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# POSTER PRESENTATIONS' ABSTRACTS

# Androgen receptor as potential drug response biomarker in endometrial cancer

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BACKGROUND: Endometrial cancer (EC) is a gynaecological malignancy that affects the uterine inner lining. Epidemiological studies associate particular androgen hormones with greater EC risk, however the molecular mechanisms of androgen receptor (AR) signalling are not well understood. Better comprehension of the latter might lead to uncovering AR as potential prognostic and drug response biomarker in EC.

STUDY AIMS: To evaluate AR biomarker potential in EC.

MATERIALS AND METHODS: The Cancer Genome Atlas (TCGA1) and the Clinical Proteomic Tumour Analysis Consortium (CPTAC2) cohorts were explored to assess AR expression and its correlation with tumour grade, molecular class and survival status. AR expression was also evaluated in 39 matched tumour/adjacent tissue pairs. Gene expression data from CPTAC were used to assess signalling pathway activity, using the GSVA package in R. Least square regression analysis was performed to calculate the correlation coefficient (r) between AR expression and pathway activity.

RESULTS: AR expression differs between EC grades and molecular classes. More specifically, low grade POLE hypermutated, microsatellite instability (MSI) high and copy number variation (CNV) low endometrioid tumours have higher AR expression comparing to adjacent control tissue and high grade, CNV high tumours. Furthermore, AR expression correlates negatively with several DNA repair mechanisms, such as base excision repair and mismatch repair ($r= 0.41$, 0.41, respectively), as well as with telomere extension ($r= 0.44$). Likewise, AR expression is inversely correlated with M G1 and G1 S cell cycle phase transition ($r= 0.5$, 0.48, respectively). Finally, AR expression is associated with better overall survival in EC patients (t test, $p= 0.009$).

CONCLUSIONS: AR expression is associated with lower tumour grade and better overall survival; thus, it might represent a positive prognostic indicator in EC. Moreover, higher AR expression is associated with lower DNA repair potential and impaired cell cycle progression in endometrial tumour cells, therefore it might be used as biomarker of response to DNA repair and cell cycle checkpoint inhibitors.

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## Beneficial effects of fish and evening primrose oil on inflammatory response and fatty acid profile in breast cancer women undergoing chemotherapy

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BACKGROUND: Breast cancer is the most common malignant tumor and one of the leading causes of cancer-related death in women in the world and in Serbia, too. Cancer and inflammation are closely related, and an exacerbated inflammatory process can lead to tumor progression and a worse prognosis for the cancer patient. Omega-3 fatty acids are considered immune nutrients and are commonly used in the nutritional therapy of cancer patients, due to their numerous biological effects.

STUDY AIMS: This study aimed to evaluate the action of fish oil and evening primrose oil, both rich in polyunsaturated fatty acids (PUFA) on plasma fatty acid profiles and levels of inflammatory cytokines, in breast cancer patients undergoing chemotherapy.

MATERIALS AND METHODS: In this randomized control trial, we included 29 postmenopausal women, aged 50-70, with ER+ and/or PR+ breast cancer. All patients underwent surgical treatment and were qualified for chemotherapy (doxorubicin and cyclophosphamide). The first group of 14 patients was taking, two gel capsules of Omega-3 Cardio, containing a total of 1 g docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), and two gel capsules of evening primrose oil, containing 234 mg of gamma-linolenic acid in 12 weeks, during their chemotherapy. The second group of 15 patients was taking placebo capsules.

RESULTS: The results of this study showed an increase in plasma levels of docosapentaenoic acid (22:5n-3), DHA, total n-3PUFA, vaccenic acid (18:1n-7), and a decrease in n-6/n-3 PUFA ratio ($p<0.01$) in the intervention group. An increase in the level of dihomo-gamma-linolenic acid (DGLA, 20:3n-6) was observed in the placebo group during the study. There was no difference in levels of examined cytokines (IL-8, IL-10, and TNF-alpha), except for IL-6 which was decreased in both groups. However, intergroup comparisons at the end of the study revealed a significantly ($p<0.01$) lower level of IL-6 in the intervention group.

CONCLUSION: In conclusion, this supplementation improves the PUFA status and decreases the level of IL-6 in breast cancer patients undergoing chemotherapy and may help in reducing those cancer complications linked to lipid metabolism and inflammation.

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## Bone Scan with Tc99m-MDP, the Missing Link in the Initial Staging of Muscle-Invasive Bladder Carcinoma

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BACKGROUND: Accurate staging is crucial to determine the type of treatment for patients with bladder cancer, especially in high-risk cases. We aimed to assess the role of bone scan in the initial staging of muscle-invasive bladder carcinoma.

MATERIAL AND METHODS: 45 patients with muscle-invasive bladder cancer were referred to our tertiary clinic to perform a Tc99m-MDP bone scan from January 2019 to March 2020. The patients

underwent bone scintigraphy with pelvic SPECT/CT before radical cystectomy. Whole-body scanning was performed four hours after injection of Technetium 99m-methyl diphosphonate (MDP) in both anterior and posterior views. Since the most common bone involvement site in these patients is the pelvic bones and the spine, pelvic SPECT/CT was performed in all patients.

RESULTS: frequency of skeletal metastasis was 26.7%. Only 19% of the metastases were detected by previous pelvic CT/MRI images performed for routine staging. All the reported skeletal metastases by previous anatomical imaging methods were detected in the bone scan. There was no statistically significant correlation between bone metastasis and the patient's age, lymph nodes metastasis, hydronephrosis, and muscle-invasive type. The mean serum calcium level was 8.7 ± 0.57 in patients with bone metastasis and 8.87 ± 0.99 in patients without bone metastasis which was not statistically significant. Serum ALP levels in patients with and without bone metastasis were 271.4±11 and 276.7±98.9, respectively. Bilateral hydronephrosis was significantly associated with lymph node metastasis.

CONCLUSION: Bone scan has higher diagnostic performance than conventional imaging methods for detecting bone metastases. It changed the management plan in 8.8% of our patients, so we conclude that performing a whole-body bone scan in the initial staging of muscle-invasive bladder carcinoma would be helpful.

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## Circulating miRNAs as potential biomarkers in patients with early stages of malignant melanoma

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BACKGROUND: Melanoma is a highly invasive form of skin cancer. Advanced melanoma is associated with a poor prognosis and significant risk of disease relapse. Non-invasive circulating tumor biomarkers in liquid biopsy, such as microRNAs (miRNA), provide the chance for better personalization of treatment strategies. The aim of this study was to evaluate the circulating miRNAs for the identification of patients with a higher risk of melanoma recurrence after surgery, and for prediction of the disease outcome.

PATIENTS AND METHODS: A total number of 22 patients with stage I to III of melanoma were enrolled in this study. Plasma samples were obtained from pre-surgery and early post-surgery peripheral blood draws. The panel of 23 candidate miRNA were designed and miRNA expressions were analyzed by qRT-PCR technique with miRNA39 as endogenous control. The IBM SPSS software was used for the statistical analysis and data presentation.

