Candidate Gene Resequencing in a Large Bicuspid Aortic Valve-Associated Thoracic Aortic Aneurysm Cohort: SMAD6 as an Important Contributor

Gillis E, Kumar A, Luyckx I, Zhurayev R, Zerbino D. et al.

Front Physiol. *2017, Jun 13;8:400. doi: 10.3389/fphys.2017.00400. (IF=4,134)*

Bicuspid aortic valve (BAV) is the most common congenital heart defect. Although many BAV patients remain asymptomatic, at least 20% develop thoracic aortic aneurysm (TAA). Historically, BAV-related TAA was considered as a hemodynamic consequence of the valve defect. Multiple lines of evidence currently suggest that genetic determinants contribute to the pathogenesis of both BAV and TAA in affected individuals. Despite high heritability, only very few genes have been linked to BAV or BAV/TAA, such as NOTCH1, SMAD6, and MAT2A. Moreover, they only explain a minority of patients. Other candidate genes have been suggested based on the presence of BAV in knockout mouse models (e.g., GATA5, NOS3) or in syndromic (e.g., TGFBR1/2, TGFB2/3) or non-syndromic (e.g., ACTA2) TAA forms. We hypothesized that rare genetic variants in these genes may be enriched in patients presenting with both BAV and TAA. We performed targeted resequencing of 22 candidate genes using Haloplex target enrichment in a strictly defined BAV/TAA cohort (n = 441; BAV in addition to an aortic root or ascendens diameter ≥ 4.0 cm in adults, or a Z-score ≥ 3 in children) and in a collection of healthy controls with normal echocardiographic evaluation (n = 183). After additional burden analysis against the Exome Aggregation Consortium database, the strongest candidate susceptibility gene was SMAD6 (p = 0.002), with 2.5% (n = 11) of BAV/TAA patients harboring causal variants, including two nonsense, one in-frame deletion and two frameshift mutations. All six missense mutations were located in the functionally important MH1 and MH2 domains. In conclusion, we report a significant contribution of SMAD6 mutations to the etiology of the BAV/TAA phenotype.

KEYWORDS: *SMAD6; bicuspid aortic valve; targeted gene panel; thoracic aortic aneurysm; variant burden test*

Partial Characterization of Tick-Borne Encephalitis Virus Isolates from Ticks of Southern Ukraine

Yurchenko OO, Dubina DO, Vynograd NO, Gonzalez JP.

Vector Borne Zoonotic Dis. *2017 Jun 27. doi: 10.1089/vbz.2016.2094. (IF=1,956)*

Tick-borne encephalitis (TBE) is the most common tick-borne viral infection in Eurasia; thousands of human cases are annually reported from several European countries. Several tick species are vectors of the tick-borne encephalitis virus (TBEV), while TBE appears to be spreading from the Eurasian continent westward to Europe. Fifteen study sites were chosen from five territories of southern Ukraine, including Odessa, Mykolaiv, Kherson Oblast, the Autonomous Republic of Crimea, and Sevastopol. Tick collection was performed in spring season of three consecutive years (1988-1990) using either flagging technique or direct collection of specimens feeding on cattle. A total of 15,243 tick imagoes and nymphs were collected from nine species, including Dermacentor marginatus, D. reticulatus, Haemaphysalis parva, H. punctata, Hyalomma marginatum, Ixodes ricinus, Rhipicephalus bursa, R. rossicus,

and R. sanguineus, pooled in 282 monospecific samples. Supernatant of grinded pool was used for inoculation to suckling mice for virus isolation. Eight TBEV isolates were identified from ticks among six study sites. Ticks showed a minimum infection rate from 0.11% to 0.81%. Phylogenetic analysis of the envelope (E) protein gene of seven isolates, assigned all to the European subtype (TBEV-Eu) showing a maximum identity of 97.17% to the "Pan" TBEV-Eu reference strain. Compared to 104 TBEV-Eu isolates they clustered within the same clade as the Pan reference strain and distinguished from other TBEV-Eu isolates. Amino acid sequence analysis of the South Ukrainian TBEV-Eu isolates revealed the presence of four amino acid substitutions 67 (N), 266 (R), 306 (V), and 407 (R), in the ectodomains II and III and in the stem-anchor region of the E protein gene. This study confirmed TBEV-Eu subtype distribution in the southern region of Ukraine, which eventually overlaps with TBEV-FE (Far Eastern subtype) and TBEV-Sib (Siberian subtype) domains, showing the heterogeneity of TBEV circulating in Ukraine.

