTEN,* or *IDH2* genes. In 3 patients, the causal alteration was of germline origin. So far, 13 patients with either *TEK* or *PIK3CA* mutations were administered targeted treatment using selective PI3K alpha subunit inhibitor, alpelisib. For all patients, marked improvement in quality of life, lesion reduction, and normalization of coagulation parameters was achieved.

Molecular-genetic analyses in patients with vascular anomalies hold great potential for diagnosis refinement. They are also a necessary starting point for new therapeutic strategies, such as targeted cancer therapeutics administration within the concept of drug repositioning. The application of this innovative approach is especially important in patients with extensive debilitating lesions, for whom standard treatment options bring only minimal benefit.

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Targeting mitochondrial iron metabolism: Selective killing of cancer cells via mitochondrially targeted deferasirox

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Deferasirox (DFX) is a cell permeable iron chelator approved for the treatment of chronic iron overload. We have designed and synthesized its mitochondrially targeted derivative, called mitoDFX, by coupling it with a triphenylphosphonium group. The resulting compound shows marked cytostatic, cytotoxic, and migrastatic properties in vitro at nM concentrations while not affecting non-malignant cells, and it significantly suppressed tumor growth and metastasis in vivo. The underlying molecular mechanisms include impairment of [Fe-S] cluster/heme biogenesis leading to destabilization and loss of activity of [Fe-S] cluster/heme containing enzymes. This results in dysfunctional mitochondria with markedly reduced respiration, disassembly of respiratory supercomplexes, increased mitochondrial ROS production and induction of mitophagy. The agent further induces significant alterations in the metabolic pathways such as TCA cycle and eventually leads to depletion of reduced glutathione levels, pointing towards an oxidative cell death, which is further supported by synergistic effect of glutathione synthesis inhibitor BSO with mitoDFX. Mitochondrial targeting of DFX, therefore, represents a way to deprive cancer cells of biologically active iron, which is incompatible with their proliferation, growth and invasion, while at the same time it exhausts their antioxidant defense mechanisms, leading to their death. Our findings highlight the importance of mitochondrial iron metabolism for cancer cells and demonstrate repurposing of deferasirox into an extremely effective anti-cancer drug via mitochondrial targeting.

Water-soluble polymer carriers for tumor treatment

Lenka Kotrchová[1], Libor Kostka[1], Weibo Cai[2], Milada Šírová[3], Tomáš Etrych[1]

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The copolymers based on *N*-(2-hydroxypropyl)methacrylamide (HPMA) represent one of the most intensively studied synthetic carriers for drug delivery. The conjugates based on HPMA copolymers containing cytostatics, e.g. doxorubicin, pirarubicin or docetaxel bound via pH sensitive hydrazone bond are highly potent drug-delivery systems for cancer treatment. The accumulation of water-soluble polymer carriers in tumor tissue is molecular-weight dependent. Nevertheless, not only the size, but also the shape of polymer carriers is crucial for their biological behavior, thus star-like and linear architectures of HPMA copolymers with different molecular weights were designed and synthesized.

For high-molecular-weight polymer carriers, their degradation to products with molecular weight below the renal threshold is necessary to facilitate their removal from the body. Therefore, we focused on the design and synthesis of high-molecular HPMA structures with various biodegradable “cores”.

We successfully tested the accumulation of polymer carriers for various star-like polymer conjugates. Various polymer carriers without attached drug were also traced in the organism via positron emission tomography. An in vivo antitumor study was performed where these new conjugates showed higher efficacy than the first generation of carriers, even at very low drug doses.

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Structure-assisted design of enzyme inhibitors

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Structure-assisted ligand design, a method used as an alternative to screening random compounds, is the process of identifying new drugs through rational design of molecules based on knowledge of the structure of their biological target. Structural information is obtained through experimental methods such as X-ray crystallography and NMR spectroscopy, or through homology modeling. Enzymes involved in pathologies are good targets for rational design. Enzymes usually

have a well-defined active site to bind substrates, and many have allosteric sites that bind regulators. These sites can be targeted by small molecules that mimic the structure of the substrate or regulator. Knowledge of the 3D structure of the enzyme, especially in complex with its natural ligand, is beneficial to lead the design. During the iterative process of rational design, the structure of the ligand is progressively altered to maximize the shape and charge complementarity to the enzyme binding site. Design also can be guided to ensure that the ligand has little to no affinity towards other off-target enzymes to prevent undesirable side effects.

Structure-assisted design of inhibitors targeting enzymes involved in cancer will be presented.

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Pátek / Friday — 2. prosince 2022 / 2nd December, 2022

Chairs: Jan Bouchal, Fred Santer

How to diagnose and induce HRD in prostate and ovarian cancer

Fred Santer

Division of Experimental Urology
Department of Urology
Medical University of Innsbruck

PARP inhibitors such as olaparib have been approved for the treatment of patients with advanced ovarian (OC) and, more recently, metastatic castration-resistant prostate cancer (mCRPC) diagnosed with homologous recombination deficiency (HRD) or pathogenic mutations in BRCA1/2, respectively. In OC, the cornerstone of somatic HRD diagnosis is based on a SNP array enabling the visualization of the genomic scar caused by HRD. In mCRPC, more patients could be stratified to olaparib therapy if diagnostic procedures could be extended to a SNP array-based protocol. However, PC tissue availability is hampered by insufficient quality, incorrect representation of the actual tumor stage and low tumor cell content issues of archived tissue specimen from prostatectomy done years before stratification to olaparib therapy. I will present at the meeting a current approach how to improve diagnosis of HRD in mCRPC.

Since only a fraction of patients is diagnosed with somatic HRD caused by (epi)genetic aberrations, efforts are undertaken to induce HRD in HR-proficient patients by combination therapies. CDK12 was found to have a function in modulating gene transcription by regulating the recognition of intronic polyadenylation sites. We have tested in a pre-clinical study in OC the impact of a small molecule inhibitor against CDK12/13 leading to the downregulation of important HR genes due to premature transcription termination. Additive effects of CDK12 inhibition with cisplatin or olaparib were noted consequently.

The hidden potential of malignant ascites for ovarian cancer research

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Ovarian cancer (OC) ranks among the deadliest cancers in women. Lack of symptoms, rapid spread of metastases and common chemoresistance contribute to unfortunate fate of majority of OC patients, especially those having high-grade serous carcinoma of the ovary, fallopian tube and peritoneum (HGSC), the most common type of OC. Many HGSC patients have excess fluid in the peritoneum at the stage of diagnosis called ascites. Malignant ascites is a complex tumor microenvironment (TME) providing a niche for disseminated cancer cells that survive there mostly in form of spheroids, surrounded by other infiltrating cells and extracellular vesicles (EVs). As such, malignant ascites is basically a biopsy in a liquid form that has a great, yet so far unnoticed, potential for translational research. Therefore, we have been collecting malignant ascites from the cytoreductive surgery of HGSC and we investigate how this TME affects disease progression. In our first study we showed, that WNT signaling inducing activity in ascites predicts poor outcome for patients.

Next, we have been creating database of proteomic profiles of EVs and other functional characteristics of ascites as well as organoids derived from tumor cells present in ascites. This database allows us to study the functional involvement of EVs in the progression of HGSCs on top of their potential as biomarkers. EVs are small membrane-bound particles that convey proteins, lipids and nucleic acids between cells and their cargo reflects the cell of origin. EVs play important role in cancerogenesis and hold great promise as disease biomarkers as well as potential therapeutic targets. Small size and polydispersity of EVs brings various challenges to their isolation and characterization, including method-dependent enrichment of different EV subtypes as well as contaminants.

In the current study, we isolated EVs from ascites of 11 patients by two different methods (ultracentrifugation coupled to sucrose cushion and size-exclusion chromatography) and analyzed them by mass spectrometry. We identified core ascitic EV proteins present in all patients that contain typical EV markers and are devoid of method-dependent contaminants. To cover interpatient heterogeneity, we expanded these “core proteins” with proteins found in majority of patients. Next, we compared them with proteins of EVs from related control fluids and found proteins present only on EVs from HGSC patients. We believe this list of proteins contain both important players of HGSC progression as well as potential biomarkers. Using single cell RNA sequencing data we mapped the origin of EVs

to different types of cells present in malignant ascites. Our results suggest that EVs in ascites do not come predominantly from tumor cells, but rather from variety of non-malignant cell types including cancer-associated fibroblasts and tumor-associated macrophages, which presence in ascites we confirmed by flow cytometry. This emphasizes the recently appreciated role of TME in the progression of HGSC. To conclude, this is the first study attempting to link EV composition to the cell types producing it. As such it opens numerous avenues both for better understanding of EV role in tumor promotion/prevention and for the improved HGSC diagnostics. Additionally, we believe our results may increase the awareness of the research potential of malignant ascites and might encourage its more comprehensive documentation and biobanking, which would ultimately lead to fundamental discovery science, and personalized medicine approaches in HGSC.

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Ligands targeting prostate-specific membrane antigen (PSMA) for prostate cancer imaging and therapy

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PSMA is a high value biomarker for the diagnosis and treatment of prostate cancer (PCa), as the enzyme is overexpressed in high grade, androgen independent, and metastatic PCa tumors. Our laboratory has a longstanding interest in the development of ligands specifically targeting PSMA. We have recently developed murine monoclonal antibodies (mAbs) recognizing human PSMA with nanomolar affinity and high specificity. The lead 5D3 mAb was next humanized using structure-assisted CDR grafting and optimized variants further characterized in-depth using an array of biochemical and biophysical assays. Collectively, our data provide an experimental basis for the further development of 5D3 as a research reagent as well as for potential future clinical use.

In vivo evaluation of enhanced blood retention and tumor uptake PSMA-targeting ^{225}Ac-labeled radioconjugates

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Introduction: The prostate-specific membrane antigen (PSMA) is overexpressed in prostate cancer tissues at significantly higher levels compared to healthy organs. Therefore, PSMA has emerged as very suitable target for molecular imaging as well as targeted radionuclide therapy of metastatic castration-resistant prostate cancer (mCRPC). In this study we investigated the *in vivo* behavior of two novel macropa-based PSMA ligands (namely [^{225}Ac]FR94, [^{225}Ac]FR96) modified with albumin binding moieties. Methods: Performed *in vivo* studies involved *ex vivo* biodistribution studies and subsequent immunohistochemical examinations of selected organs from LNCaP-tumor bearing mice. Organs were dissected, weighed, and the accumulated activity was quantified in a gamma-counter at 1, 4, 24, 48, 72, and 120 h (plus 168 h for [^{225}Ac]FR96) post-injection to determine the radiotracer uptake as a percentage injected activity (dose) per gram of the corresponding organ (%ID/g). Kidneys, liver and tumor were examined using immunohistochemical staining methods to detect PSMA expression, DNA damage (γH2AX), proliferation status (Ki67) and necrosis (H&E). Immunohistochemical results were quantified as the histoscore values.

Results: The highest accumulation of radioactivity was measured in the LNCaP tumors at 168 h p.i. for [^{225}Ac]FR96 (153.48 ± 37.76 %ID/g) and at 128 h p.i. for [^{225}Ac]FR94 (46.04 ± 7.77 %ID/g). The second most prominent organ of radioligand uptake were kidneys with the highest accumulation of 67.92 ± 20.67 %ID/g (4 h p.i.) for [225Ac]FR94 and 59.90 ± 6.46 %ID/g (48 h p.i.) for [^{225}Ac]FR96. Blood clearance of radioactivity was slower compared to the corresponding counterparts without albumin binders. The γH2AX staining revealed significant DNA damage in tumor cells of mice applied with [^{225}Ac]FR94 as well as [^{225}Ac]FR96 compared to untreated controls. Insignificant DNA damage was observed in the kidney tissue compared to the untreated controls.

Conclusion: *In vivo* experiments in tumor-bearing animals confirmed slower pharmacokinetic behavior of studied PSMA ligands i.e. longer retention in blood and kidneys, caused by the presence of albumin binding moieties in their structures. More importantly, the mentioned improvement of the ligand structure increased the tumor uptake significantly. Histological examination of the organs confirmed substantial DNA damage in the tumor tissue of mice injected with both studied ^{225}Ac-compounds, on the other hand the same parameter revealed only low DNA harm in the kidneys.

Pátek / Friday — 2. prosince 2022 / 2nd December, 2022

Chairs: Karel Smetana, Zdenek Kolar

Cancer-associated fibroblasts

Karel Smetana, Jr.[1,2], *Lukáš Lacina*[1,2,3], *Michal Kolář*[4]

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Cancer-associated fibroblasts (CAFs) represent the most numerous non-malignant cell type in the majority of solid cancers. CAFs seem to be essential in the formation of the ecosystem supporting cancer stem cells. CAFs originate from numerous cell types, such as local fibroblasts, mesenchymal stem cells, circulating fibrocytes, pericytes, and endothelial cells. These populations can be recruited by malignant cells via intercellular signalling. Many signalling pathways participate in the transition of these cells to CAFs; however, the TGF-β family seem to be the most prominent. Participation of exosomes produced by cancer cells in CAFs formation has also been demonstrated. No specific single marker distinguishing CAFs from normal fibroblasts is recently available. Of note, smooth muscle actin expression is broadly accepted as a particularly helpful marker. CAFs represent an intrinsically heterogeneous collection of cells. Some CAFs produce extracellular matrix (e.g., nestin or podoplanin), while other cells produce pro-inflammatory factors. In the majority of tumours, CAFs release chemokines such as CXCL-1 and CXCL-8 and interleukins IL-1 and IL-6. These soluble factors affect the phenotype of cancer cells and enhance their migratory activity. CAFs also participate in the vascularization of the tumour. The secretory activity of CAFs is also responsible for the induction of systemic manifestations of cancer, such as cancer cachexia. Because CAFs are so remarkably influential in tumour biology, implementing CAFs detection for improved diagnostics and their therapeutic targeting can be a promising direction in future oncology research.