RESULTS: We observed upregulation of miRNA182 (P=0.07), miRNA19a (P=0.05), and miRNA21 (P = 0.05), while miRNA1260 (P = 0.05), and miR145 (P = 0.04) were downregulated in post-surgery compared to pre-surgery plasma samples. Higher pre-operative expression levels of miRNA1980 (P = 0.001), miRNA99a (P = 0.008), miRNA320 (P = 0.009), miRNA494 (P = 0.018), and miRNA4487 (P = 0.048) were associated with shorter disease-free interval (DFI),

whereas, higher pre-operative plasma levels of miRNA494 (P = 0.009), miRNA320 (P = 0.016), miRNA99a (P = 0.017), miRNA1980 (P = 0.024), miRNA1260 (P = 0.026), and miRNA221 (P = 0.026), were associated with worse overall survival (OS).

CONCLUSIONS: Liquid biopsy and circulating biomarkers provide the possibility to assess patients by a minimally-invasive approach, which could lead to a better understanding of cancer behavior. Our study showed that assessing miRNA levels in plasma of early stages melanoma patients, may have the potential of being a reliable non-invasive biomarker to estimate the risk for melanoma relapse as well as to improving disease prognosis.

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## Do EMT and Wnt markers predict lymph node involvement in endometrial cancer?

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BACKGROUND: Two of most important and partially intertwined molecular pathways, linked to the carcinogenesis and progression of endometrial cancer, are Wnt signalling and epithelial-to-mesenchymal transition (EMT). Wnt signalling leads to nuclear accumulation of $\beta$-catenin and transcription of cell-cycle regulator genes and is regulated by various Wnt inhibitors, such as Dkk proteins. EMT is a process, characterized by loss of epithelial (E-cadherin) and gain of mesenchymal (N-cadherin) markers and has been associated with the process of metastasis. Better understanding of mechanisms, leading to lymph node involvement (LNI) in endometrial cancer is important for adequate treatment planning and can influence the prognosis of the patient.

STUDY AIMS: We aimed to compare the expression of markers, associated with Wnt signalling ($\beta$-catenin and Dkk1) as well as EMT (E-cadherin and N-cadherin) with LNI, to determine their influence on lymphovascular metastasis in endometrial cancer.

MATERIALS AND METHODS: We analysed samples of tumour tissue from women who underwent hysterectomy for the treatment of endometrial cancer. In addition to standard histopathological report, molecular classification of tumours was done by determining POLE mutation status as well as p53 and MMRd expression, which was done by immunohistochemistry (IHC). To assess the influence of Wnt signalling, $\beta$-catenin, Dkk1, N-cadherin and E-cadherin expression was evaluated, using IHC methods. We compared the expression of beforementioned tumour characteristics with LNI, using non-parametric statistical tests.

RESULTS: 6 (9,2%) out of 56 patients had LNI. Our results show that women with LNI had lower expression of $\beta$-catenin (U=136.5, p=0.720) as well as E-cadherin (U=86.5, p=0.092) but slightly higher expression of N-cadherin (125.0, p=0.507) and Dkk1 (U=146.5, p=0.926). However, none of our results were statistically significant. Furthermore, comparison of nuclear $\beta$-catenin expression and LNI gave equivocal results with 3 cases of positive lymph nodes in either group of tumours (p=0.397).

CONCLUSIONS: Our results did not show significant correlation with the expression of either of the assessed molecular markers. However, we observed a pattern of lower expression of E-cadherin and higher expression of N-cadherin in tumours with LNI, suggesting the role of EMT the process of metastasis.

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# Dramatic Cessation of gross sustained treatment resistant hematuria after lu177-psma therapy, a case report

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We report a patient with locally invasive metastatic castration resistant prostate adenocarcinoma, which has massive invasion to the bladder and caused frequent gross hematuria. The patient had received more than 10 units of pack cell at the time he was referred for lu177-psma therapy, but despite frequent transfusions his hemoglobin was always under 8 g/dl.

He had received first generation androgen depreviation therapy (ADT) from 3 years ago and the second generation from 1 year ago and had undergone multiple procedures for cessation of hematuria, such as multiple cystoscopies, bladder irrigations and angioembolizations in the urology department.

We performed Tc99m-PSMA whole body and SPECT/CT scan, which demonstrated a large PSMA avid prostate mass with massive invasion to the bladder. All components of the locally invasive tumor showed high PSMA avidity, so he was scheduled for Lu177-PSMA therapy.

1 week after the diagnostic scan, Lu177-PSMA was administered and the patient reported no hematuria 4 days after the lu177-PSMA injection. In the follow up, no recurrent hematuria was reported, too. The PSA level declined from 40.5 ng/ml to 18.7 ng/ml, 1 month after the first treatment.

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# Elimination of falsely elevated hCG immunoreactivity in serum due to heterophilic antibodies

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BACKGROUND: Positive Interference by heterophilic antibodies is a rather common problem in immunometric assays. Manufacturers of diagnostic kits have usually added various components to their reagents in order to reduce this problem. However, this may not be enough to eliminate interference in all samples. Thus, the laboratory must have efficient techniques and strategies to tackle this problem.

STUDY AIMS: We aimed to study samples from our routine hCG- and hCGbeta assays where both direct analysis and removal of possible heterophilic interference had been ordered. hCG and hCGbeta were quantitated with assays before and after heterophilic antibody removal with Heterophilic Blocking Tube (HBT).

MATERIALS AND METHODS: We analyzed the measurement data of serum hCG and/or hCGbeta taken during routine clinical follow up, from five patients. hCG and/or hCGbeta was analyzed before and after treatment with HBT at least once during follow-up. HBT was obtained from Scantibodies Laboratory. The assays used were an in-house immunofluorometric hCGbeta assay, Abbott Architect total hCG assay and PerkinElmer AutoDELFIA intact hCG assay.

RESULTS: Cases #1-4. Four testicular cancer patients during follow-up with no clinical evidence of disease. In ten moderately elevated serum samples (hCGbeta 2.2 - 8.7 pmol/L) treatment with HBT decreased hCGbetato normal. Intact hCG was <1 IU/L in all samples. Case #5: Suspicion of ectopic pregnancy. hCG was quantitated by Abbott Architect in 18 samples obtained during a period of 3 months. hCG concentrations ranged between 62 - 151 IU/L. After treatment with HBT hCG in

one sample (hCG 71 IU/L) became undetectable (hCG <1.2 IU/L). The same untreated sample was analyzed with AutoDELFIA which gave a result of 0.5 IU/L and after treatment with HBT 0.1 IU/L.

CONCLUSIONS: In this study the Heterophilic Blocking Tube was shown to be effective in eliminating falsely elevated hCG immunoreactivity in 13 samples from five different patients. The use of HBT as a confirmation is a simple and convenient way to verify if a result is actual or falsely elevated by interference of heterophilic antibodies.

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## EP4 receptor agonist is cytotoxic to chronic lymphocytic leukemia cells and acts synergistically with ibrutinib and idelalisb

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BACKGROUND: Prostaglandin EP4 receptor signaling was shown to prevent B cell receptor-mediated proliferation and represents a novel approach toward improving the therapy of B cell malignancies. In our previous research we have shown that selective EP4 receptor agonists inhibited NF-$\kappa$B signaling pathway, resulting in an increased caspasemediated apoptosis of malignant B cells.

STUDY AIMS: The aim of our preclinical study was to evaluate the EP4 receptor as a potential therapeutic target and EP4 receptor agonist PgE1-OH as a drug candidate for the treatment of chronic lymphocytic leukemia (CLL), the most common hematological malignancy diagnosed in adults.