KEYWORDS: *Ukraine; envelope protein; nucleotide sequencing; phylogenetic analysis; tick-borne encephalitis virus*

CHEMICAL COMPOSITION OF SELECTED COMMERCIAL HERBAL REMEDIES IN RELATION TO GEOGRAPHICAL ORIGIN AND INTER-SPECIES DIVERSITY

Konieczynski P, Viapiana A, Lysiuk R, Wesolowski M.

Biol Trace Elem Res. *2017 Jun 21. doi: 10.1007/s12011-017-1078-z. (IF=2,339)*

Infusions prepared from medicinal herbs that are rich in flavonoids are very popular herbal remedies in societies of Eastern Europe. Therefore, the content of essential elements together with total flavonoids was analyzed in 65 commercially available samples of herbal drugs originating from Ukraine, Romania, and Belarus. The results showed that metallic elements (in mg kg^{-1} d.w.) have occurred in the following order: Fe > Mn > Zn > Cu, both for total and water-extractable species. Total flavonoids were determined in the range from 10.0 to 191.8 mg g^{-1} d.w. Several significant correlations have been found between the analytes, especially among water-extractable Fe with other metals, and total flavonoids and Fe, Zn, and Mn. Analysis of variance has revealed significant differences among studied samples due to their origin from different countries, especially between Belarussian samples and others. Differences owing to belonging to various plant species were also found, as it was noticed in the case of Polygoni aviculare herba in comparison with other botanical plant species. Moreover, multivariate statistical techniques, such as cluster analysis (CA) and principal component analysis (PCA) were used to gather herbal drugs based on similarity of chemical composition. CA grouped the samples into clusters with similar level of elements and total flavonoid contents, and PCA has indicated Hyperici herba, Tiliae flores, and Crataegi fructus as herbal remedies with close concentration of studied elements and flavonoids.

KEYWORDS: *Essential elements; Herbal remedies; Statistical evaluation; Total flavonoids*

EUROPEAN CONTRIBUTION TO THE STUDY OF ROS: A SUMMARY OF THE FINDINGS AND PROSPECTS FOR THE FUTURE FROM THE COST ACTION BM1203 (EU-ROS).

Egea J, Fabregat I, Frapart YM, Kaminskyy D. et al.

Redox Biol. *2017 May 18;13:94-162. doi: 10.1016/j.redox.2017.05.007. (IF=6,337)*

The European Cooperation in Science and Technology (COST) provides an ideal framework to establish multi-disciplinary research networks. COST Action BM1203 (EU-ROS) represents a consortium of researchers from different disciplines who are dedicated to providing new insights and tools for better understanding redox biology and medicine and, in the long run, to finding new therapeutic strategies to target dysregulated redox processes in various diseases. This report highlights the major achievements of EU-ROS as well as research updates and new perspectives arising from its members. The EU-ROS consortium comprised more than 140 active members who worked together for four years on the topics briefly described below. The formation of reactive oxygen and nitrogen species (RONS) is an established hallmark of our aerobic environment and metabolism but RONS also act as messengers via redox regulation of essential cellular processes. The fact that many diseases have been found to be associated with oxidative stress established the theory of oxidative stress as a trigger of diseases that can be corrected by antioxidant therapy. However, while experimental studies support this thesis, clinical studies still generate controversial results, due to complex pathophysiology of oxidative stress in humans. For future improvement of antioxidant therapy and better understanding of redox-associated disease progression detailed knowledge on the sources and targets of RONS formation and discrimination of their detrimental or beneficial roles is required. In order to advance this important area of biology and medicine, highly synergistic approaches combining a variety of diverse and contrasting disciplines are needed.

KEYWORDS: *Antioxidants; Oxidative stress; Reactive nitrogen species; Reactive oxygen species; Redox signaling; Redox therapeutics*

COMPUTER-AIDED PREDICTION AND CYTOTOXICITY EVALUATION OF DITHIOCARBAMATES OF 9,10-ANTHRACENEDIONE AS NEW ANTICANCER AGENTS.