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Smoldering inflammation and inflammatory threshold in tissue remodeling and cancer niche development

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Long-lasting chronic inflammation induces progressive fibrosis of the involved tissues. Chronic inflammation, a leading factor in many pathological processes, can address cancer establishment and evolution. Local changes in immune activation inside a tissue, if maintained and supported by the environment, can induce structural remodeling. Reciprocally, collagen accumulation can affect local immunity. Immune activation induced by conventionalization (contamination) of germ-free (GF)mice quickly modifies the local and systemic immunity and, contemporarily, induces a fast remodeling of the collagen scaffold in the intestinal mucosa. Induced colitis (by DSS) or colorectal carcinogenesis (by AOM) leads to inflammation with the active remodeling of the collagen scaffold organization, even when the mucosa appears recovered from the acute induction. Multi-photon confocal microscopy of CV and GF animal mucosa showed higher complexity in structure in the CV rats (continuously challenged by microbiome). The results indicated that the collagen scaffold adapts to the immune microenvironment conditions, and quickly it can be altered if the „inflammatory threshold“ (intended as the regulatory limit of tolerance of inflammatory signals in a tissue) is overcome. The dis-balance between proinflammatory and regulatory signals found even under apparently normal o reduced levels of microenvironmental cytokines, generates a smoldering inflammation still capable to damage the tissue structure. Moreover, in a mouse pancreatic cancer model, IL-17 expression resulted important to specifically address the evolution of profibrotic collagen organization. Influence in the cell behavior is also associated with the scaffold modification in its 3D structure, as it appears also in tumor spheroids. Concluding, inflammatory-threshold changes producing smoldering inflammation predispose to chronic inflammation and/or cancer niche establishment. Cytokine levels and collagen scaffold remodeling measured in the tissue can offer new diagnostic markers.

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Mesenchymal cells associated with glioblastomas and brain metastases: Characterization and relationship to immune infiltrate

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Background: Cancer-associated fibroblasts (CAFs) belong among phenotypically heterogeneous mesenchymal cell population and represent important component of the tumor microenvironment contributing to several hallmarks of cancer. They characteristically express a serine protease Fibroblast Activation Protein (FAP). Mesenchymal cells in glioblastomas (GBM) and brain metastases (BM) remain poorly characterized and their putative immunomodulatory role in the microenvironment of these malignancies is currently largely unexplored. The aim of this study was to determine the quantitative and qualitative differences in immune cell populations in GBMs and BMs in relation to the presence of FAP+ mesenchymal cells and to decipher their possible immunosuppressive effect.

Methods: MCP Counter was used to evaluate proportion of immune cell subpopulations in GBMs divided according to the expression of the CAF marker FAP. mRNA expression was analyzed by qRT-PCR and immunohistochemistry (IHC) was used to detect immune cells in paraffin-embedded tissue sections. Localization of FAP+ cells, T-lymphocytes and macrophages was evaluated by IHC double labeling. Mesenchymal cells derived from human GBMs and BMs were characterized by immunocytochemistry (ICC) and RNA-sequencing. Mononuclear cells were isolated from healthy donors´ buffy coats. Markers of M2-polarized macrophages (CD163, CD206, CD14) were analyzed using flow cytometry in monocytes co-cultured with FAP+ mesenchymal cells or exposed to their conditioned media. T-lymphocyte proliferation and expression of activation markers CD69 and CD25 were analyzed using flow cytometry after cultivation of T-lymphocytes in conditioned media from FAP+ mesenchymal cells. 3D co-culture spheroids were prepared from FAP+ mesenchymal cells and GBM stem cells embedded in extracellular matrix.

Results: T-lymphocytes, monocytes/macrophages and other immune cell subpopulations were more abundant in GMBs with a higher proportion of FAP+ mesenchymal cells. In addition, T-lymphocytes and macrophages were frequently in close contact with FAP+ mesenchymal cells. FAP+ mesenchymal cells derived from GBMs and BMs expressed various fibroblast markers as determined by ICC and RNA sequencing. However, transcriptomic analysis revealed substantial differences in comparison with extracranial fibroblasts. In addition, GSEA showed enrichment of immune related GO terms in comparison with mesenchymal cells derived from non-tumorous brain tissue. After exposure of monocytes to FAP+ mesenchymal cell conditioned media, the expression of M2-macrophage markers was increased and this was even more pronounced after direct co-culture. T-lymphocyte proliferation and expression of the CD69 activation marker were decreased after exposure to FAP+ mesenchymal cell conditioned media. In a 3D spheroid model, the number of surviving cells after addition of activated T-lymphocytes was higher in spheroids containing FAP+ mesenchymal cells in comparison with spheroids without them.

Conclusion: FAP+ mesenchymal cells are an integral part of the GBM and BM microenvironment and may induce M2 polarization of macrophages and impair the T-lymphocyte response by limiting the activation, proliferation and cytotoxicity of T-lymphocytes.

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Pátek / Friday — 2. prosince 2022 / 2nd December, 2022

Chairs: Jan Bouchal, Juan Bautista de Sanctis

Perturbations of the sphingolipid and glycosphingolipid metabolism in colon cancer tissue

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Sphingolipids (SLs) and glycosphingolipids (GSLs) contribute to the formation of membrane microdomains, regulation of membrane-bound proteins or formation of extracellular vesicles, and they are involved in intracellular signaling. The alterations of SL and GSL production, or their accumulation, are suspected to play a significant role in cancer development, including colon cancer. They are formed via three closely interconnected metabolic networks: fatty acid synthesis, SL and GSL metabolic pathways. However, our current understanding of molecular basis of the links between tumor development and SL/GSL metabolism is still far from complete. Here, we aimed to characterize in detail changes of a wide range of SLs/GSLs, and the genes related to SL/GSL metabolism, within colon cancer tissue, as compared with the adjacent normal colon mucosa. Two major types of alterations were identified in colon tumor samples: 1) increased levels of SLs that are known to support cancer progression (sphingosine, sphingosine-1-phosphate, ceramide-1-phosphate); 2) increased levels of a series of GSLs (glucosylceramide, lactosylceramide, globoside Gb3 and gangliosides GA2, GM3, GM2 and GD3). While upregulation of CERS, ASAH1 and SPHK1 appeared to be potentially responsible for cancer-related changes in SL levels, upregulation of B4GALT6 (and probably also some hydrolases such as GBA2 and GlcCer transporter FAPP2), and consecutive increased production of LacCer (without apparent changes in glucosylceramide synthase and ganglioside synthases) might represent a key step in increased production of Gb3 and gangliosides. Additionally, increased concentrations of lysosphingomyelin and hexosylsphingosine (produced by increased ASAH1) merit further investigation. The epithelial cells forming a bulk of colon adenocarcinoma tissue appeared to be the primary source of increased levels of SLs and GSLs. The altered profiles of SLs and GSLs, as well as differences in expression of the genes being involved in SL/GSL metabolism may help to identify novel CRC-specific lipid/gene biomarkers, as well as potential therapeutic targets, and contribute to a better understanding of specific lipid-based regulatory mechanisms linked with cancer progression. Besides ceramidase ASAH1 and sphingosine kinase SPHK1, in particular lactosylceramide synthase B4GALT6 appeared to be a relevant candidate gene associated with increased levels of GSLs, with a potential to serve as a biomarker in colon adenocarcinoma. [Supported by the Czech Ministry of Health, grant no. NU21-03-00421.]

Clinical lipidomics: applications in cancer and beyond

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Lipidomics as a branch of metabolomics provides unique information on the complex lipid profile in biological materials. In clinically focused studies, hundreds of lipids together with available clinical information proved to be an effective tool in the discovery of biomarkers and understanding of pathobiochemistry of various diseases. We provide an insight into the application of clinical lipidomics focusing on cancer and other clinical areas with the potential of future

implementation into the routine analysis.

RCC, the most common type of kidney cancer, is associated with high mortality. A non-invasive diagnostic test remains unavailable due to the lack of RCC-specific biomarkers in body fluids. We have previously described a significantly altered profile of sulfatides in RCC tumour tissues, motivating us to investigate whether these alterations are reflected in collectable body fluids and whether they can enable RCC detection. We collected and further analysed 143 plasma, 100 urine, and 154 tissue samples from 155 kidney cancer patients, together with 207 plasma and 70 urine samples from 214 healthy controls. For the first time, we show elevated concentrations of lactosylsulfatides and decreased levels of sulfatides with hydroxylated fatty acyls in the body fluids of RCC patients compared to controls. These alterations are emphasized in patients with the advanced tumour stage. Classification models are able to distinguish between controls and patients with RCC. In the case of all plasma samples, the AUC for the testing set was 0.903 (0.844–0.954), while for urine samples it was 0.867 (0.763–0.953). The models are able to efficiently detect patients with early- and late-stage RCC based on plasma samples as well. The test set sensitivities were 80.6% and 90%, and AUC values were 0.899 (0.832–0.952) and 0.981 (0.956–0.998), respectively. Similar trends in body fluids and tissues indicate that RCC influences lipid metabolism and highlight the potential of the studied lipids for minimally-invasive cancer detection, including patients with early tumour stages, as demonstrated by the predictive ability of the applied classification models.

The intensive development of new non-invasive microsampling technologies is paving the way for a new era of patient-friendly precision medicine. We have designed a concept of 3D-printed attachment with porous glass filter disks called SLIDE (Sweat sampLIng DevicE) for easy sampling of AS. By applying pseudotargeted LC(C8)-MS(MRM) lipidomics the relevant lipids present in AS have been selected and semiquantified to evaluate the reproducibility and robustness of this novel approach. SLIDE was tested on 10 healthy individuals for three time points over a week for the evaluation variability and reproducibility. The applicability of SLIDE for cancer screening was tested on 20 clinically defined patients and 23 controls in parallel with the standard screening process in the clinic. Patients were found to have increased concentrations of ceramides and free fatty acids and conversely decreased hexosylceramides. The main advantages of AS microsampling include the non-invasiveness of the procedure, speed of collection and patient comfort, while the application of SLIDE as an AS sampling technique brings new perspectives for use in modern clinical practice.

Finally, several of our clinically focused projects will be shown where the potential of clinical lipidomics provided how it can be used to describe the pathobiochemistry of various diseases or to stratify groups of patients.

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Molecular profiling of cardiovascular diseases applying an untargeted lipidomics approach

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Cardiovascular diseases (CVDs) are the leading cause of death in the developed and developing world, taking almost 17.9 million lives each year. Estimates are that by 2030 the annual mortality of CVDs is expected to reach 23.6 million. Up to 80% of all cardiovascular deaths can be prevented by risk assessment and proper implementation of preventive measures. Thanks to this, it is crucial to detect the cardiovascular disease as early as possible so that management with counselling and medications can start and prevent these premature deaths. Cardiovascular disease risk assessment using new lipid biomarkers should be essential to individualise target cholesterol levels or other markers of lipid metabolism. This issue is topical in targeted therapy with PCSK9 inhibitors or small interfering RNA molecules.

Lipidomics (considered a subset of metabolomics) studies and measures lipid molecule levels across phenotypes. Lipids are a very diverse group of molecules playing an essential role from the cellular level up to the organism level. Besides their structural function of forming membranes, they can act as messenger molecules in autocrine, paracrine signalling, and autophagy.

Disruption of lipid metabolism plays a critical role in many pathobiochemical processes. Promising results of recent lipidomics studies have defined several CVD-related lipid classes (mainly ceramides) and individual plasma lipids, which are slowly getting the attention of clinicians, given that they are already part of the Mayo clinic test catalogue (CERAM test). We can measure hundreds of lipids in a single analysis by liquid chromatography coupled with mass spectrometry.

The approach of untargeted lipidomics is a tool for hypothesis generation and delivers the

semiquantitative type of data. Here in our work, we present data from analyses of lipid profiles of patients after STEMI (ST-elevation myocardial infarction) compared to the control group. Since there is no focus on a particular group of lipids, we are not eliminating the possibility of revealing the unanticipated changes, including unknown metabolites and pathways. With the intelligent acquisition of fragmentation spectra and the application of neural networks, we were able to group molecules based on their structure similarity, thus revealing new biomarker candidates. Many of those compounds were successfully annotated using machine learning algorithms (SIRIUS).

Results suggest that molecular profiling of lipids seems to be a promising tool for diagnosing CVDs.