MATERIALS AND METHODS: Malignant B cells were isolated from whole blood obtained after informed consent from CLL patients in accordance with the ethical approval of Republic of Slovenia National Medical Ethics Committee (Nr. 93/12/10). The viability of cells was assessed using resazurin based assay. Cytokine production was measured by cytometric bead array.

RESULTS: PgE1-OH induced a concentration and time dependent cytotoxicity in CLL cells. The anti-cancer effects were mediated via EP4 receptor as evident from stronger cytotoxic effects of PgE-1OH compared to endogenous ligand PgE2 and the fact that EP4 receptor antagonist prevented PgE1-OH induced apoptosis. We evaluated the selectivity of PgE1-OH towards CLL cells using LCLs and peripheral blood mononuclear cells (PBMCs) obtained from healthy individuals. The average EC50 values for PgE1-OH after 24 h were 13.56 μM on CLL cells (N=151), 55.43 μM on LCLs (N=24) and 46.36 μM on PBMCs (N=14), indicating that PgE1-OH was significantly more cytotoxic to malignant B cells compared to immune cells isolated from healthy individuals. Moreover, PgE1-OH acted synergistically with fludarabin, ibrutinib and idelalisib in CLL cells. Since the microenvironment in CLL is a potent driving force for the malignant B cell survival, we assessed the immunomodulatory properties of EP4 receptor agonist by measuring cytokine production. PgE1-OH significantly decreased levels of IL-2, IL-10, TNF$\alpha$ and IFN$\gamma$ compared to untreated activated LCL cells and diminished IL-2 and TNF$\alpha$ in activated CLL cells, further confirming the EP4 receptor agonist as a promising drug candidate for the treatment of CLL.

CONCLUSIONS: Prostaglandin EP4 receptor was identified as a promising therapeutic target in CLL.

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# Expression and Activity of Proteasome Subunits in Platelets Differs Between Individual Donors

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BACKGROUND: Proteasomes are large protein complexes responsible for intracellular proteolysis. They have three specific subunits responsible for the catalytic activity (ß1, ß2, ß5). Hematopoietic cells assemble a specialized form of proteasomes, known as the immunoproteasomes, in which the constitutive catalytic sites are replaced by ß1i, ß2i and ß5i. Proteasomes play a key role in MHC class I antigen processing and in shaping the immune response through regulation of cytokine production, cell differentiation, survival, and proliferation. In addition, platelets have an important role in cancer, recruiting granulocytes to the platelet-tumor cell aggregates by chemokine secretion, thus foster tumor cell survival. However, the role of (immuno)proteasome modulation in platelets and their potential role in shaping the tumor microenvironment remains unclear.

STUDY AIMS: Characterization of differences in proteasome and immunoproteasome subunit expression and activity in platelets.

MATERIALS AND METHODS: Venous blood was obtained from informed healthy donors and platelets were isolated and afterwards treated with selective inhibitors of immunoproteasome subunits. Catalytically active subunits were identified in the isolated platelets by western blotting. Assessment of proteasomal activity was determined by irreversible fluorescent probes. Chemokine release was demonstrated with flow cytometer in supernatants of platelets pre-treated with selective inhibitors (LMP7-IN-1, KZR-504) and activated with ADP or TRAP-6.

RESULTS: Our research identified differences in the expression of proteasome subunits $\beta$1c, $\beta$2c, and $\beta$5c as well as the immunoproteasome subunits $\beta$1, $\beta$2i and $\beta$5i in platelets from healthy donors. In addition, we found differences in the activity of proteasome and immunoproteasome subunits in human individuals, where $\beta$1c, $\beta$1i and $\beta$5i were more prominently expressed. Furthermore, preliminary results showed that selective inhibitors of $\beta$1i and $\beta$5i subunit can up-regulate the chemokine RANTES (CCL5) by itself or after the addition of platelet aggregation activators (ADP, TRAP-6), suggesting that proteasomes play a role in shaping platelet function.

CONCLUSIONS: The catalytically active subunits of the proteasome and the immunoproteasome in platelets of individual donors differ in expression and activity. If the subunits are modulated by the use of selective inhibitors, altered in vitro platelet function can be observed in terms of aggregation and chemokine release. However, further research is needed to determine the essential role of the proteasomes in platelets.

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# How a novel web application can help in Urinary Bladder Cancer risk management: The BLUCAB Index®

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BACKGROUND: The urinary UBC® Rapid Test is a quantitative point-of-care test for the detection of bladder cancer (BC). It has a sensitivity of 53–71% and a specificity of 61–96%, and is not yet recommended for screening patients for the presence of BC.

STUDY AIMS: Our aim was to develop and validate nomograms that combine the predictive power of the UBC® Rapid Test and established risk factors to help identify patients at high-risk for primary BC as well as to provide an online tool for rapid risk determination.

MATERIALS AND METHODS: We retrospectively reviewed data from 1035 patients from three study centers in Germany tested between 2013 and 2021. 746 patients were included in the analysis, comprising 377 patients with primary BC and 369 healthy controls with no history of BC. Urine samples were analyzed with the UBC® Rapid Test (IDL Biotech, Bromma, Sweden), applying a cutoff of 9.9 µg/L. We used a multivariate logistic regression model (LRM) to predict BC in a development cohort of 394 patients and validated the LRM in a cohort of 352 patients. Discrimination and calibration were assessed using the receiver operating curve and calibration curve.

RESULTS: Age, smoking status, UBC® Rapid Test results and hematuria were identified as independent predictors that can also be easily assessed at the point of care. After external validation, the nomograms based on these predictors achieved 0.76 (95% CI: 0.70–0.83) and 0.93 (95% CI: 0.90–0.96) accuracy in predicting LG-BC and HG-BC, respectively. Excellent calibration accompanied higher net benefit compared to using the UBC® Rapid Test alone for all risk thresholds in decision curve analysis.

CONCLUSION: The results of the UBC® Rapid Test in combination with other established BC risk factors offer a simple and non-invasive tool for the detection of primary LG-BC and especially primary HG-BC. High accuracy indicates that the prediction models own high disease recognition ability. Decision curve analysis proved the clinical usefulness of the nomograms. The web-based access-free tool BLUCAB Index® can be used to determine the risk of BC and is available under the link https://blucab.shinyapps.io/Blucab-Index-2/.

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## HSD17B14 as potential biomarker of chemoresistance of high-grade serous ovarian carcinomas

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BACKGROUND: High-grade serous ovarian carcinoma (HGSOC) is the most frequent ovarian cancer, which commonly develops resistance to chemotherapeutics. To improve HGSOC management, specific biomarkers for early detection of chemoresistance are needed. Our previous study of HGSOC (Pavli et al., 2022, Cancers, 14(11):2583) showed increased HSD17B14 and CYP1A2 in chemoresistant versus chemosensitive tissues, revealing their potential as chemoresistance-specific markers of HGSOC.

STUDY AIMS: To evaluate HSD17B14 and CYP1A2 as candidate chemoresistance biomarkers using HGSOC cells differing in carboplatin resistance (COV362, Caov-3, OVCAR-3, OVCAR-4). To develop higher resistance (R) of parental cells (COV362/R, Caov-3/R, OVCAR-3/R, OVCAR-4/R) and to examine the effects of carboplatin resistance on HSD17B14 and CYP1A2 expression. To evaluate HSD17B14 and CYP1A2 in chemoresistant and chemosensitive tissues of the HGSOC molecular subtypes (immunoreactive, differentiated, proliferative, mesenchymal).