Comparative effects of fatty acid supplementation on human NK cell cytotoxic activity of healthy and overweight young and old adults

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NK cells are essential in cancer immune surveillance. Their efficiency is affected by obesity and ageing. The study aimed to analyse NK cytotoxic efficiency of young and elderly individuals, overweight versus having a normal BMI, against susceptible tumour cells (K562) and resistant (Daudi) cell lines. The effect of fatty acid supplementation before the cytotoxic challenge was analysed. Eighty normolipemic donors of both genders were divided into four aged-matched groups. Significant differences were observed in lymphocyte subpopulations among groups ($p<0.01$). When purified NK cells were pre-incubated with saturated fatty acids (C16, C18, C20, C22 and C24), the cells were more cytotoxic, independently of the group and the target ($p<0.001$). K562 and Daudi cells supplemented with saturated fatty acid were more resistant to NK lysis ($p<0.001$). When NK cells were incubated with EPA or DHA, NK cytotoxic response against K562 increased ($p<0.05$), not affecting Daudi cell lysis. However, when both tumour cell lines were incubated with EPA or DHA, they were more susceptible to NK lysis ($p<0.001$). Thus, saturated fatty acids may induce tumour survival despite increasing NK cytotoxic activity, and ω3 fatty acids increase K562/Daudi cell susceptibility to NK cell killing. Mitochondrial metabolism could be critical in tumour resistance and NK cell killing.

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Pátek / Friday — 2. prosince 2022 / 2nd December, 2022

Chairs: Ondrej Slaby, Jozef Skarda

Impact of the comprehensive genomic profiling on the individual therapeutic planning in high-risk/refractory tumors: real-world precision medicine in pediatric oncology

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Background: Despite major improvements in the survival of pediatric cancer patients that were achieved through the intensification of chemotherapy and the perfection of supportive care in the past decades, treatment outcomes for high-risk, relapsed, and refractory solid cancers remain unsatisfactory. Accelerating the progress of pediatric oncology requires both therapeutic advances and attention to reducing the long-term cytotoxic treatment-related side effects. This could be achieved by targeting specific molecular changes that drive pediatric malignancies, which have been identified through rapidly progressing large-scale molecular profiling techniques. Material and methods: From September 2016 to August 2022, a total of 192 patients with pediatric high-risk solid tumors successfully underwent comprehensive genomic profiling. Since more than thirty patients had two or more biopsies from recurrent relapses, the total number of samples examined was 295. In the cohort, there were 78 cases of central nervous system tumors, 68 sarcomas, 14 neuroblastomas, 10 lymphomas, and 22 tumors of other histology. Whole-exome sequencing was performed in all patients, fusion gene analysis in 96% of patients, whole-transcriptome profiling in 84% of patients, and CNV analysis in 63% of patients. Results: The diagnostic yield of therapeutically actionable findings was 40%, with single-nucleotide variants and small insertions/deletions being the most common actionable alteration types. In 23% of patients, a clinically relevant gene fusion was identified. The majority of the identified fusions were of diagnostic significance, and 18% of those were therapeutically targetable gene fusions involving BRAF, RAF1, ALK, FGFR1, or NTRK2. Four patients were eligible for immunotherapy based on high tumor mutational burden (>10 mut/Mb). Lymphomas and CNS tumors showed the highest rate of patients with therapeutically actionable findings (60% and 56%, respectively), followed by neuroblastomas (36%), sarcomas (25%), and other solid tumors (23%). All results and individual treatment plans were discussed at multidisciplinary molecular tumor boards. Conclusion: Precision medicine in pediatric oncology has rapidly developed over the last decade and resulted in new therapeutic options based on molecular biomarkers and increased our understanding of the complexity of pediatric malignancies. Further implementation of comprehensive genomic profiling into clinical practice is a necessary step to advance the overall management of pediatric cancer patients.

Automated NGS and its strong positive effect on an oncology patient survival rate and improvement on the healthcare service

Filip Držík

Clinical Account Manager, ThermoFisher Scientific

The importance of fast and accurate diagnosis is nowadays an absolute key factor when working with oncology patients. Next generation sequencing technology, which provides sufficient accuracy, depth and robustness, has long been perceived to be one of the best methods in this area. However, what this technology lacked until recently was time efficiency. In 2021 and 2022, several studies were published that dealt with the use of rapid NGS diagnostics to improve patient survival. It turns out that with the right laboratory setup, the improvement after implementing the NGS is enormous. One of the latest studies on this topic is a study by the author Robert E. Smith and his team published in *The Journal of Clinical Oncology*, in which the authors examine and evaluate the results of the use of early targeted diagnostics based on NGS. This early NGS adoption made it possible to implement targeted treatment for patients with non-small cell lung cancer in stage 4. The results point to the enormous influence of such diagnostics approach used as one of the primary diagnostics approaches. The results of this study are further enhanced by another article by Brandon S. Sheffield et.al. published in *The Current Oncology journal*, which talks about the implementation of rapid NGS diagnostics within the standard conditions of medical care and its impact on the quality and speed of response in clinical practice. The results after the inclusion of a fully automated NGS approach with standard methods, such as IHC, for the diagnosis of various types of tumors, including NSCLC, melanomas, colorectal carcinomas and others, speak of a rapid acceleration in results provision and subsequent correct therapy within three working days from the initial ,more than two weeks time frame, standard response turn around time. This significant impact of fully automated NGS not only increases the quality of the services provided in healthcare, but also significantly boosts the probability of longer survival of patients with serious conditions.

Applications and challenges of artificial intelligence in digital pathology

Mariam Gachechiladze

ARTIDIS AG, Basel, Switzerland

Artificial intelligence (AI) refers to the simulation of the human mind in computer systems that are programmed to think like humans and mimic their actions such as learning and problem-solving. AI has become an integral part of everyday life, solving problems in many different areas and pathology is no exception. AI neuronal networks entered in digital pathology for their ability to perform exceptionally well in image recognition and classification tasks. Currently, it is perceived that AI based digital pathology approaches may improve the clinical workflow efficacy, diagnostic quality and ultimately create personalised diagnosis and treatment plans for patients, by recognising clinically meaningful tissue features, far beyond the capacity of human based visual assessment. Therefore, there is rigorous research is ongoing in the field. Proposed talk will include the overview of the history of AI in digital pathology, significant developments, current status and challenges of the implementation of AI in everyday pathology practice.

The future of pathology

Agnieszka Ciesielska

10xGenomics, Leiden, Netherlands

Using high-throughput single cell analysis to understand cellular diversity in tissues has opened new avenues of investigation and fueled new discoveries. These insights are only made richer when combined with spatial information. Examining spatial cellular patterns reveals details of the organization of normal and diseased tissue, the immune response and the complex arrangement of neural tissue.

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The drugs' mechanism of action identification based on digital-phase contrast images analyzed by AI

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The mechanism of action identification is a crucial phase of drug development; thus, developing high-throughput screening techniques for that purpose is very important. In this study, we show an analysis of topoisomerase inhibitors' effect on living cells. The study was performed assuming that compounds with different effects impact cell phenotypes differently. We used digital-phase contrast images to observe cell phenotypes; this is easier and faster than traditional fluorescence microscopy. Although these images could be challenging to analyze due to complex cell appearance and imaging artifacts, we overcome this by developing the CNN trained end-to-end directly on the input images without requiring manual segmentations or any other auxiliary data. Our method can distinguish between tested cytotoxic drugs with an accuracy of 98%, provided that their mechanism of action differs, outperforming previous work. The results are even better when substance-specific concentrations are used. We show the benefit of sharing the extracted features over all classes (compounds) and 2D visualization with cluster annotation. The experimental workflow could be fully automated using a robotics platform with Cell Voyager CV8000 as a High-Content Screening System.

This work was supported by European Union – Programme EXCELES, ID Project No. LX22NPO5102, the Czech Ministry of Education, Youth and Sports (CZ-OPENSCREEN - LM2018130, EATRIS-CZ - LM2018133), and by the internal grant of Palacky University Olomouc (IGA_LF_2022_033) and European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868).

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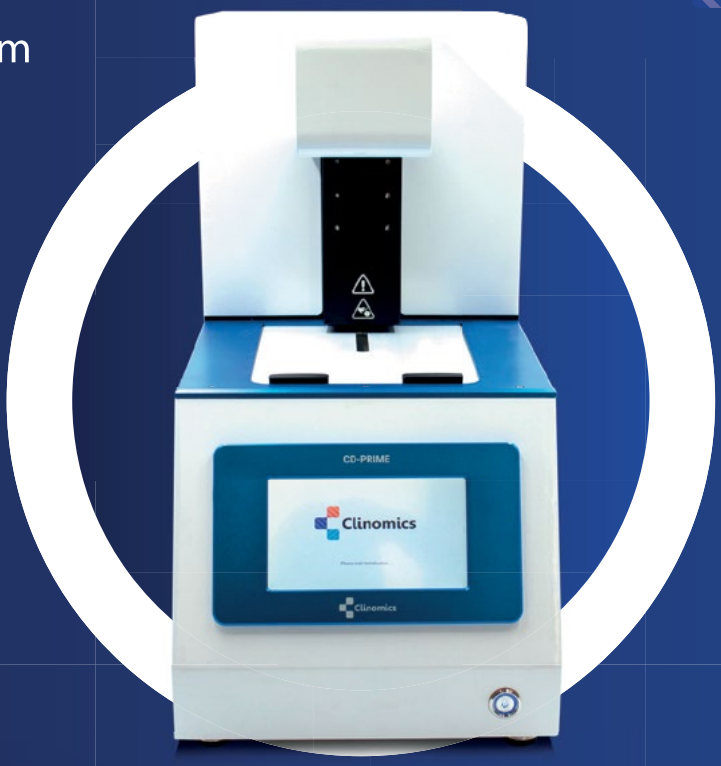

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KOPRETINA study: Pilot self-sampling device distribution to Czech women who should be participating in the cervical cancer screening program

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Introduction: The implementation of primary HPV screening and increasing cervical screening attendance are major challenges to cervical cancer screening in the Czech Republic. The offering of self-sampling to cervical screening non-attenders could significantly increase participation as was shown in several European countries.

The objective of this study was to bring the pilot data about the acceptance of self-sampling by Czech women and to find out the high-risk HPV (hrHPV) prevalence in the screening population.

Methods: Evalyn® Brush self-sampling kits (Rovers Medical Devices) were distributed by mail between October 2019 and March 2020 to 6388 women aged 30-65. Women were chosen from the database of Naturamed, Ltd. clients regardless of their cervical cancer attendance. After the self-sampling, samples were returned free of charge by regular mail. All samples were tested for hrHPV DNA using the Qiascreen HPV PCR Test (Qiagen). Results were delivered to women by mail or e-mail with the recommendation to schedule the check-up regardless of their HPV status.

Results: The response rate in this study was 7.61% (486/6388). All samples were suitable for analysis using Qiascreen by which hrHPVs were detected in 7.47% (36/486) self-samples. Seven HPV16 (19.4%), one HPV18 (19.4 %), and 28 other (77.7 %) high-risk HPV-positive cervical/vaginal samples were identified.

Conclusion: HrHPV was detected in 7.61% of women chosen from the cervical cancer screening population. The offering of self-sampling could significantly increase the attendance of Czech women in the cervical screening program.

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Analysis of ccfRNA markers of prostate cancer progression and ARTA therapy failure in liquid biopsy samples of patients with advanced prostate cancer

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Prostate cancer (PCa) is the second most commonly occurring cancer as well as the second leading cause of cancer-related deaths in men in Western countries. Advanced PCa can be treated by androgen deprivation therapy in combination with androgen receptor targeted therapy (ARTA). However, after some time PCa develops an ability to survive in a low androgen environment resulting in the therapy resistance (CRPC, castration resistant prostate cancer). Our goal is to monitor changes that may indicate therapy failure, disease progression, and metastatic activity of cancer. Therefore, we collect liquid biopsy samples of advanced PCa patients before, during, and after ARTA therapy treatment. The circulating cell-free RNA is isolated from 2 ml of plasma and is used for RT-PCR examination of the presence and quantity of selected mRNA and miRNA markers. We were able to analyse ccfRNA markers: AMACR, AR, Arv7, EpCAM, synaptophysin, and TBP and also miRNA-375 in baseline plasma samples of mCRPC patients (n=29). Our data suggest that patients with high expression of miR-375, as well as patients with high levels of PSA benefited from ARTA therapy for a significantly shorter time (p=0.0023 and p=0.0336, respectively), which is in line with other studies. Also, correlations of circulating AMACR with AR and EpCAM were found, however, without relation to clinicopathological parameters. These results are currently being validated in a larger cohort which contains approximately 360 samples from 120 patients. Analysis of miRNA-375 can be useful tool for ARTA therapy monitoring and can help clinicians with a difficult treatment switch decision.

Anticancer effects of steroid cardiac glycoside derivatives

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Cardiac glycosides are bioactive

natural compounds well-known mainly for their potency to induce a cardiotonic effect by sodium-potassium ATPase (NKA) inhibition. For many years, cardiac glycosides have been utilized to treat heart failure and arrhythmias; however, according to novel research studies, these compounds have an enormous potential also as medicinally promising compounds for cancer treatment. This study describes the effects of cardiac glycoside derivatives on prostate cancer cells. The experiments were performed with DU-145 and LNCaP prostate cancer cell lines in comparison with RWPE-1 non-cancer prostate cell line. Only one active cardiac glycoside derivative (oleandrigenin-related androstane derivative) from the group of tested compounds was shown to strongly influence cell viability, proliferation, cell cycle distribution, apoptosis, nuclear steroid receptor expression and localization and molecular pathways responsible for these processes in prostate cancer cells without an effect on a non-cancer prostate cell line. The cancer and normal cells respond differently to certain aspects of the structure of cardiac glycoside derivatives. At present, only a few natural agents are known to offer potential for the selective/preferential elimination of cancer cells without affecting the growth of normal cells. Cardiac glycoside derivatives are promising for the development of new anticancer drugs.

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Comparison of Age Prediction from Blood by Pyrosequencing and Massively Parallel Sequencing

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Probably the most pursued and the most rapidly developing phenotyping tool all over the world is age prediction. Most of the published methods rely on Massively Parallel Sequencing (MPS) while one of the commercially available epigenetic typing kits, AgePlex from Qiagen, is based on pyrosequencing. In this project, we tried to develop an MPS age prediction model that would yield better performance characteristics than AgePlex for the Czech population.

We tested 81 CpGs in areas previously published as differentially methylated depending on age using methyl-specific qPCR in duplexes followed by MPS on Illumina platform MiSeq and NovaSeq. The statistical model was developed using the 7 most informative CpGs in the testing set of 100 samples and was validated using the set of another 100 samples. For benchmarking, the same set of samples was tested by the AgePlex kit.

The final prediction model consists of individual CpGs in genes *CCDC102B, ELOVL2, PDE4C, FHL2* and *C1orf132 (MIR29B2CHG)*. The rate of successful prediction was 89 % in the range of 4 years, with a mean absolute error of 1.79 years and a mean square error of 3.0 years.

Here, we present more detailed results of prediction model validation and external quality control with the University of Cologne and the Czech Criminalistic Institute in Prague. Additionally, we present a performance comparison for both our MSP-based prediction method and commercially available pyrosequencing-based prediction method AgePlex.

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Anticancer effect of novel 1,4-benzodiazepine derivatives through tubulin polymerization inhibition

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Benzodiazepines are well known as a key scaffold in medicinal chemistry, providing bioactive compounds for a wide variety of biological targets. Among their primary use as anxiolytics and sedatives, the 1,4-benzodiazepine derivatives and their metabolites play a role as antitumor, antithrombotic, antiviral and antimalarial agents.

The antiproliferative activities of novel derivatives were tested against a panel of 10 cancer cell lines and 2 non-cancer cell lines. The compounds with IC50<10 μM were further analyzed for the cell cycle. Flow cytometry and fluorescent cell cycle indicator (FUCCI) assays were performed to evaluate the effects of derivatives on the cell cycle phases. The immunofluorescence alpha-tubulin assay and tubulin polymerization assay were accomplished to detect the changes in the mitosis. Implementation of molecular docking revealed the specific binding site of the derivatives.

All tested derivatives reveal the G2/M block in the cell cycle and 3 of them were identified as M-phase blockers. The immunofluorescence alpha-

tubulin assay detected the disruption of the cellular microtubule network, and the tubulin polymerization assay uncovered the potential of the tested compounds to inhibit tubulin polymerization. Molecular docking confirms that benzodiazepine derivatives fit the colchicine site best and are partially overlaid with the native colchicine binding pose. This suggests that these compounds may adapt to the tubulin binding site in a conformation induced by colchicine.

The inhibition of tubulin polymerization by novel derivatives is one of their possible mechanisms of action on antiproliferative effect on cancer cell lines. More rigorous studies will be performed further to establish pose stability and explain structure-activity relationships.

The role of transcription factor Sp1 in the active demethylation process of multiple myeloma cells

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Trascription factor Sp1, which is expressed in all mammalian cells, plays an important role in regulating the transcriptional activity of genes involved in most cellular processes including oncogenesis. By binding to respective gene promotores, Sp1 is able to up-regulate gene expression, and its dysregulation is associated with several cancer types and diseases. TET proteins are enzymes responsible for the conversion of 5-methylcytosine to 5-hydroxymethylcytosine and its further oxidation to 5-formylcytosine and 5-karboxylcytosine during the active demethylation process. These demethylation enzymes are probably involved in tumor progression through the epigenetic reprogramming. The role of Sp1 transcription factor in the active demethylation process in multiple myeloma cells is still underscribed. Sp1 could be involved in this process by binding to CpG islands located in TET promoters, with subsequent changes in the expression of the respective genes: TET1, TET2, TET3. The occurrence of Sp1 in complex with TET promoter regions in treated myeloma cell lines KMS12-PE and KMS12-BM was determined using chromatin immunoprecipitation method followed by quantitative real-time PCR. RNA for the cDNA expression profile analyses of TET enzymes was isolated from myeloma cells treated with two demethylation agents: 5-azacytidine (AZA) and/or 5-aza-2´- deoxycitidine (DAC). Moreover, the expression profile of studied TET genes was performed in triplicate from two independent repeats using quantitaitve real-time PCR. Relative increase in the formation of the Sp1 complex containing the promoter sequences of all three studied genes TET1, TET2 and TET3 was detected in KMS12-PE myeloma cells after the AZA treatment, but not in DAC-treated myeloma cells. In addition, multiple myeloma cells treated with both demethylation agents showed significantly increased normalized expression of TET2 gene compared to TET1 and TET3 genes. Based on our results, Sp1 participates in the formation of a complex containing the demethylation genes TET1, TET2 and TET3 in KMS12-PE myeloma cells preferentially after treatment with the demethylation agent AZA, and thus apparently affects their transcriptional activities. Furthermore, in treated myeloma cells we detected a significant increase of TET2 gene expression, after the both used AZA and DAC treatments. Finally, treatment of multiple myeloma cells with AZA causes specific binding of Sp1 to TET promoter sequences, while the demethylation effect of both used AZA and DAC treatments used leading to a significant increase in TET2 gene expression could indicate the presence of methylated sequences in the TET2 gene promoter in multiple myeloma cells.

Discrimination of resected glioma tissue using surface enhanced Raman spectrosco py and Au@ZrO_2 nanocomposite

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Gliomas present one of the most prevalent malignant tumors related to the central nervous system. Diagnosis and follow-up classification of gliomas mostly rely on tumor histopathologic properties, which provide limited information on the response to a follow-up therapy or different patient prognosis. A surgical extraction is still a preferred route for glioma treatment. Nonetheless, neurosurgeons still have a considerable challenge to intraoperatively and correctly detect actual margins of the targeted glioma because of its great infiltrate nature. The residues of the original tumor or existing tiny satellites being left aside are one of the main reasons leading

to recurrences of the disease and a poor prognosis for the respective patients.

Here we evaluated the possibility of using surface-enhanced Raman spectroscopy to analyze freshly resected brain tissues. The developed method is based on the application of Au@ZrO_2 nanocomposite, which dramatically lowers the fluorescence present in the Raman data, and thus considerably improves the quality of the measured signal. The developed method allows for rapid discriminating between the glioma's periphery and central parts, which could serve as a stepping stone toward high-precise neurosurgery.

Nová laboratoř *in situ* hybridizace ve Fakultní nemocnici Ostrava

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S rozvojem diagnostiky a péče o onkologické pacienty došlo ke spojení ústavu patologie a oddělení lékařské genetiky ve Fakultní nemocnici Ostrava s cílem vyšetřování genetických aberací u onkologicky nemocných pacientů. V důsledku tohoto spojení byla otevřena i laboratoř fluorescenční *in situ* hybridizace (FISH), kde se pro vyšetření standardně využívají formalínem fixované histologické řezy nádorových tkání. Výsledky je tak možno korelovat s imunohistochemickým nálezem patologa.

Pro vyšetření se používají specifické přímo značené fluorescenční sondy k identifikaci nejčastějších genetických aberací typických pro dané nádorové onemocnění. Hodnotí se počet signálů (amplifikace/delece genů) nebo jejich uspořádání (translokace) v interfazických jádrech.

V laboratoři fluorescenční *in situ* hybridizace jsou vyšetřovány prediktivní markery pro karcinom prsu a NSCLC a další tumor specifické genetické změny.

Synthesis, cytotoxicity and mechanism of action of triterpenoid pyrazines and pyridines and their prodrugs

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In this work, we have prepared a set of fifteen triterpenoid pyrazines and pyridines from parental triterpenoid 3-oxoderivatives (betulonic acid, dihydrobetulonic acid, oleanonic acid, moronic acid, ursonic acid, heterobetulonic acid, and allobetulone) and subjected them to the biological evaluation. First, cytotoxicity of all compounds was tested on the panel of eight cancer and two fibroblast cell lines. Thereafter, we performed small SAR study, based on which we revealed that the triterpenoid core determines whether the final molecule is active or not, while the heterocycle substituent is able to improve the activity and modulate the specificity. Interestingly, five compounds (1b, 1c, 2b, 2c, and 8) were found to be preferentially and highly cytotoxic (IC50 ≈ 1 μM) against leukemia cell lines (CCRF-CEM, K562, CEM-DNR, or K562-TAX). Moreover, compounds 1c, 2b, and 2c proved 10-fold higher cytotoxic activity against leukemia cell lines with MDR phenotype (CEM-DNR and K562-TAX) compared to their non-resistant counterparts (CCRF-CEM and K562). Further, pharmacological parameters were evaluated for the most promising candidates and two types of prodrugs were synthesized: The first group comprises sugar-containing conjugates, most of which had improved cell penetration and retained high cytotoxicity toward CCRF-CEM cell line. On the other hand, addition of sugar moiety led to the loss of selectivity against resistant cells. Second group of prodrugs comprises medoxomil derivatives, among which compounds 26-28 gained activities ranging between IC50 0.026-0.043 μM in K562 cells. Finally, compounds 1b, 8, 21, 22, 23, and 24 were selected for the detailed study of the mechanism of action based on their lowest IC50 in CCRF-CEM cell line. Several experiments showed that the majority of them induce apoptosis via the mitochondrial pathway. Interestingly, compounds 1b, 8, and 21 inhibit growth and disintegrate spheroid cultures of HCT116 and HeLa cells, which would be important for the treatment of solid tumors. In summary, compounds 1b, 1c, 2b, 2c, 24, and 26-28 are highly and selectively cytotoxic against cancer cell lines and were selected for further development of anticancer drugs.

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New antimitotics derived from 4-thiazolidinone interfere with microtubule dynamics

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Drugs possessing 4-thiazolidinone scaffold display a wide range of biological activities including anticancer, antiinflamatory and antiviral effects. 5-ene-2-arylimino/amino-4-thiazolidinones are of special interest as one of the most perspective groups among 4-thiazolidinones. Newly synthesized 5-arylidene-2-(4-hydroxyphenyl)aminothiazol-4(5H)-ones derivatives had a significant antimitotic activity and were shown to induce mitotic arrest by interfering with microtubule polymerisation. In silico predictions located binding site of the compounds to the colchicine binding site.

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Effect of opioid and cannabinoid receptors gene expression on survival of patients with colorectal cancer

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Introduction: Colorectal cancer (CRC) is one of the leading causes of cancer death in the world. CRC patients are often treated with opioid (e.g., morphine, or synthetic opioid piritramide) analgesia for pain, nausea, and side effects of chemotherapy. These analgesics act through opioid and cannabinoid receptors, which pathways are involved in tumor progression and metastases and thus can negatively affect the survival of patients.

In our previous studies, we analyzed the presence of circulating tumor cells (CTCs) in two groups of CRC patients. One group received morphine analgesia (MA) and second piritramide analgesia (PA). We found that MA associated with increased CTCs levels and significantly shorter cancer-specific survival (CSS). These findings lead to further analysis of the opioid and cannabinoid receptors tumor gene expressions. We expected that the different expression of specific receptors in tumor tissue makes a difference between the effects of MA and PA on patients´ survival.

Methods: Gene expression of opioid receptors μ (OPRM), κ (OPRK), δ (OPRD), nociceptin (OPRL) and cannabinoid receptors 1 and 2 (CB1, CB2) was analyzed in RNA purified from tumor tissues in 131 patients with CRC. Expression of markers was detected using real-time RT-PCR on LightCycler 1536 from Roche. B-actin gene expression was used for gene expression normalization. Specific cut-off values were calculated for each marker using maxstat R software, ver. 3.3.1. Relationship between expression of receptors in tumor tissue and patients' survival was analyzed using COX regression, Kruskal-Wallis/ANOVA test, and Kaplan-Meier method.

Results: In total, 118 patients (45 females and 73 males, average age 68 years), of clinical stage-III were analyzed. It was found that MA in CRC patients was associated with significantly shorter cancer-specific survival (CSS; p=0,027). The univariate survival analysis revealed that CRC patients with high OPRM tumor tissue gene expression had significantly longer overall survival (OS; p=0.017) and patients with CB2 gene expression had significantly longer disease-free survival (DFS; p=0,029). All other tested opioid receptors expressions had no statistically significant effect on patients' survival.

Conclusion: The diverse opioid receptors' expressions on tumor and immune cells can affect the survival of cancer patients treated with opioid analgesia. Morphine perioperative analgesia seems to shorten the CCS in CRC patients in our study. However, further research is required to elucidate the effect of opioids on different cell populations.

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New NPL4 protein inhibitors as possible anticancer therapeutics and their mechanism of action

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NPL4 protein is one of the cofactors of p97 complex which is connected with the Ubiquitine Proteasome System [1] and is a possible target in antitumour therapy, especially when its higher expression was observed in bladder cancer [2]. Dithiocarbamates (DTCs) are small organic compounds that easily form complexes with metals, especially copper [3]. One of these complexes – bis(diethyldithiocarbamate)-copper complex (CuET) – acts as NPL4 inhibitor and causes NPL4 aggregation in cells, heat shock response and accumulation of polyubiquitinated proteins [4]. To see whether this effect can be observed also in other DTCs, 23 other DTCs that were either synthesized or commercially available, were tested. For basic screening for the effect of DTC-copper complexes on NPL4 protein a developed flow cytometric assay was used, where the NPL4 GFP signal was measured after pre-extraction. During the screening 14 of 23 DTC-copper complexes immobilized NPL4 in cells and the cytotoxic screening identified the same number of complexes with anticancer activity. A positive correlation between the effect on NPL4 protein and cytotoxicity in DTC-copper complexes was found (R2 = 0,75). In addition, the interaction between DTC-copper complexes and purified NPL4 was confirmed in vitro using DARTS method. NPL4 protein is a promising target in antitumour therapy, and its inhibition leads to severe damage in cancer cells. DTC-copper complexes are capable of this inhibition and have the potential to become possible future anticancer drugs.