METHODS: We established higher resistance HGSOC cells COV362/R, Caov-3/R, OVCAR-3/R, OVCAR-4/R by cultivating parental cells with increasing carboplatin concentrations. The HSD17B14 and CYP1A2 expression was studied by qPCR using TaqMan assays and by using public mRNA

expression data (cBioPortal) for cell lines and tissue samples of different molecular subtypes of HGSOC.

RESULTS: QPCR analysis revealed increased HSD17B14 expression with higher carboplatin chemoresistance in all parental cells, except in OVCAR-4. The established resistant cells COV362/R, Caov-3/R, OVCAR-3/R showed higher HSD17B14 expression versus parental cells, while in OVCAR-4/R the opposite trend was seen. The CYP1A2 expression was the highest in highly chemoresistant COV362 cells and differentially expressed in others. In COV362/R, Caov-3/R, and OVCAR-3/R, the CYP1A2 was lower, and in OVCAR-4/R higher versus the corresponding parental cells. Subanalysis of HSD17B14 and CYP1A2 expression in tissues of the four molecular subtypes of chemoresistant versus chemosensitive HGSOC revealed increased HSD17B14 in differentiated subtype, whereas CYP1A2 showed higher expression trend only in mesenchymal HGSOCs.

CONCLUSIONS: Our results indicate the potential of HSD17B14 as a predictive tissue marker of tumor chemoresistance of differentiated HGSOC and as biomarker candidate of disease progression. However, further validation studies are needed before these results can be translated into treatment practice.

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## Influence of alginate-based microcapsule permeability on 3D cancer cell cluster proliferation

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BACKGROUND: Functional interaction between cancer cells and the surrounding microenvironment is still not sufficiently understood, which motivates the tremendous interest in the development of numerous in vitro and in vivo tumor models.

STUDY AIMS: To study the influence of different permeability of microcapsules (MCs) on different cancer cell proliferation, and to design and engineer the formation of 3D tumor clusters in MCs.

MATERIALS AND METHODS: A fluidics-based low-cost methodology was used to reproducibly generate alginate (AL) and alginate-chitosan (AL-CS) MCs in a cross-junctions water-in-oil system. The diffusion through the shell of AL and AL-CS MCs was monitored using fluorescein sodium (376 Da), FITC-Dextran 70 (70 kDa), and FITC-Dextran 2000 (2000 kDa) as fluorescent probes representing small molecules, proteins, and macromolecules, respectively. HepG2 Red FLuc (human hepatoma cell line) and A375 (human melanoma cell line) cultured in DMEM medium were used to study the proliferation differences in dimensions and geometries in AL and AL-CS MCs. The metabolic activity of tumor clusters in MCs was confirmed by tracking the turnover of testosterone to androstenedione with liquid chromatography-tandem mass spectrometry.

RESULTS: HepG2 Red FLuc and A375 cells show different proliferation properties in AL and AL-CS MCs. A375 tumor clusters grow faster in more permeable AL MCs and slower in less permeable AL-CS MCs. In the case of HepG2 Red FLuc, a significant difference in proliferation rate was not observed between AL and AL-CS MCs at the early stage (1 week). Interestingly, it was observed that different loose and tight cell cluster morphologies can form, also including cell proliferation along radial

directions in both MC types and both cell lines. Cytochrome P450 (CYP)-dependent metabolization of testosterone by both HepG2 Red FLuc and A375 tumor clusters in the AL and AL-CS MCs showed the same trends in good agreement with their proliferation stages.

CONCLUSIONS: A low-cost cross-junction-based microfluidic droplet system was constructed and used to generate AL MCs and AL-CS MCs with different permeability for culturing HepG2 Red FLuc and A375 cells. As the permeability differences between MCs influence tumor cluster formation, cell proliferation, and metabolic ability of cells, engineering of MC is an effective method for the targeted design of 3D tumor clusters.

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## Inter-component immunohistochemical assessment of proliferative markers in uterine carcinosarcomas

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In the scientific literature, a selected number of reports have investigated the impact of proliferative activity on the development and progression of uterine carcinosarcomas (UC). The aim of the present retrospective study was to compare the immunohistochemical proliferation markers [Ki67, proliferating cell nuclear antigen (PCNA), minichromosome maintenance complex component 3 (MCM3), and topoisomerase IIα (topoIIα)] assessment in both components of UC. A total of 30 paraffin-embedded slides of UCs, obtained from patients who underwent surgery between January 1, 2006, and December 31, 2020, were analyzed. Medical records and clinicopathological data of patients were reviewed. Formalin-fixed, paraffin-embedded tissue sections were immunostained with monoclonal antibodies against Ki67, PCNA, MCM3 and topoIIα. Ki67-positive nuclear immunoreactivity was reported in 20 (67%) and 16 (53%) UC carcinomatous and sarcomatous components, respectively. In the epithelial component, Ki67 positive staining was related to the International Federation of Gynecology and Obstetrics (FIGO) stage (P=0.025), and histological grade (G1 vs. G2/G3, P=0.031). Nuclear PCNA reactivity was observed in 18 (60%) and 16 (53%) carcinomatous and sarcomatous components, respectively. Notably, all four cases with omental metastases were PCNA-positive, and a relationship between staining pattern and the existence of metastases was of significant value (P=0.018). MCM3-positive nuclear staining was found nearly twice as high in the carcinomatous (n=19; 63%), compared with the sarcomatous (n=11; 37%) component, respectively, and MCM3 expression in the epithelial component was related to clinical stage (P=0.030), and the existence of omental metastasis (P=0.012). In addition, out of the 30 UCs, 17 (57%) and 13 (43%) showed topoIIα positivity in the carcinomatous and sarcomatous UC components, respectively. A significant relationship between protein immunoreactivity and FIGO stage (P=0.049), and omental metastasis (P=0.026) was revealed to exist. However, no significant differences between expression of proliferation markers and clinicopathological features in the sarcomatous UC component were identified. Finally, a significant correlation between each protein immunohistochemical staining was demonstrated, particularly in the sarcomatous UC component. Collectively, a combined analysis of Ki67, PCNA, MCM3, and topoIIα may provide more detailed information of cell-cycle alterations determining the heterogeneity of uterine carcinosarcomas.

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## JAG1 intracellular domain enhances AR expression and signaling and promotes stem-like properties in prostate cancer cells

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The expression of androgen receptor variants (AR-Vs) is associated with the development of advanced castration-resistant prostate cancers (CRPCs), while prostate cancer stem cells (PCSCs) have been evaluated as the most dangerous malignant seeding cells. JAG1 expression is upregulated in high-grade and metastatic prostate carcinomas, and its high expression is associated with poor disease-free survival of prostate cancer patients. Intriguingly, all JAG1-positive prostate carcinoma tissues express JAG1 cytoplasmic intracellular domain (JICD), although its function in prostate cancer (PC) cells is poorly understood. In this study, we investigated a role of JICD in AR expression and signaling in prostate cancer cells, which is associated with CRPC progression. JICD overexpression increased both mRNA and protein expression of AR, especially AR-Vs in PC cell lines. JICD also significantly enhanced androgen-independent function of ARs. In addition, JICD increased PC cell mobility, while it caused a quiescence in cell proliferation. Intriguingly, JICD overexpression upregulated the expression of PCSC marker CD133, self-renewal marker NANOG, and anti-apoptotic BCL-X(L) protein, while it downregulated the expression of apoptotic BIM protein. Therefore, JICD may play a crucial role in enhancing androgen independence and promoting stem-like properties in prostate cancer cells, driving PC cells to be AR-positive and CD133high with high self-renewal and survival ability.