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The role of ABCB1 and NOTCH3 in the resistance to taxanes and mitochondrially targeted iron chelators

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Conventional taxanes paclitaxel (PTX, Taxol®) and docetaxel (Taxotere®) show high antitumor efficacy and are widely used in the treatment of breast cancer. However, the appearance of resistance of tumor cells limits the clinical use of conventional taxanes. Therefore, the laboratory of Prof. I. Ojima developed second and third generation taxoids called Stony Brook Taxanes (SB-Ts). SB-Ts overcome PTX resistance and reduce drug efflux by ABCB1 (P-glycoprotein; P-gp). In the present work, we show that SB-Ts are more effective in killing cancer cells with high ABCB1 level, acquired by either genetic means or by continuous culture in the presence of PTX. Furthermore, we have revealed the existence of cross-resistance to mitochondrially targeted iron chelators, a new class of anticancer compounds recently introduced by our group, in PTX resistant cells.

Our patient data suggested alterations in the Notch signaling pathway especially in patients carrying breast and ovarian tumors that were refractory to the treatment, being in line with the already published evidence that Notch signaling plays a multiple role in carcinogenesis. Therefore, in order to delineate the role of Notch signaling, we generated NOTCH3 KO MCF7 breast cancer cells and tested the effect of PTX and SB-Ts on their viability and cell death. Our results show that NOTCH3 KO does not significantly alter the cytostatic effect of all compounds in MCF7 cells. However, NOTCH3 KO cells were much less sensitive to cell death induced by the taxanes. Interestingly, we observed that the deletion of the NOTCH3 locus also provided protection against the cytotoxic effect of mitochondrially targeted iron chelators. Altogether, our results further strengthen the notion of SB-Ts as potential alternatives in the treatment of chemoresistant tumors and opens the door to further research in the role of NOTCH3 signaling in the sensitivity of cancer cells to cytotoxic agents.

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Development of new PSMA-specific antibody-based tools for immunotherapy of prostate cancer

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Prostate cancer is one of the most common death-causing malignancies in man population. Serious consequence of progressed disease leads to innovations and development of new highly specific tools and medical treatments that would efficiently target prostate tumor and derived metastases. Research interest is mainly focused on biomarker Prostate-specific membrane antigen (PSMA) that is present in high number on the surface of progressed prostate cancer cells. Engineered antibody-derived molecules oriented to PSMA thus would specifically target prostate cancer cells with minor toxicity in non-target tissues.

Our team developed and characterized PSMA-specific antibody 5D3 that recognizes native PSMA with high specificity and affinity under in vitro as well as in vivo conditions. Furthermore, engineered fragments of 5D3 antibody were evaluated in mouse model of xenografted prostate cancer. The fragments were further manipulated into the form of bispecific molecules that would engage host immune cells in the site of prostate cancer. Namely, engineered molecule-driven linkage of cells is supposed to trigger specific immune anti-tumor activity potentially resulting in the elimination of cancer cells. In vitro study showed that 5D3 fragment fused to anti-CD3 fragment was able to link T lymphocytes with PSMA-positive cancer cells and transmitted cancer cell death. Moreover, 5D3 fragment linked to cyclic peptide 33 triggered production of reactive oxygen species in activated monocytes when PSMA antigen was present. The data show high potential of 5D3-derived molecules for immunotherapy treatments of PSMA-positive tumors.

A high-throughput screening campaign to identify inhibitors of carbonic anhydrase IX

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Human carbonic anhydrase IX (CA IX) plays a crucial role in cancer cell proliferation and metastases. Its overexpression in hypoxic tumors is associated with malignant progression and poor treatment outcome. Therefore the inhibition of CA IX activity could be a promising approach to novel anticancer therapies. The aim of this study was to identify new inhibitors of CA IX.

A new fluorescence-based assay was designed to identify small molecule inhibitors of carbonic anhydrases in HTS conditions. In this assay, pyranine, as a fluorescent indicator of pH change, was used. The assay was validated on the commercial libraries LOPAC and Prestwick. In the primary screen, unique compounds from IMTM proprietary library were analyzed at one concentration (10 μM) and the PI (percentage of inhibition) values were calculated. Data were analyzed by Dotmatics software. To quantify the suitability of the assay in HTS, the Z-factor was determined for each plate.

Obtained results from validation and primary screen as well as identified hits will be presented and discussed.

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In vitro cellular models of nucleoside-based drugs resistance

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Nucleosides are purine or pyrimidine analogs used in antiviral or anticancer therapy. Like other drugs, also nucleosides face the problem of drug resistance, which complicates the treatment of cancer patients. In our project, we developed in vitro cellular models resistant to nucleoside-based drugs cytarabine, fludarabine, and 6-thioguanine. We chose two leukemia cell lines (CCRF-CEM and K562) to develop resistant clones, and the selection was successful in both cases. For resistant cells, the expression level of proteins associated with the metabolism of nucleoside-based drugs (CDA, HGPRT, XO, etc.), nucleoside transporters (ENTs, CNTs) and proteins related with multidrug resistance phenotype (Pgp, MRP, BCRP, LRP) was analyzed. This information can help to understand the mechanism of the development of specific drug resistance. Resistant cell lines were also used to screen new potential anticancer drugs to test their ability to overcome such drug-resistant phenotypes.

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The identification and characterization of anticancer activities of unique, nucleoside-based A3 adenosine receptor agonists

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Adenosine receptors are G protein-coupled receptors (GPCR). There are four types of adenosine receptors (A1, A2A, A2B, and A3). They play roles in several pathological conditions, including cancer. The finding of new agonists and antagonists as potential anticancer drugs is challenging. We have used a cell-based aequorin-coupled reporter system to monitor adenosine GPCR signalling. The hits from the primary screen were evaluated in the counter-screen (calcium mobilization assay) and confirmed in concentration-dependent response experiments. Several compounds with previously observed cytotoxic activity and a selective A3 adenosine receptor agonist activity were identified in our screening. The compound (code name PNH173) has significant cytotoxic effects against several cancer cell lines derived from tumors of various histogenetic origins, with almost no cytotoxic activity against normal human fibroblasts. PNH173 demonstrates good pharmacological properties in non-clinical ADME tests, reduces tumor growth, and increases overall survival in „in vivo“ mice experiments. To study the mechanism of action of PNH173, a fluorescently labelled A3 adenosine receptor antagonist CELT-171 was used. The PNH173 binding to the orthosteric binding site on the A3 receptor was verified by competitive assay with CELT-171, measuring the fluoresce signal level at the cellular membrane of viable cells. The study's aim was also to analyze downstream signalling pathways responding to the PNH173 treatment, especially inhibition of the PI3K/Akt signalling pathway.

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Mitochondrial changes are important for the tamoxifen-resistant cells to survive the therapy

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Tamoxifen (Tam) resistance represents a major clinical issue in the treatment of ER+ breast cancer patients. Despite the continuous development of new therapeutic approaches, between 30 and 50% of responsive Tam-treated patients eventually develop resistance to the therapy. It has been shown that Tam not only affects the estrogen-signaling pathway but it is also able to alter mitochondrial function. For this reason, we hypothesized that mitochondria could play an important role in Tam-resistant cells (TamR cells). In order to answer this question, we generated MCF7 Tam5R and T47D Tam5R cells by long term cultivation in the presence of 5 μM Tam. Our results clearly show differences in terms of lower respiration and higher dependence on glycolysis in TamR cell lines, in comparison with the parental controls, as shown by the increased lactate production and the higher sensitivity to 2-deoxyglucose. Furthermore, Tam5R cells have reduced formation and activity of mitochondrial supercomplexes and higher levels of mitochondrial reactive oxygen species (ROS). Moreover, results from confocal microscopy and flow cytometry indicate variations in the network structure and the number of mitochondria between parental and TamR cells. Importantly, a very similar phenotype can be observed in a cell line with dysfunctional mitochondria (rho zero cells) which are markedly resistant to Tam-treatment. Interestingly, we also found elevated levels of sirtuin-3 (SIRT3) in the MCF7 and T47D Tam5R cells. SIRT3 is the major mitochondrial deacetylase and it is implicated in regulation of mitochondrial metabolism and antioxidant defense. Furthermore, we have identified some novel mutant variants of SIRT3 in Tam5R cells and we are elucidating the role of SIRT3 and its variants in the development and maintenance of tamoxifen resistance. In summary, we suggest that, in TamR cells, mitochondria undergo metabolic reprogramming and alter their function in order to survive Tam-treatment, suggesting that mitochondria could play an important role in the context of tamoxifen resistance.

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MicroRNA expression profile associated with recurrence in atypical meningioma patients

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Meningiomas are classified as the most common primary tumors of the central nervous system and account for over one-third of all primary intracranial malignancies in adults. Although most of these tumors are predominantly benign, meningiomas classified by the World Health Organization as Grade 2 and Grade 3 are more aggressive and are associated with poor prognosis. Atypical meningioma (AM) recurs in 40% of patients despite total resection and adjuvant radiotherapy (aRT). To this day, no consensus on optimal aRT management has been found, and it is difficult to identify patients insensitive to RT in clinical practice. Therefore, finding prognostic and treatment response predictive biomarkers is necessary. MicroRNAs (miRNAs) are short non-coding RNAs that regulate most biological processes, including cell proliferation, differentiation, and apoptosis. Differentially expressed miRNAs were proven to affect the formation, recurrence, growth, invasivity, clinical stage, and radioresistance of MNGs. This study aims to identify tissue miRNAs capable of predicting patients with AM who would benefit from the indicated aRT. We further suggest that there are miRNAs with the ability to predict the risk of recurrence independently on meningioma grade and Simpson grade of resection.

The study includes 80 patients with meningioma in the exploratory phase and 400 in the validation phase. Results indicated significantly dysregulated miRNAs among AM patients with/without recurrence ($p<0.05$). Dysregulated miRNA expression profile was also observed between AM patients with indicated RT who did/did not develop a recurrence ($p<0.05$). The performed analysis could help with a more accurate prognosis estimation of surgically treated patients and determine which patients would benefit from the aRT.

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Fusion gene analysis as a tool for diagnosis and therapeutic planning in pediatric cancer patients

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The cancer genome of pediatric patients has several characteristics that distinguish it from adult malignancies. These include a low mutational load, a significant role of epigenetic changes, and the frequency of fusion genes as drivers of carcinogenesis. Fusion genes result from several types of chromosomal rearrangements, such as translocations, deletions, insertions, or inversions, with various functional consequences. Although they have been studied mainly in the context of hematological malignancies in the past, their importance in the diagnosis and therapy of solid tumors is increasing. The study aimed to investigate fusion genes in pediatric oncological patients using targeted RNA sequencing and to evaluate their clinical potential.

A total of 258 patients with solid tumors from the Department of Pediatric Oncology of University Hospital Brno were analyzed for fusion genes by targeted RNA sequencing. Sequencing libraries were prepared using the TruSight RNA Pan-Cancer Panel kit (Illumina), which covers 1385 clinically relevant genes and allows the identification of both known and previously undescribed fusion genes. Sequencing of libraries was performed using the NextSeq Mid Output Kit (150 cycles) on the NextSeq 500 platform (Illumina). Sequencing reads were mapped to the hg38 reference genome using the STAR aligner with parameters set to allow the detection of fusion genes. The quality of the mapping was verified using QualiMap and Picard tools. Arriba and STARfusion tools were used to find fusion genes, and the identified fusion genes were manually verified in IGV software.

Clinically relevant fusion genes were identified in 25% of patients. Sarcoma-associated fusions such as *EWSR1-FLI1*, *PAX3-FOXO1*, or *SS18-SSX1/2* accounted for the largest proportion of identified fusions. The second largest group was represented by fusions typical for central nervous system tumors, particularly *KIAA1549-BRAF* or other fusions activating *Ras/MAPK* signaling. 33% of the identified fusion genes were therapeutically targetable, of which 2/3 of the patients were treated accordingly. In supratentorial ependymomas, the analysis contributed to prognostic stratification and, in selected cases, to change or refinement of the initial histopathological diagnosis.

Fusion gene analysis significantly impacts diagnostics, prognostic stratification, and therapeutic planning of pediatric oncological patients. Using high-throughput approaches such as RNA sequencing allows the identification of novel fusion genes and, thus, a deeper understanding of the complex changes accompanying the origin and development of malignant diseases.

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Emetine's anti-DNA replication activity reflects proteosynthesis inhibition not targeting Okazaki fragment formation

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Emetine is a natural product alkaloid, many decades known as a specific chemical inhibitor of Okazaki fragments synthesis. Inhibition of lagging strand can cause replication stress, which is considered as a source of genome instability. One of the replication stress markers is the formation of single-stranded DNA (ssDNA). Accumulation of ssDNA can occur on either leading or lagging strand by uncoupling the polymerases or by unregulated unwinding by helicase exposing the strands. In this work, we propose the idea that DNA replication inhibition by emetine is not caused by strand uncoupling but rather by protein synthesis inhibition. Our in vitro studies focused on strand uncoupling markers after emetine exposure in comparison to adarotene, an inhibitor of PolA. Our results revealed that emetine does not cause ssDNA accumulation followed by chromatin-bound RPA32 loading and activating DNA damage response pathway. In line with this, emetine did not activate the replication checkpoint. Moreover, inhibition of protein synthesis precedes inhibition of DNA replication after treatment with emetine. Collectively, we showed that emetine completely blocks DNA replication and should not be used as a lagging strand inhibitor.

Automatic de novo design and structural optimization

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There are multiple requirement for compounds on early stages of development of innovative drugs. Primary hits should demonstrate structural novelty, a decent activity towards a major target and the ability for further modifications/optimization (developability). Leads should have favorable ADME/Tox properties, selectivity profile, etc. Computational approaches can facilitate both stages, hit identification and lead optimization, and suggest multiple compounds with favorable properties. However, existing pipelines are not automated, require complex setup and expert knowledge. One of the major challenges in implementation of automatic computational drug design pipelines is structure generation which should result in synthetically feasible molecules. This will substantially increase the speed and quality of generated hits and leads which can be further reviewed by medicinal chemists to select more preferable compounds for synthesis and biological evaluation.

We developed fragment-based structural generator which generate chemically valid and synthetically feasible structures and can be applied to both tasks: de novo design and structural optimization. We developed several automatic pipelines which are able to generate novel molecules or optimize existing ones to satisfy user-defined criteria. Those criteria can be fitting of a ligand to a binding pocket of a particular protein, its binding energy, establishment of particular protein-ligand contacts, lipophilicity and other drug-like properties. The selected criteria can be combined in a single objective function to guide multi-objective optimization of properties of generated compounds. We demonstrated applicability of the developed tools in several case studies related to development of anticancer agents: de novo generation of CDK2 inhibitors and optimization of tubulin inhibitors.

Immunocompetent cell-infiltration of NSCLC in relation to prognosis, response to therapy and microbiome composition

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Modulation of the immune system is involved in the development of a malignant tumour. Currently, we know some key roles played by tumour-infiltrating lymphocytes (TILs) and tumour-associated macrophages (TAMs) in the genesis and progression of neoplasms. The immune-cell infiltrate consists of T and B lymphocytes, tumour-suppressive M1 macrophages and tumour-supportive M2 macrophages, in varying ratios. Their mutual proportion is an important prognostic marker in many neoplasms, and the influence of microbiome on the development of several GIT as well as airway neoplasms has been proven in many studies. In the case of lung neoplasms, including non-small cell lung carcinoma (NSCLC), the microbiome of the oral cavity can also play a role via aspiration. In our work, we focused on discovering a possible connection between the composition of the immune-cell infiltrate of NSCLC, specific clinical criteria (NSCLC subtype, patients' comorbidities, response to therapy) and the composition of oral and airway microbiome. Having analysed these data in 31 patients, in which

we assessed ratios between B and T lymphocytes and the portions of CD 204+ in all (CD 68+) macrophages, microbiome composition, correlated with the presence of diabetes, COPD and smoking, we found a significantly higher concentration of B lymphocytes and M1 macrophages in the infiltrate of squamous cell carcinomas (SC). In addition, in this group of patients, smoking and diabetes were more common than in patients with adenocarcinoma (AC). In SC, full remission after therapy was associated with high numbers of CD 20+ B lymphocytes and low number of CD 204+ macrophages in the infiltrate, whereas in AC, full remission was associated with high concentration of CD 3+ T lymphocytes and high concentration of CD 204+ macrophages. Furthermore, high concentration of carcinogenesis-supporting microorganisms was found in the microbiomes of the oral cavity and upper airways of patients with NSCLC, regardless of histological type.

Surface plasmon resonance biosensor for monitoring stimuli-triggered drug release from polymeric systems

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Controlled drug delivery systems represent a rapidly growing scientific field in which the materials in the nanoscale range are employed to deliver therapeutic drugs to targeted sites in a controlled manner. The drug is typically bound to a carrier, such as a polymer, dendrimer, liposome, or nanoparticle, and delivered to the target site in a human body, where the drug is released from the carrier via a specific stimulus (e.g., a change of pH, heat or light). The drug is bound to the carrier via cleavable linkage or weak noncovalent interactions, which enables a controlled release of drugs in the target site. In order to characterize the release of drugs from carriers, conventional methods, such as fluorescence and UV-Vis absorption spectroscopy, are typically used. Since these methods are indirect and require a multi-step preparation procedure (collection, extraction, etc.), new techniques are needed to simplify the drug release characterization.

In this study, we demonstrate for the first time the use of the surface plasmon resonance (SPR) biosensor method for monitoring the stimuli-triggered release of drugs from carriers. In particular, we investigated the release of drugs with anti-cancer and anti-inflammatory effects, which are bound to a polymer carrier via pH-sensitive linkages. In the first stage of the experiment, we attached the biotinylated drug-loaded polymers to a streptavidin-coated sensor surface via the streptavidin-biotin interaction. Then, we induced the drug release by injecting a buffer with a low pH. We studied how the pH and the drug loading level in polymer affect the drug release. We demonstrate that the SPR biosensor method enables direct and real-time monitoring of the drug release for both fast and release processes making it potentially an attractive approach for the drug release characterization.

Correlation of FOXP3, IL-35 and PD-L1 in intra and peritumoral lymphocytic infiltrate of cutaneous melanomas as important part of antitumor immunity

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The tumor microenvironment is an important mediator enabling tumor growth and progression. It is composed of extracellular matrix and stromal cells. An important component are cells of the immune system, tumor infiltrating lymphocytes (TILs), At the same time, tumor cells develop mechanisms by which they can escape the action of the immune system. An immunosuppressive mechanisms suitable for tumor growth are very complex, cooperating with each other, involving a whole series of events involving cells of the immune system, the tumor microenvironment itself, or chemokines and cytokines.

In our work, we focused on monitoring the expression of FOXP3, IL-35 and PD-L1 proteins, which are involved in immunosuppression mechanisms in some tumors, including melanoma. They are also associated with progression, early metastasis and a generally poor prognosis.

We examined 95 cutaneous melanomas and 25 melanocytic nevi as a control group. Melanomas were divided into four groups according to the TNM classification – pT1 (35), pT2 (21), pT3 (21), pT4 (18).

PD-L-1 expression on lymphocytes was increased in pT3 and pT4 stage melanomas, also especially in the periphery of the lesions ($p < 0,001$). The amount of FOXP3 lymphocytes was positively correlated with the stage of the disease, with higher values being recorded in the center of the tumors ($p < 0,001$). Likewise, the expression of IL-35 ($p < 0,001$) increased together with the advanced stage of the tumor.

Our work demonstrates that stimulation of the immunosuppressive environment develops in proportion to the stage of melanoma. The most significant changes occur at the periphery of the tumor, which

confirms the heterogeneity of the tumor stroma, which is more pronounced in more advanced tumors and which may contribute to the higher aggressiveness of advanced stages in these places.

"Molecular" resection margins in oral squamous cell carcinoma – report of the first results from the multidisciplinary view

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Malignant tumors of the head and neck represent approx. 5 % of the total number of diagnosed malignant tumors in Europe among them squamous cell carcinoma is the most common, representing more than 90 % of all malignant tumors of the oral cavity. The therapy of oral squamous cell carcinoma (OSCC) has significantly intensified in the last decade. Here, we focused on sensitive mutation analysis of resection margins that could improve the prediction of relapse and/or sensitivity to specific drugs.

DNA was isolated from 38 patients (tumor, margins and peripheral blood from all patients, in total 3 samples/patient). We performed Illumina sequencing using a panel of 88 cancer genes containing tumor suppressors and oncogenes. Only non-synonymous variants in tumor/margin that were reported in the ClinVar database as "pathogenic", "likely pathogenic" or "of uncertain significance", and were simultaneously not present in the peripheral blood, were selected for our analysis.

In total, we identified 29 mutated genes, among them mainly tumor suppressor genes involved in DNA repair. We detected mutations in 28 DNA samples isolated from 34 tumors tissue and in 6 DNA samples from 38 tumor margins. Gene TP53 was the most commonly mutated gene in tumor and margins followed by CDKN2A and BRCA2 and BRCA1. 14 genes were mutated in 2 and more patients. The median tumor load was 1,5 pathogenic mutation per patient on average (range 0-15). This parameter did not correlate with the presence of histological markers like perineural invasion probably due to small cohort size. Similarly, we did not observe association of mutations in resection margins and probability of disease relapse.

The spectrum of the tumor mutations is similar as in other studies with the exception of mutations in the BRCA genes. The presence of mutations in these genes are not investigated in OSCC so frequently. The data in the literature suggest that BRCA 1 mutations in OSCC examined by sequencing are 3% (55 cohort) and 7% in BRCA2 (176 cohort). Variants identified in our dataset are often introducing stop codons leading to truncated and non-functional proteins.

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Transcriptome analysis of small extracellular vesicles derived from blood sera of colorectal cancer patients

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Introduction: Colorectal cancer (CRC) accounts for about 10% of all cancers and is the second most common cancer-related death worldwide. In recent years, small extracellular vesicles (sEVs) emerged as potential reservoirs of clinically valuable biomarkers and promising drug delivery systems. sEVs are 30-200 nm-sized membranous vesicles endogenously produced by most cell types. They serve as intercellular messengers by delivering proteins, microRNAs, mRNAs, or long non-coding RNAs to recipient cells. This study aimed to analyze the transcriptome of sEVs derived from blood sera of CRC patients and healthy controls.

Methods: Two precipitation-based methods and size-exclusion chromatography were used to extract sEVs from blood sera. The concentration of vesicles was measured by dynamic light scattering, the size of the isolated vesicles was evaluated by electron microscopy, and sEV-specific content was analyzed by western blot and qRT-PCR. High-throughput transcriptome analysis of sEVs extracted from samples of CRC patients (N=77) and healthy controls (N=29) was performed using next-generation sequencing (NGS). Differential expression analysis was carried out in R using DESeq2 package.

Results: The extracted vesicles

were confirmed to be sEVs according to ISEV guidelines. Size-exclusion chromatography yielded the purest sample of sEVs, and extraction of sEVs from blood serum with this technique was optimized. Extraction of RNA from sEVs and sequencing library preparation was optimized. Subsequent NGS transcriptome analysis of sEVs derived from CRC patients and healthy individuals sera identified coding and non-coding RNAs to be differentially expressed (FC>1.5, p-value < 0.01).

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Comprehensive metabolomic and lipidomic study of tauopathy and Alzheimer‘s disease patients

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Alzheimer‘s disease (AD) belongs to the group of tauopathies, which are categorized as neurodegenerative disorders. AD is manifested by dementia, cognitive functions loss, and other neurological impairments. Currently, more than 55 million people worldwide have dementia and 60-70% of them have been diagnosed with AD. One of the causes of AD development is the accumulation of structurally disrupted tau protein, which aggregates into insoluble neurofibrillary tangles that disrupt neurons.

The aim of this study was to characterize the pathological processes in AD patients and different five cohorts of tauopathy and to compare them with the profile of healthy individuals. The tauopathy cohorts were as follows: Progressive supranuclear palsy (PSP), Semantic variant/ Non-fluent agrammatic variant of primary progressive aphasia (svPPA/ nfaPPA), Corticobasal degeneration (CBD) and Behavioural variant of frontotemporal dementia (bvFTD) and. Cerebrospinal fluid samples were obtained from patients and controls. To objectively assess the study, a combination of targeted metabolomic and lipidomic approaches was chosen.

Metabolic-lipidomic analysis revealed statistically significant differences in the profiles of patients compared to controls. The changes found were most pronounced in patients suffering from one of the tauopathy cohorts. Elevated levels in tauopathies dominated in (un) saturated short- and medium-chain acylcarnitines in metabolome. The lipid profile was mainly influenced by increased levels of sphingomyelins, phosphatidylcholines and their lysoforms. These observations are probably associated to anti-inflammatory processes [1] or to the release of phospholipid membranes due to neurodegeneration [2]. In addition, mitochondria and other cellular components may have been disrupted due to oxidative stress [1,3]. This was reflected by the accumulation of purine catabolism intermediates in patients with tauopathy. Upregulation of these reactions leads to increased production of oxygen radicals. All these findings will contribute to the understanding of the pathobiochemistry of neurodegenerative diseases and their diagnosis.

Refereces:

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Combination of extracellular matrix proteins potentiate oncogenic pathways and advanced disease in prostate cancer

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Introduction. Prostate cancer initiation and progression is largely dependent on tumor microenvironment and extracellular matrix proteins that could serve as stromal markers for disease prognosis and prediction. In this regard, periostin (POSTN) and versican (VCAN) seem promising. Versican is a chondroitin sulfate proteoglycan expressed at high levels during organogenesis, inflammation, tissue repair processes and progression of the tumor into a metastatic one. Interestingly, association between the Docetaxel response and high expression of versican has been also demonstrated. Periostin, also called osteoblast-specific factor 2 is a unique ECM protein that interacts with integrins to support cell adhesion. Periostin has been shown to be highly expressed in osteoblastic cells, in periosteum and in periodontal ligaments to allow formation and structural maintenance of bones and teeth. It plays an

important role in the development of the heart valves and is re-expressed during recovery from myocardial, vascular and skeletal muscle injuries. Increased expression of POSTN accompanies the formation of metastases, since POSTN activated signaling pathways and promote cell survival, angiogenesis, and resistance to hypoxia-induced cell death. We and others showed that POSTN expression is an important independent prognostic factor for patients with prostate cancer. POSTN promotes tumorigenesis by the process of epithelial-mesenchymal transition via affected phosphatidylinositol 3-kinase (PI3K) and the serine-threonine protein kinase AKT/PKB pathways, resulting in the loss of expression of epithelial markers and in an increased expression of mesenchymal markers.