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## Logistic Regression Using a Combination Of CA-62, CA15-3, And Age for Detecting Early Stages Of Breast Cancer In Screening

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BACKGROUND: The logistic regression model is widely used in medical science for predictive analytics and diagnostics. The main advantage of this type of statistics is its ability to combine several continuous, binary, or categorical independent variables to predict the probability of a given binary outcome such as 'sick' or 'healthy'. In a previous communication (Cancer Biomarkers v.35 (2022) p. 57–69), the cutoff value for each marker was set so that there would be no false positives (100% specificity). Then, the positive cases for each marker were combined to calculate the sensitivity, which was 75%.

OBJECTIVES: To determine if a Logistic Regression using the CA 15-3 and CA-62 original values from the abovementioned article plus the age of each individual improved the method's diagnostic efficacy.

METHOD: The serum samples were blinded and had measurements of CA 15-3 (ELISA) and CA-62 (CLIA) as well as their corresponding TNM classification. The study included 300 breast cancer patients (254 at Stages I and II, 20 with ductal carcinoma in situ (DCIS), and 26 Stage III and IV patients), 47 patients with breast benign diseases, and 141 healthy controls.

The Logistic Regression analysis was done with the Real-Statistics Excel® Add-in and included as continuous predictors, CA 15-3, CA-62, and Age.

RESULTS: Implementation of the binary logistic regression analysis including age allowed to estimate the probability of having breast cancer with a sensitivity = 95% and specificity = 99%

(AUC = 0.99) as compared to 75% sensitivity with 100% specificity (AUC = 0.985) with the previous method.

CONCLUSIONS: The obtained results show that the application of Binary Logistic Regression on the original dataset considerably improves the prediction of a breast cancer diagnosis from 75% with 100% specificity to 95% sensitivity with 99% specificity.

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## Machine learning algorithms including angiogenic factors as potential tool for enhanced endometrial cancer diagnostics

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BACKGROUND: Endometrial cancer (EC) is the most frequent gynaecological malignancy in developed countries. It requires a relatively invasive diagnostic evaluation and operative therapy. Angiogenesis is one of the main processes needed for cancer growth and spread. The production of angiogenic factors (AFs) appears early in the process of carcinogenesis. The detection of AFs in plasma may contribute to earlier diagnosis and prognosis and more patient oriented therapeutic approach. Machine learning algorithms help developing cancer risk stratification systems with great precision and might help create robust diagnostic model using multiple EC specific risk factors.

STUDY AIMS: To analyse the potential of AFs as diagnostic and prognostic biomarkers as a part of machine learning models.

MATERIALS AND METHODS: Our study analysed 202 patients, of whom 91 were diagnosed with EC and 111 were control patients with benign gynaecological disease. Using Luminex xMAP™ multiplexing technology, we measured the pre-operative plasma concentrations of six previously selected angiogenic factors – leptin, IL-8, sTie-2, follistatin, neuropilin-1, and G-CSF. Besides basic statistical methods, we used a machine-learning algorithm to create a diagnostic model based on the plasma concentration of tested AFs.

RESULTS: The plasma levels of leptin were significantly higher in EC patients than in control patients. Leptin was higher in type 1 EC patients, and IL-8 was higher in type 2 EC, particularly in poorly differentiated endometrioid EC grade 3. IL-8 plasma levels were significantly higher in EC patients with lymphovascular or myometrial invasion. Among univariate models, the model based on leptin reached the best results on both training and test datasets. A combination of age, IL-8, leptin and G-CSF was determined as the most important feature for multivariate model, with ROC AUC 0.94 on training and 0.81 on test dataset. The model utilizing a combination of all six AFs, BMI and age reached an ROC AUC of 0.89 on both the training and test dataset, strongly indicating the capability for predicting the risk of EC even on unseen data.

CONCLUSIONS: According to our results measuring plasma concentrations of angiogenic factors could represent an important supplementary tool for early detection and prognostic characterization of EC, which could guide the decision-making regarding the extent of treatment.

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# Potential of AKR1B1 as diagnostic biomarker of endometrial cancer

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BACKGROUND: Endometrial cancer (EC) is one of the most common malignancies experienced by women worldwide. Currently, there are no screening methods for EC and diagnosis relies on minimally invasive or invasive surgical procedures with histopathology examination. Discovery of specific biomarker(s) for EC would enable early diagnosis of asymptomatic patients and appropriate treatment before progression of this disease. Aldo-keto reductase family 1 member B1 (AKR1B1) is known to affect progression of different cancer types. Our previous studies showed higher levels of this protein in adjacent non-neoplastic endometrial tissue compared to endometrioid EC suggesting it has a protective role[1].

STUDY AIMS: This study aimed to investigate whether AKR1B1 has a potential as a non-invasive diagnostic biomarker of EC.

MATERIALS AND METHODS: In this study, we have included 72 postmenopausal women. Selected patients were divided into two groups after histopathological analysis of endometrial tissue biopsy: 1) women with diagnosed EC (cases, n=36) and 2) women with benign pathologies (controls, n=36). There was no significant difference in age and BMI between cases and controls. Additionally, for each EC patient, different clinical data were gathered including histological grade and EC type, depth of myometrial invasion (MI), lymphovascular invasion (LVI) and presence of metastasis (MET). Plasma samples of patients were collected at the Department of Obstetrics and Gynecology at the University Medical Centre Ljubljana in Slovenia following a strict SOP. Only once frozen/thawed samples were used for the analysis. Undiluted samples were analysed using AKR1B1 ELISA kit (Novus Biologicals, USA) according to the manufacturer's instructions.

RESULTS: Although slightly higher plasma levels of AKR1B1 were detected in controls compared to cases, difference was not confirmed as statistically significant (Mann-Whitney, p=0.162). ROC curve analysis showed low diagnostic potential of AKR1B1 with area under the curve (AUC) value of 0.602. Data analysis according to EC type (endometrioid EC and serous EC vs controls), EC gradus, depth of MI, presence of LVI and MET showed no differences in plasma levels of AKR1B1 between examined groups.

CONCLUSIONS: AKR1B1 showed low potential in separating cases from controls in the plasma samples of the selected cohort. Further analysis to examine its prognostic potential in a larger patient cohort is currently ongoing.

References:

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# Preclinical evaluation of prostaglandin EP4 receptor as a novel biomarker and therapeutic target in chronic lymphocytic leukemia cells

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BACKGROUND: Chronic lymphocytic leukemia (CLL) is currently incurable disease with high inter-individual variability, which is reflected in the development of the disease, prognosis and response to the therapy. Prostaglandin EP4 receptor signalling was shown to restrain B cell receptor (BCR)-mediated proliferation, and represents a novel strategy toward improving the therapy of B cell malignancies, known to be depend on BCR signals for survival.

STUDY AIMS: The aim of this study was to evaluate prostaglandin EP4 receptor as a potential novel biomarker and therapeutic target for the treatment of CLL.