Materials and methods.101 FFPE prostate carcinoma archival samples were reviewed for clinical and pathological data. Slide samples were stained immunohistochemically for periostin, versican, vimentin, E-cadherin, b-catenin, Skp2, Slug, Ki67, p53, AR, PSA and scored. Slides were reviewed for the presence of tertiary Gleason and samples were classified into localized, advanced and metastatic groups, and ISUP 2014 Gleason grade groups. Using the Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) data that were processed by Gene set enrichment analysis (GSEA) and other bioinformatics tools, we aimed to identify hallmark genes/pathways associated with prostate cancer with elevated expression of periostin and versican independently and in combination. Associations with clinical and pathological variables were also studied.

Results. Periostin and versican were overexpressed in prostate cancer. Periostin stromal positivity correlated with versican stromal expression (Rs 0.368, p<0.001). Periostin stromal expression positively correlated with tertiary Gleason and Gleason grade group ((Rs 0.276 and 0.269, both p=0.008) and was higher in group with lymph node metastases (p=0,05). High versican stromal expression was independent predictor for high Gleason grade group and the trend of higher expression of versican was observed in metastatic group (p=0.08), however, both periostin and versican were significantly higher in patients with seminal vesicle involvement (p=0.024 and 0.032, repectively). To investigate periostin and versican prostate cancer tissue expression, we have previously checked the expression of genes coding those proteins in different tumors by the web based tool called tumor immune estimation resource (TIMER) where POSTN and VCAN were upregulated in various tumors in comparison to normal tissues. GSEA analysis showed enrichment of genes involved in angiogenesis, epithelial-mesenchymal transition, inflammatory response and TGF-beta signaling in VCAN high group, while genes involved in cell cycle regulation, MYC-targets, IL6-JAK-STAT3 signaling and epithelial mesenchymal transition were enriched in POSTN high group. In a group with combined higher RNA levels of both POSTN and VCAN we observed significant enrichment of genes invoved in oncogenic pathways and epithelial mesenchymal transition.

Discussion. We showed for the first time association of periostin and versican with pathological parameters of advanced prostate cancer and revealed associated gene groups and signalling pathways that can serve as potential anticancer targets.

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MicroRNAs in Brain Metastases - Promising Diagnostic Biomarkers for Known and Unknown Origins

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Brain metastases (BMs) represent a heterogenous group of the most frequent intracranial tumors in adults, significantly influencing the morbidity, survival, and life quality of the patient. Despite the recent advances in imaging methodology providing earlier BM identification together with progress in treatment strategies, BMs remain lethal, leaving patients with short survival time. Moreover, BMs of unknown primary origin diagnostics represent a demanding challenge for modern medicine. MiRNAs are endogenously expressed small non-coding RNAs, well-known as post-transcriptional regulators of gene expression, modulating cell physiology. Their dysregulation has been confirmed in many pathological processes, including the complex and not fully understood metastatic cascade. Based on the data, these molecules are extensively studied as potential diagnostics biomarkers in tissue and easily obtainable liquid biopsies due to their high stability.

We performed high-throughput miRNA profiling (Illumina small RNA sequencing) on 3 types of samples (metastatic tissue, blood plasma, cerebrospinal fluid) from a cohort of 30 patients – 5 patients from each BM type (colorectal carcinoma, melanoma, lung carcinoma, breast cancer, renal cell carcinoma; 90 samples total). We identified specific miRNA signatures of all BM types tested.

We observed a significantly dysregulated miRNA expression profile in metastatic tissue with the ability to differentiate between primary origins with high specificity and sensitivity. MiRNAs could clearly identify metastasis originating from breast cancer, melanoma, colorectal, and renal cell carcinoma. On the other hand, the heterogeneity of lung carcinoma was also reflected in the metastasis, making it difficult to characterize precisely. Even though tissue-specific miRNA signature was the most accurate, our results presented a diagnostic potential of blood plasma and cerebrospinal fluid of metastatic patients. In conclusion, the miRNAs obtained from liquid biopsies from metastatic patients have the potential to be a new non-invasive diagnostic biomarker of metastasis of known and unknown origin.

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Clonal somatic variants in immune cells involved in atherosclerotic plaque formation

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Clonal hematopoiesis of indeterminate potential (CHIP) has recently been described as a common age-related condition manifested by the accumulation of somatic mutations in cells of the hematopoietic system. This state is a potential precursor of malignant transformation, but more interestingly, it can also increase the risk for diseases such as atherosclerosis and ischemic stroke.

In this study, we investigated somatic mutations of immune cells in 30 patients with symptomatic or asymptomatic carotid stenosis, who underwent carotid endarterectomy. For mutational analysis, DNA was obtained from peripheral blood and three different parts of the carotid plaque of each patient, resulting in 120 sequencing libraries in total. Mutations were identified by a sensitive method of massively parallel sequencing using a targeted DNA custom panel containing 38 CHIP-related genes.

Mutations found in the blood samples were in most cases confirmed also in the patient's plaques. Findings from different parts of carotid plaques suggest that mutated immune cells are not distributed equally across the atherosclerotic tissue. No mutation was present in the plaques of patients, who had no mutation detected in the blood.

Diblocks polymer conjugates for tumor treatment

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Biodegradable and biocompatible polymer-based drug delivery systems fulfill the requirements of successful nanomedicines because they enable to obtain the polymer systems with optimized blood circulation, pharmacokinetics, biodegradability, and renal excretion. One of these polymers is based on N-(2-hydroxypropyl) methacrylamide. We designed and successfully synthesized four novel chain transfer agents (CTA) for the RAFT polymerization, allowing the straightforward synthesis of hydrolytically labile diblock structures. The hydrolytic degradation of diblock precursors in buffers is in the range of 5 hours to 21 days depending on CTA's structure. The antitumor drug pirarubicin was successfully conjugated to the diblock copolymers via a pH-sensitive hydrazone bond and in vitro and in vivo experiments were performed. Diblock nanomedicines demonstrated superior antitumor efficacy in comparison to basic linear polymer-based conjugates.

Expression of PIWIL1-4 in glioblastoma stem-like cells

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Glioblastoma stem-like cells (GSCs) are considered important for glioblastoma (GBM) progression, treatment resistance and relapse. PIWI proteins, encoded by *PIWIL1-4* in humans, are piRNA-binding proteins that belong to the Argonaute family. They are involved in maintaining genome stability and are thought to be important for stemness maintenance in cancer stem-like cells. Our aim was to assess the expression of *PIWIL1-4* in GSCs and non-GSCs.

Freshly resected GBM tissues were enzymatically dissociated to obtain a single cell suspension. Serum-free medium with EGF and bFGF was used to expand GSCs, whereas serum-containing medium was used to propagate the non-GSCs. Expression of stem cell markers (CD133 and SOX2) was evaluated using flow cytometry, expression of differentiation markers (GFAP and beta III-tubulin) was analyzed by immunocytochemistry. GSCs or non-GSCs were orthotopically implanted in immunodeficient mice to assess their tumorigenicity. Expression of *PIWIL1-4* transcripts was analyzed by RT-qPCR.

GSC cultures typically formed spheres *in vitro*, upregulated differentiation markers in serum-containing medium, formed tumors in vivo and compared to paired non-GSC cultures had higher expression of stem cell markers. *PIWIL2* expression was significantly higher in GSCs compared to paired non-GSCs

(N=12), whereas we did not find a statistically significant difference in the expression of *PIWIL4*. *PIWIL1* and *PIWIL3* transcripts were not detected in any of the tested paired GSC and non-GSC cultures.

In conclusion, our serum-free derived cultures showed stem cell-like properties and expressed higher levels of *PIWIL2* transcript than non-GSC cultures. Further studies are aimed at elucidating the importance of PIWI proteins in GSCs.

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Study on the long non-coding RNA expression profiles in glioblastoma and characterization of structure and function of LINC00634

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Despite the significant efforts put into the research of glioblastoma (GBM), the effectiveness of conventional therapy is still lacking, and only a few clinically relevant molecular biomarkers are currently in use. Therefore, more studies in recent years have been focusing on largely unexplored groups of molecules that could serve as novel biomarkers or therapeutic targets. One such group is long non-coding RNAs (lncRNAs) which are thought to be directly involved in GBM biology as regulators of gene expression on all levels. We sequenced transcriptomes of 60 human GBM specimens and 17 non-tumor brain tissue samples. The differential analysis of lncRNA expression revealed 2,387 significantly dysregulated lncRNAs between GBMs and non-tumor samples (adjusted p-value < 0.05, |log2FC| > 1.5). The expression levels of 11 significantly dysregulated lncRNAs were successfully validated in an independent sample cohort of 157 GBMs and 29 non-tumors using RT-qPCR ($p < 0.0001$). The lncRNAs with the most dysregulated expression in GBM also included LINC00634 (NGS: log2 fold change in expression -2.58, an adjusted p value less than 0.0001; qPCR: $p<0.0001$), which we selected for further functional characterization. The plasmid construct, containing the LINC00634 transcriptional variant sequence named ENST00000381348.4, was transfected by lipofection into the GBM cell lines U251MG and T98G. The increased expression of LINC00634 was confirmed in stable transfectants, and its effect on clonogenicity, migration, and viability was analyzed in cells. During the study, the small peptide SMIM45 was predicted to be potentially translated from the short open reading frame in the transcript LINC00634. The structure and function of LINC00634 were further characterized in the laboratory and bioinformatically, particularly in relation to the protein-coding potential, polyadenylation, cellular localization, and interaction of the transcript with microRNAs.

Pilot data: Current incidence of HPV-driven oropharyngeal cancer and the possible role of liquid biopsies in recurrence monitoring

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Background: High-risk human papillomaviruses (HPVs) are etiological agents of several human malignancies, including oropharyngeal squamous cell carcinomas (OPSCCs). HPV-driven OPSCCs are newly recognized as a distinct subgroup of head and neck squamous cell carcinomas (HNSCCs) with a unique epidemiological and clinical profile. Although HPV-driven OPSCCs are more treatment responsive and have a favorable prognosis, a certain subgroup of HPV-positive OPSCCs retains a higher risk of later recurrence with limited possibilities for early detection. Liquid biopsies represent a promising strategy for post-treatment monitoring and early detection of disease recurrence. This study aims to evaluate liquid biopsy collection methods and monitoring of HPV persistence in OSPCC patients for recurrence risk stratification.

Materials/Methods: In this study, newly diagnosed OPSCC patients and patients in remission were enrolled. HPV tumor status was determined based on the results of HPV DNA testing in fresh or FFPE tissue samples and the results of p16 immunohistochemistry. Pre & post-treatment HPV testing in gargle lavage (GL), oropharyngeal swabs (OPS), and plasma samples was performed, followed by regular sampling according to the standard follow-up protocol.

Results/Conclusion: In total, 30 OPSCC patients have been enrolled. In a prospective cohort, 9/12 (75%) of newly diagnosed OPSCC patients were HPV(+)/p16(+), 2/12 (16.7%) were HPV(-)/p16(-) and 1/12 (8.3%) was HPV(+)/p16-incompleted. Positive agreement between HPV test-

ing in tumor tissue compared to OPS and GL samples in newly diagnosed OPSCC was 100% and 78%, respectively. HPV16 genotype was found in 100% of HPV(+) OPSCC cases. In conclusion, these preliminary data show a predominant incidence of HPV-positive OPSCC cases compared to HPV-negative OPSCCs. At the time of diagnosis, HPV testing in OPS showed higher sensitivity.

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CVID - vzácnost či opomíjená jednotka?

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Ústav klinické a molekulární patologie LF UPOL a FN Olomouc, Olomouc, Czech Republic

Běžná variabilní imunodeficience neboli CVID je označení pro heterogenní skupinu primárních imunodeficiencí. Přesná příčina onemocnění dosud není známa, mimo genetických faktorů se předpokládá také vliv vnějšího prostředí. V klinickém obraze dominují zejména recidivující bakteriální infekce dýchacích cest a laboratorně lze zjistit nízké hladiny protilátek. Nemocní mohou být náchylnější k alergiím a autoimunitním chorobám. Onemocnění se může manifestovat v dětství či dospívaní, ale i ve vyšším věku. V histologickém obraze onemocnění dominuje zejména absence plazmocytů, dále mohou být přítomny granulomy. Svým průběhem může onemocnění napodobovat sarkoidózu či v případě postižení střeva také IBD. Terapie zahrnuje v první řadě substituci imunoglobulinů (i.v., i.m.), často je nutné také podávání kortikosteroidů, cytostatik či antibiotik.