MATERIALS AND METHODS: Malignant B cells were isolated from whole blood obtained after informed consent from CLL patients in accordance with the ethical approval of Republic of Slovenia National Medical Ethics Committee (Nr. 93/12/10). The viability of cells treated with PgE1-OH was assessed using by resazurin based assay. CLL samples were genotyped for the single nucleotide polymorphisms (SNPs) rs4495224 and rs7720838. Ptger4 mRNA expression was determined.

RESULTS: EP4 receptor agonist PgE1-OH exerted cytotoxic effects in all CLL cells (N = 151) with EC50 values ranging from 2 to 55 $\mu$M indicating inter-individual variability in response. PgE1-OH was comparably cytotoxic in all subgroups of CLL cells, which is of prime importance, especially for the patients with progressed disease with Binet stages B and C and with unfavourable prognostic factors, such as del11q and del17p, the latter leading to aberration of the tumor suppressor gene TP53 and predicting an aggressive disease course. PgE1-OH may thus show potential also in patients with TP53 aberration. The analysis of the results revealed sex-dependent sensitivity of CLL cells to PgE1-OH, which was more cytotoxic to the cells of male compared to female donors. PgE1-OH was also more cytotoxic toward the cells of the carriers of the variant A allele of EP4 receptor expression-modulating polymorphism rs4495224. Moreover, male patients had higher expression levels of Ptger4, coding for EP4 receptor, compared to female patients as did the donors with the rs4495224 AA genotype compared to those with rs4495224 AC/CC genotype.

CONCLUSIONS: In conclusion, EP4 receptor was identified as a promising therapeutic target and potential biomarker in CLL.

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## Prediction of overall survival in metastatic prostate cancer patients by using AroCell TK 210 ELISA as a tool

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BACKGROUND: Thymidine kinase 1 (TK1) is a salvage pathway enzyme that plays an important role in the synthesis of DNA precursors. TK1 has been established as a biomarker for prognosis and therapy monitoring of different malignancies. Most of the commercial TK1 assays such as TK-REA and TK-Liaison measures for TK1 activity have limited applicability for routine clinical use. A commercial ELISA-based measurement (TK 210) has recently been developed by AroCell based on two monoclonal antibodies against human TK1.

STUDY AIM: In this study, we explored the prognostic role of serum TK1 protein levels as determined by TK 210 ELISA in patients with prostate cancer (PCa).

MATERIALS AND METHODS: Serum samples from patients with metastatic prostate cancer (n=43) and localized disease (n=40) were included in this study for a total of 83 samples. In 43 men with de

novo metastatic PCa, the disease stage was confirmed by bone scan. All participants were diagnosed and treated at the Tampere University Hospital, Finland, between 2000-2010. The serum sample was obtained at diagnosis before treatment. Information on deaths was obtained from the comprehensive national registry. The protein levels of TK1 in serum samples were determined using the AroCell TK 210 ELISA (AroCell AB, Uppsala, Sweden) according to the manufacturer's instructions (www.arocell.com).

RESULTS: The median serum TK1 protein levels in patients with metastatic PCa were significantly higher compared to patients with localized disease (Median = 0.61 vs 0.28 μg/l). During the median follow-up of 67 months, 36 of the 43 men died, 24 due to metastatic PCa. Serum TK1 level above the median was associated with worse prostate cancer-specific survival (HR 2.47 95% CI 1.05-5.82) and overall survival (HR 3.08, 95%CI 1.49-6.36) compared to lower TK1. The random forest classification model including TK1 predicted PCa death more accurately than any other model.

CONCLUSIONS: Serum TK1 protein levels predicted survival after diagnosis of metastatic PCa, demonstrating additional predictive value over established clinical risk factors. If confirmed in larger studies, TK1 could be used for clinical risk assessment to select optimal treatment and surveillance schedule in metastatic PCa.

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## PrediPet measures TK1 protein and is an efficient serum biomarker for dogs with malignancies

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BACKGROUND: Serum biomarkers are non-invasive and inexpensive methods in cancer diagnostics both in human and veterinary medicine. Thymidine kinase 1 (TK1) is a protein released from rapidly proliferating cells such as tumour cells. TK1 activity determinations have been used to diagnose malignant disease for many years.

STUDY AIMS: PrediPet is a new ELISA method for determination of TK1 protein concentrations in blood samples from dogs based on two highly specific monoclonal antibodies. Here we report result demonstrating that PrediPet is highly efficient for detection dogs with several types of cancer diseases.

MATERIALS AND METHODS: The study was performed with serum samples from healthy dogs (117 sera) and dogs with T and B cell lymphoma (92 cases), and several other tumour diseases such as Histiocytic sarcoma, Hemangiosarcoma and other solid tumours (111 cases). TK1 protein was determined with PrediPet from Alertix and TK1 activity with the tritiated Thymidine phosphorylation assay. The cut off values were determined using the data from healthy dogs participating in the blood donor service at the Swedish University of Agricultural Sciences, Uppsala, Sweden.

RESULTS: The sensitivity for differentiation between the healthy dogs from those with lymphoma was 73% and from the solid tumour group it was 58% with 95% specificity. When using the TK1 activity assay the lymphoma group showed 55% sensitivity while with the solid tumour group no significant difference to the control group could in this case be detected.

CONCLUSIONS: These results for dogs with hematologic cancer are similar to what has been described earlier using TK1 activity measurements. However, here we demonstrate that PrediPet shows higher sensitivity for solid tumours and higher sensitivity also for the lymphoma group. Thus

PrediPet have several advantages as a diagnostic tool in veterinary medicine and furthermore it is an immunodiagnostic method that can be performed in most laboratories.

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## Prognostic and predictive models in oncology and cardiology. It is easier to predict death than the recurrence of disease or metastasis

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A prognostic factor is a characteristic of a tumour or disease that identifies patients with a specific risk of progression or death. These factors act independently of the treatment administered. They are particularly useful for dividing patients into groups for which differently intensive treatment is indicated, or treatment is omitted entirely in patients with a favourable prognosis.

Prognostic factor

Marker status Median survival (months)

No therapy Therapy A Therapy B

high 5 10 15

low 10 20 30

High prognostic factor values retain their prognostic significance despite the different types of treatment (hazard ratio 0.5). Treatment B is more effective than treatment A (hazard ratio 1.5).

A predictive factor is a sign that predicts different efficacy of a particular therapy. It may therefore serve to individualize anticancer treatment. In order to find such a sign, it should first be identified in pilot studies, then validated in a randomized study.

Predictive factor

Marker status Median survival (months)

No therapy Therapy A Therapy B high 5 10 10

low 5 10 30

High and low values of the marker have no prognostic significance, while low values of the marker studied predict higher efficacy of B treatment.

The author will show several studies in which he participated from the oncology and cardiology fields and compare their success rates. Risk functions in the cardiology were: - ischaemic stroke (including TIA) or embolism, - major bleeding in patients on anticoagulant treatment, - overall risk of death, - risk of developing atrial fibrillation, - risk of heart failure in patients with atrial fibrillation, - risk of developing cardiovascular disease, - risk of developing ischaemic heart disease, - risk of diabetes II. Type, - risk of fatty liver, - risk of stroke. In the oncology these were these risk function - progression, - success rates of therapy (for some tumours and therapies), - all-cause-death. It turns out that mostly ROC analysis of AUC leads to higher areas for all-cause-death risk funtions than for prediction of disease recurrence or its generalization.

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# Promoter Methylation Statuses of Wnt/β-catenin Pathway's Regulatory Genes in Astrocytic Brain Tumors

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BACKGROUND: Wnt pathway is a conserved signaling with important functions in development including nervous system. The dysregulation of this pathway has been repeatedly implicated in human tumors. Secreted frizzled related proteins (SFRPs) are modulators of Wnt signaling, DKK1 and DKK3 are negative regulators, while GSK3 can also act positively.

STUDY AIMS: To explore epigenetic changes and expression levels of key regulators of Wnt pathway in human astrocytomas of different malignancy grades in order to answer the question on their involvement in progression.

MATERIALS AND METHODS: Astrocytomas together with FFPE slides were collected with patients' consents. Classification was carried out according to recent WHO guidelines. Methylation-specific-PCR was used for promoter analyses, while immunohistochemistry and semi-quantitative score for the assessment of consequent protein expressions.

RESULTS: Promoters of selected genes displayed different methylation frequencies, DKK3 and DKK1 displayed the highest 43% and 38%, SFRP1 followed with 32% while SFRP4 and GSK3$\beta$ were less methylated in 16% and 18% of samples, respectively. SFRP1 gene was significantly more silenced in glioblastomas (grade IV, P=0.042) compared to lower grades. Contrary, SFRP4 promoter was methylated in 72.7% of grade II astrocytoma, while in higher grades methylation was not detected (P<0.001). Although grade IV comprised the lowest number of methylated GSK3$\beta$ cases and highest of DKK3, significant association was not reached. The effect of epigenetic statuses on the protein expression levels showed that samples with methylated SFRP1 promoter expressed significantly less SFRP1 protein than unmethylated (P=0.031). SFRP4 expression decreased in higher grades, i.e. grade II astrocytomas had more protein than grades III and IV (P=0,002). Immunostaining of active (pY216) and inactive (pS9) forms of GSK3$\beta$ revealed high levels of active form in all grades with strong expression in 70% of cases. Of note is a positive correlation between samples with methylated DKK3 promoter and the expression of active GSK3$\beta$ (P=0.011).

CONCLUSIONS: The oncogenic effect of GSK3$\beta$ is indicated. SFRP1 acts antagonistically and the expression of SFRP4 was not lost through promoter methylation. Our study shows that Wnt pathway is activated and emphasizes the importance of methylation for its regulation. The selected genes may serve as biomarkers of progression and future investigation toward epigenetic-modifying drugs.

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# Remarkable FDG uptake in numerous granulomatous panniculitis lesions: a case report

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A 42 y/o female patient with history of rheumatoid arthritis and Erythema Nodosum from 8 years ago, who was treated with celcept and prednisolone was admitted due to skin lesions (arrows) in the upper and lower extremities. Skin lesion's excisional biopsy was in concordance with panniculitis. 260 MBq of (F18)-fluorodeoxyglucose was administered intravenously via the right ante-cubital vein. To allow

for distribution and uptake of radio-tracer, the patient was allowed to rest quietly for 60 minutes in a shielded room. Imaging was performed on integrated 6-slice PET/CT scanner, with scanning from the skull top to toes. A low dose CT acquisition was done without contrast enhancement. MPI (A) and trans-axial PET/CT images (B) depicted FDG uptake in the numerous subcutaneous nodules in the upper and lower extremities.

Granulomatous Panniculitis in Erythema Nodosum is inflammation of the subcutaneous fat and manifest clinically as tender reddish nodules. Panniculitis is a relatively rare condition that is associated with streptococcal infection, Behçet disease, pregnancy, trauma, sarcoidosis, lymphoma, or inflammatory states. Increased cytokines expression is noted in Erythema Nodosum, with neutrophil recruitment and activation in serum and skin. As FDG is known accumulate in activated inflammatory cells, the involved sites in EN revealed increased FDG accumulation. Our case showed FDG uptake in the numerous subcutaneous nodules in the upper and lower extremities. FDG uptake in the panniculitis lesion is rarely reported in the literature.

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## STK1 concentration and STK1 activity as a diagnostic and prognostic proliferation biomarker for ovarian cancer

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BACKGROUND: Ovarian cancer is a common malignancy of the female reproductive system. It is usually diagnosed at an advanced stage because the early-stage disease is usually asymptomatic and symptoms are nonspecific. Transvaginal ultrasonography and CA125 testing are combined as a screening method, for distinguishing which women are more likely to have ovarian cancer. Unfortunately, it is known, that serum biomarkers lack adequate sensitivity (SN) and specificity (SP), and also these markers are not produced by all ovarian cancers. In our research, we aimed to investigate whether elevated levels of serum thymidine kinase 1 concentration (STK1c) or its activity (STK1a) provide a diagnostic tool that can better identify the presence of ovarian cancer at an earlier stage.

MATERIALS AND METHODS: In our research, the women were classified into the control group, benign tumor group, and malignant tumor group (borderline and malignant tumors). Among 134 included women, 72 had benign tumors, 19 had borderline ovarian tumors, and 43 had malignant tumors. Blood samples were collected from all the patients before surgery and additional information was obtained regarding their lifestyle and gynecological and clinical status. For sample collection, strict standard operating procedures were followed, and serum was aliquoted and stored at −80°C until analysis. TK1c and TK1a were analyzed using the LIASON® assay; CA125 and HE4 were measured by a Cobas e411 immunoassay analyzer (Roche Diagnostics GmbH, Germany).

RESULTS: The mean levels of serum HE4 in the control group and the ovarian cancer group were (49±11) pmol/L and (212±376) pmol/L, p<0,0001, respectively; the mean levels of serum CA125 were (15,1±8,5) kU/L and (292±799) kU/L, p<0,0001, respectively; the mean levels of serum TK1c were (0,24±0,13) $\mu$g/L, and (0,74±0,53) $\mu$g/L, p<0,0001, respectively and the mean levels of serum TK1a were (6,5±2,5) U/L and (8,0±4,6) U/L, p=0,027, respectively. Pairwise comparisons of the results were conducted, which showed that the differences in the mean levels of serum HE4, CA125, TK1c, and TK1a in the ovarian cancer group were statistically significant (p<0,05) as compared to those in the control group.

CONCLUSIONS: The results showed that the joint detection of four tumor markers CA125, HE4, TK1p, and TK1a could significantly increase the sensitivity and specificity in the diagnosis of ovarian cancer and improve the screening program for early detection of ovarian cancer.

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## Successful amplifications of the GC region within EGFR: -191C/A (rs712830) and 181946 G/A (rs2293347) in lung cancer patients using Biomaster mix

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Polymerase chain reaction (PCR) is worldwide used technique for detection of EGFR mutation in both molecular biology research and various clinical applications including NSCLC patients sample. However, unique optimization of each PCR protocol is required especially when guanine-cytosine (GC) rich regions are amplified. In this study we have tested for first time two available kits Biomaster (Biolab Mix) LR HS PCR 2x and HS Taq-PCR Color 2x for amplification of these EGFR polymorphisms (SNPs): -191 C/A (rs712830) and 181946 G/A (rs2293347) according to recommendations of manufacturer and adjusted with the same PCR-RFLP protocols for both SNPs. Restriction enzymes BseRI (New England Biolabs, Ipswich, MA) and Cfr42I (Fermentas/Thermo Fisher Scientific, Vilnius, Lithuania) were used in this protocols. PCRs were performed in total volume of 25 $\mu$l, with 0.4 or 0.5 $\mu$l genomic DNA and 0.4 $\mu$ of each primer. PCR products for 191C>A polymorphism were detected by gel electrophoresis on 3% agarose gel, and PCR products for -216G>T were detected on 8% polyacrilamide gel electrophoresis. Sequence analysis of the templateDNAshowed that the region is extremely extremely GC rich, with 75.45% G+C content in a sequence of 660 bp (sum C + G = 421). The examined region contains a CpG island region spanning 558 bp (–450/+108 from translation start site), with an observed-to-expected ratio of CpG 0.97. Results indicated that these two available kits were effective in amplification of these difficult regions. Since, biomaster HS Taq-PCR Color 2x reaction mixture contains all of the components needed for PCR, including highly processive recombinant HS-Taq DNA polymerase, deoxynucleoside triphosphate mixture, 2× PCR buffer, Mg2+ and aditive significantly reduce preparation time as weel as decrease contamination risk during the preparation of PCR reaction solution compared to manually protocol. Our experience shows success in proving these GC regions in patients with NSCL especially in every day clinical work where we have a lot of samples.