Unique reporter model for c-Myc protein level monitoring under physiological conditions

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Although c-Myc is well known as a proto-oncogene, its structure and function as a transcription factor make it a problematic therapeutic target. To identify c-Myc inhibitors, we have developed a high-throughput screening (HTS) system based on a combination of CRISPR/Cas9 and NanoLuc technologies. This system uses a cell-based assay to detect c-Myc inactivation in an HTS format generated from two pure clones of a stable osteosarcoma cell line. In each clone, one of the major c-Myc isoform is tagged p64 or p67. A very small tag known as HiBiT, consisting of only a few amino acids, was incorporated at the C-terminus of the c-Myc protein using CRISPR/Cas9, resulting in the expression of the two tagged protein isoforms from the endogenous c-Myc locus. Using chemiluminescence readout as a surrogate for c-Myc expression, we validated the obtained cellular models using siRNA and known small molecule modulators of c-Myc expression. We will use this system to perform quantitative HTS against approximately 2,600 existing bioactive compounds from two different libraries. Our work has also demonstrated the unique advantage of combining novel technologies in accelerating drug discovery for c-Myc-targeted anti-cancer therapies.

Fully synthetic antibody mimetics selectively target CD64-expressing cells

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Monocytes and macrophages are necessary for proper function of the immune system. However, these cells can be involved in autoimmune diseases or fail to respond to tumor development. Therefore, therapeutic approaches to modulate the activity of these myeloid cells are being examined. One option, explored in the field, is targeting myeloid cells through FcγRI (CD64).

We have recently described a development of stable, highly-modular synthetic polymer conjugates called iBodies, which, compared to antibodies, excel in their versatility and low cost. These antibody mimetics based on N (2 hydroxypropyl)methacrylamide (HPMA) polymer are decorated simultaneously by a specific ligand targeting CD64 and a cytotoxic moiety connected to the HPMA polymer by a suitable linker.

Compared to the ligand alone, iBodies showed a significant improvement in binding potency to CD64. We detected specific targeting of iBodies to CD64-expressing cell lines and primary monocytes. Using viability assays, we studied specific cytostatic elimination of CD64-expressing cells in vitro using anti CD64 cytotoxic iBodies.

Thus, iBodies could be potentially utilized as a versatile therapeutic system of synthetic conjugates that targets and eliminates

specific cells involved in the tumor microenvironment or in the etiology of autoimmune diseases.

Establishing cancer comprehensive genomic profiling in University Hospital Olomouc

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Introduction: Increases in the prevalence and incidence of different forms of cancer and availability of novel targeted anti-cancer drugs boost the cancer diagnostics market. Nowadays in clinical genomics, somatic variants profiling and tumor mutation burden (TMB) assessment become very important tool utilized to understand the mutational landscape of tumors. TMB may have utility as a prognostic biomarker in immunotherapy-naïve patients, with a protective effect at higher TMBs. In this project, we aimed to implement TMB prediction analysis into clinical practice. For TMB assessment, we chose QIAseq Tumor Mutation Burden Panel (Qiagen). This comprehensive panel covers 486 genes and 27 microsatellite instability (MSI) markers.

Materials and Methods: Samples: DNA isolated from formalin fixed paraffin embedded (FFPE) blocks mainly of colorectal carcinoma, lung cancer, ovarian cancer and samples from other cancer types; SeraSeq standards (Seraseq® FFPE TMB RM Score 20; Seraseq® FFPE TMB RM Score 7), Horizon Diagnostics reference samples (KRAS Gene Specific Multiplex Reference Standard - HD301; Quantitative Multiplex Reference Standard fcDNA (severe) - HD803); Quantitative Multiplex Reference Standard fcDNA (moderate) - HD799).

NGS library preparation: QIAseq Tumor Mutation Burden Panel (Qiagen) library preparation was introduced into laboratory with combination of UMI and QIAseq 96-Unique Dual Index Set A.

Sequencing: Pair-end (PE) mode on Illumina NovaSeq 6000 was used. Expected data amount per sample is 40 M PE reads.

Data analysis: CLC Genomics Workbench (GWB, Qiagen) was used for data analysis. Pipeline includes mapping to reference genome, unique molecular index (UMI) calculation, trimming, variant calling, artifacts identification, and filtration. Bioinformatic analysis involves TMB score calculation (number of mutations found within a tumor per megabase). TMB score can be classified as TMB low (<10 mut/Mb) or TMB high (>10 mut/Mb). High TMB score correlates with response to checkpoint blockade immunotherapy.

Results: Reference standards for genotypization: The results of determination of variants (n=74, variant allele fraction (VAF) > 2.5 %) declared by the manufacturer of Horizon reference standards in all types of samples were correct in all cases.

The number of detected variants in reference samples was used to calculate sensitivity and specificity. Where we obtained a value of 100 % ± 0 and 100 % ± 0 respectively.

Reference standards for TMB score determination: Seraseq reference standard values with a known mutational load value for both samples were detected. Both measurements obtained within TMB method are within the range Average ± 3 SD from the values given by the manufacturer.

Reproducibility: Data from the repeated determination of oncogenic variants of samples 20-0852-DG-12271 and 21-0023-12304 were used for reproducibility assessment.

Variant detection results were reproducible in all cases. Three variants were identified as artifacts based on the analysis of qualitative parameters and manual evaluation of the sequencing data. The method is reproducible, but it is necessary to take into account possible artifacts and, if necessary, identify them manually.

Application into clinical practice: So far, we analyzed 110 samples utilizing TMB panel, in which we can see the prevalence of detected mutations. Average UMI consensual sequencing depth of 110 samples was 825 X, that allows us to detect variants present at as low as 5 % VAF.

Conclusion: Overall, the specificity of the validated TMB method was calculated to 100 % ± 0 and the sensitivity of the method was 100 % ± 0. The method is valid, reproducible and suitable for diagnostic predictive determination of the genotype for the analyzed genes in DNA samples isolated from paraffin blocks of tumors. Method was successfully performed on 110 samples determined for TMB profiling. In 27 patients high TMB score was detected, so treatment with checkpoint blockade immunotherapy could be recommended.

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Endometrial Tumor Predictive Testing Using fastGEN Technology for Deep Amplicon Sequencing of *POLE*

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Introduction and aims: A prerequisite for tumor risk-group assessment and personalized treatment in endometrial carcinoma is tumor DNA testing of POLE gene, which encodes central catalytic subunit of DNA polymerase epsilon. POLE mutated tumors lower the risk and, thus, adjuvant chemotherapy should be deescalated. POLE mutations occur in 7 – 12 % of endometrial cancers and have been associated with high tumor mutation burden. Retrospective analysis showed that pathogenic POLE mutations are associated with clinical benefit to immune checkpoint inhibitor therapy. Thus, further thorough prospective studies are warranted to validate POLE mutation as a predictive biomarker.

Methods: Deep amplicon sequencing (DAS) has a potential to be suitable method for simultaneous detection somatic mutation within hotspot regions with a defined detection limit down to 1 % minor allelic frequency (MAF). We have developed and validated a unique fast method known as fastGEN for genotyping of POLE exonuclease domain (exons 9, 11, 13, and 14) and other hotspot cancer mutations for RAS, BRAF, EGFR, IDH1/2, and PIK3CA based on DAS using Illumina platform.

Results: We have analyzed 65 endometrial tumors with 98.5% success rate. Pathogenic variants were found in 6 samples (2 with POLE p.P286R, 2 with p.V411L, 1 with p.M444K, and one with p.S459F). Results of fastGEN were validated using larger somatic NGS panel (Nonacus Pancancer TMB/MSI; Qiagen QIAseq TMB Panel; Archer VariantPlex GyNcore and Illumina TSO 500). Using samples (n = 10), where results of both methods were available, we observed perfect concordance with 100% specificity and sensitivity. Variant detection using fastGEN POLE was highly reproducible (n = 4, POLE p.S459F, MAF = 29.8 % ± 1.7 % [mean ± SD]). The minimum turn-around-time (sample to final report) was less than 24 hours.

Discussion and conclusion: fastGEN technology used for somatic mutation of POLE (and other genes validated in the past) is routinely used in tumor diagnostics in our lab and showed excellent performance; therefore, it was licensed and thus could be used in other labs as a kit. The partner BioVendor Group is able to produce the kit in a high amount, perform rigorous quality controls, ensure the certification, and distribute it worldwide. Bioinformatics pipeline for a successful diagnostic product based on Genovesa fastGEN POLE platform is under development.

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A-ring-fused pyrazoles of dihydrotestosterone targeting prostate cancer cells via the degradation of the androgen receptor

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High expression of the androgen receptor (AR) and the disruption of its regulation are strongly responsible for the development of prostate cancer (PCa). Therapeutically relevant non-steroidal or steroidal antiandrogens are able to block the AR effect by eliminating AR-mediated signalling. Herein we report the synthesis of novel steroidal pyrazoles derived from the natural sex hormone 5a-dihydrotestosterone (DHT). 2-Ethylidene or 2-(hetero) arylidene derivatives of DHT obtained by regioselective Claisen-Schmidt condensation with acetaldehyde or (hetero)aromatic aldehydes in alkaline ethanol were reacted with monosubstituted hydrazines to give A-ring-fused 1,5-disubstituted pyrazoles as main or exclusive products, depending on the reaction conditions applied. Transcriptional activity of the AR in a reporter cell line was examined for all novel compounds, and several previously synthesized similar DHT-based pyrazoles with differently substituted heteroring were also included to obtain information about the structure-activity relationship. Two specific regioisomeric groups of derivatives diminished the transcriptional activity of AR in reporter cell line and displayed reasonable antiproliferative activity in AR-positive PCa cell lines. Lead compound (3d) generally suppressed AR signalling, moreover, it also led to a sharp decrease in wt-AR protein level probably caused by proteasomal degradation. We confirmed the antiproliferative activity selective for AR-positive PCa

cell lines, cellular interaction of 3d with AR, and described the binding in ligand-binding domain by the flexible docking. Moreover, compound 3d was shown to be potent even ex vivo in patient-derived tissues, which highlights the therapeutic potential of A-ring-fused pyrazoles.

Poznámky / Notes:

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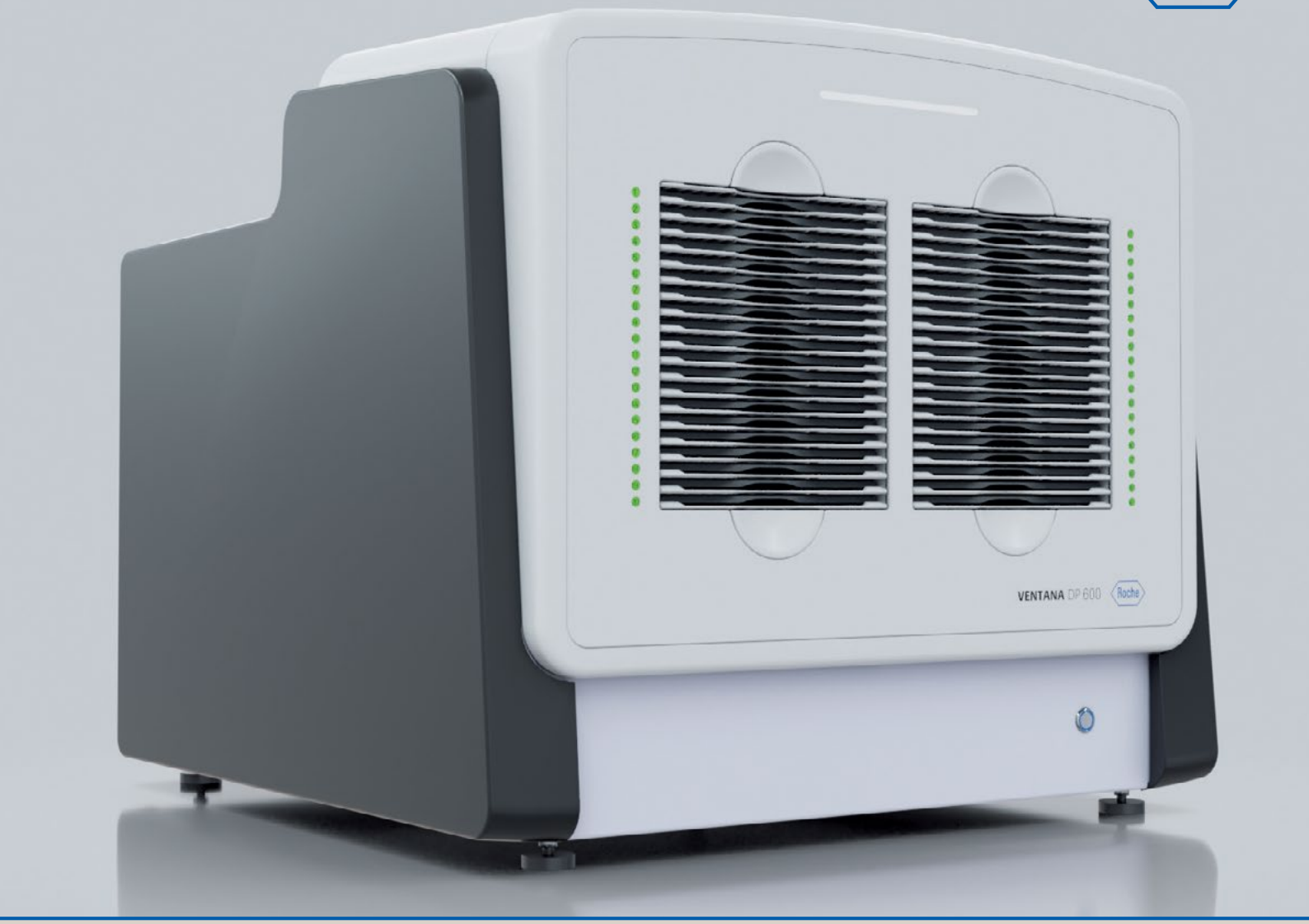

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