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## Synchronous endometrioid-type carcinomas of the uterine corpus and uterine cervix proceeded by different precancerous lesions

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The incidence rate of two synchronous endometrioid-type carcinomas originated from uterine corpus and uterine cervix is exceerdingly rare. They constitute up to 5% of all female genital tract neoplasms reported up to now. Only a few case reports were reported in english literature. In this case

report, we presented synchronously occurring early-staged EC of adenocarcinoma-type with cervical adenocarcinoma. Interestingly, although both neoplasms diplayed similar histological subtypes, they differ significantly either with histological grading or with clinical stage of the disease. Finally, it is worth to emphasize both synchronous neoplasms were proceeded by different precancerous lesions, atypical endometrial hyperplasia with nuclear atypia concomitantly with adenomyosis and endometriotic lesions within the uterine cervix. Although the atypical endometrial hyperplasia is a well-known condition leading to EC, the mechanism resulting the malignant transformation of endometrioid foci into the endometrioid-type cancer located within the uterine cervix, is still a matter of dysputy. Brief literature summary has been presented underlying the impact of different precancerous lesions on the development of synchronous endometrioid-type carcinomas within the uterine corpus and uterine cervix.

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## Temozolomide exposure results in elevated FREM2 protein expression levels in glioblastoma and astrocyte cells

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BACKGROUND: Glioblastoma (GBM) is the most common primary human brain tumor with a life expectancy of less than 18 months. Since GBM is still considered incurable identification of new biomarkers and treatments is crucial. Here we explore a potential new GBM biomarker FRAS1-related extracellular matrix protein 2 (FREM2) which is overly expressed on the plasma membrane of GBM cells, and is associated with cell motility and migration. Literature also suggests that the commonly used chemo-therapeutic drug temozolomide (TMZ) affects FREM2 expression level. Namely, the higher frequency of variant allele of FREM2 present in TMZ-treated cell cultures implies a connection between FREM2 and GBM progression after TMZ treatment. This could be linked to the hyper-mutator phenotype associated with TMZ treatment.

STUDY AIMS: To establish TMZ-resistant GBM cell lines and to quantify the changes in FREM2 protein and gene expression levels in resistant compared with control non-treated cells.

MATERIALS AND METHODS: We exposed two stem-like (NCH644, NCH421K), 2 differentiated (U87MG, U251MG) GBM cells, and astrocytes to increasing concentrations of TMZ (1 to 50 μM) for 5 weeks. Cell viability was monitored with metabolic and apoptotic assays twice per week. After the treatment, FREM2 gene and protein expression levels were analysed using qPCR and western blotting.

RESULTS: All cell lines survived the maximal concentration of TMZ for 1 week. Western blot showed an overall increase in FREM2 protein levels in cells treated with TMZ. Both qPCR and western blotting showed higher FREM2 gene and protein expression in stem-like GBM cells compared with differentiated GBM cells and astrocytes. U87MG showed a similar protein expression as the human astrocytes, while U251MG had a higher expression level of FREM2 protein compared with human astrocytes. On the other hand, only NCH644 showed an increase in relative FREM2 mRNA expression level after TMZ treatment.

CONCLUSIONS: TMZ-resistant cell lines can be successfully established using increasing concentrations of TMZ. Cells are viable for at least 1 week in the presence of 50 μM TMZ. Our results show that TMZ treatment results in increased FREM2 protein expression levels. Since the mRNA expression does not follow the same trend, we reason there is a complex underlying mechanism of FREM2 expression regulation that needs further exploring.

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## The intratumor bacteria and neutrophilic inflammation in squamous cell vulvar carcinoma

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OBJECTIVE: A causal link between microbiota composition and oncogenesis has been demonstrated for several types of cancer. Neutrophils play a role both in immune protection against bacterial threats and in carcinogenesis. This study aimed to characterize intratumoral bacteria in vulvar squamous cell carcinoma (VSCC) and their putative effect on neutrophil recruitment and cancer progression.

METHODS: Clinical material was obtained from 56 patients with VSCC. Next-generation sequencing of 16S rRNA and quantitative polymerase chain reaction were used to detect bacterial species in VSCC. Subsequently, immunohistochemical analyzes by anti-CD66b antibody staining of tumor specimens were performed to verify neutrophil activation.

RESULTS: Fusobacterium nucleatum and Pseudomonas aeruginosa were found to be associated with a shorter time to progression in our cohort of VSCC patients. Furthermore, a high abundance of CD66b, the neutrophil activation marker, was found to be associated with poor survival in patients with VSCC.

CONCLUSION: Our study suggests that the presence of F. nucleatum and P. aeruginosa and neutrophilic inflammation may be tumor-promoting in VSCC. These findings provide new therapeutic opportunities, such as shifting the balance of neutrophil activities to antitumorigenic.

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## Xpert Bladder Cancer Monitor Test – is this a good screening test for bladder cancer?

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BACKGROUND: Cystoscopy in complement with urinary cytology represents the gold standard for the follow-up of patients with urinary bladder tumours. Xpert Bladder Cancer Monitor Test (XBC) is a novel mRNA-based urine test for bladder cancer surveillance. The aim of the study was to evaluate the performance of the XBC and voided urinary cytology (VUC) in the follow-up of bladder tumours

PATIENTS AND METHODS: The XBC was performed on stabilized voided urine and VUC was performed on urine samples. The results were compared to cystoscopic findings and histopathological results after transurethral resection of the bladder lesion

RESULTS: For the prediction of malignant histopathological result sensitivity, the specificity and negative predictive value were 76.9%, 97.5% and 93.0% for the XBC and 38.4%, 97.5% and 83.3%, respectively for VUC. For the prediction of suspicious or positive cystoscopic finding sensitivity, the specificity and negative predictive value were 75.0%, 95.2%, and 93.0% respectively for the XBC

and 41.7%, 97.6%, and 85.4% for VUC. The sensitivities for papilary urothelial neo-plasms of low malignant potential (PUNLMP), low- and high-grade tumours were 0.0%, 66.7% and 100.0% for the XBC and 0.0%, 66.7% and 42.9%, respectively for VUC

CONCLUSIONS: The XBC showed significantly higher overall sensitivity and negative predictive value than VUC and could be used to increase the recommended follow-up cystoscopy time intervals. Complementing the XBC and voided urinary cytology does not improve performance in comparison to the XBC alone.

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