Stasevych M, Zvarych V, Lunin V, Deniz NG, Gokmen Z, Akgun O, Ulukaya E, Poroikov V, Gloriozova T, Novikov V

SAR QSAR Environ Res. *2017 May;28(5):355-366. doi: 10.1080/1062936X.2017.1323796. (IF=1,897)*

Anticancer activity as an associated action for a series of dithiocarbamates of 9,10-anthracenedione was predicted using the PASS computer program and analysed with PharmaExpert software. The predicted cytotoxic activity of the dithiocarbamate derivatives of 9,10-anthracenedione was evaluated in vitro on cancer cells of the human lung (A549), prostate (PC3), colon (HT29) and human breast (MCF7) using the sulforhodamine B (SRB) cell viability assay. Among these compounds, 9,10-dioxo-9,10-dihydroanthracen-1-yl pyrrolidin-1-carbodithioate and 9,10-dioxo-9,10-dihydroanthracen-2-yl pyrrolidin-1-carbodithioate were identified as the most potent anticancer agents with cytotoxic activity against the MCF-7 human breast cell line with GI_{50} values of 1.40 μM and 1.52 μM, whereas the GI_{50} value for the reference anticancer drug mitoxantrone was 3.93 μM. Thus, anticancer activity predicted by PASS with a probability Pa > 30% was

confirmed by the experiment. Relatively small Pa values estimated by PASS indicated the novelty of the considered derivatives comparing to the compounds from the PASS training set.

KEYWORDS: *Cell Line Cytotoxicity Predictor; Dithiocarbamates of 9, 10-anthracenedione; PASS; PharmaExpert; anticancer activity; computer-aided prediction*

GUT MICROBIOTA CHANGES AS A RISK FACTOR FOR OBESITY

Kvit KB, Kharchenko NV

Wiad Lek. *2017;70(2):231-235. (IF=0,08)*

INTRODUCTION: The number of obese people in recent decades is increasing significantly. Among the many aspects of obesity in the last decade, the role and importance of changes in the gut microbiota (GM) attracts special attention. The aim of the review was to analyze the results of studies, focused on the role of gut microbiota in the obesity development.

MATERIALS AND METHODS: Screening was conducted on 33 researches, which examined the role of the gut microbiota balance in the development of obesity. Among them, 13 studies were selected for more detailed analysis.

RESULTS: Obesity revealed typical changes in GM: an increase in the number of microbes of the genus Firmicutes and a decrease in the number of microbes of the genus Bacteroeidetes, which is particularly vividly demonstrated by studies of rodents. In obese mice, the microfamilies of the genus Firmicutes account for 80% of all GM (in control animals 60%), and the number of microorganisms of the genus Bacteroeidetes decreases by half (from 40 to 20%), compared to mice with normal weight.

CONCLUSION:Despite the complexity of the question of the relationship between GM and obesity, the totality of the data received, especially the results of experimental studies, affirm the thesis that changes in GM may contribute to the development of obesity.

KEYWORDS: *body weight ; lipid metabolism; obesity; gut microbiota*

FEATURES OF THE IMMUNOHISTOCHEMICAL CHARACTERISTICS OF PRIMARY TUMORS AND RECURRENCES OF BREAST CANCER AFTER RADICAL TREATMENT

Prystash YY

Wiad Lek. *2017;70(2):227-230. (IF=0,08)*

INTRODUCTION: Appearance of Recurrence (RC) of breast cancer (BC) is associated with a high risk of distant metastases, needs re-treatment and indicates the tumor aggressiveness. It has been remained unclear the molecular characteristics both of the RC and primary tumors in patients with invasive forms of breast cancer after mastectomy by Madden.

THE AIM: To explore the changing of the receptor status of the primary tumor and local RC in patients with breast cancer.

MATERIALS AND METHODS: Immunohistochemical study were conducted on 262 patients with

invasive breast cancer. Patients were divided into two groups: only local RC - 131 women and primary tumors of patients without local RC - also 131 persons.

RESULTS: The difference of the receptor status of the tumors is presented. In the group of patients with recurrent "triplet negative" cancer and patients with positive reaction of epidermal growth factor (HER2neo) is more than 15.2%. In patients where RC (control group in the study) was not observed we have the mass greater proportion of tumors with positive hormone receptors in various combinations.

CONCLUSIONS: Relapses are accompanied by lower levels of hormone receptors and increasing the frequency of "triplet negative" cancer as well as increasing of epidermal growth factor.

KEYWORDS: mastectomy ; recurrence; breast cancer

FEATURES OF SOME CLINICAL EXAMINATION PARAMETERS IN PATIENTS WITH PSORIATIC ARTHRITIS

Syzon O, Voznyak I, Dashko M

Wiad Lek. *2017;70(2):205-207. (IF=0,08)*

INTRODUCTION:The relationship of cause-and-effect mechanisms of exacerbation and progressing with defining of the role of stress reaction in the psoriatic arthritis (PA) development has been examined in the article by analyzing the characteristics of medical history, current dynamics as well as clinical, instrumental and laboratory examinations. The objective was to study the role of basic indices of laboratory examinations of patients used clarify their role in the pathogenesis of PA.

MATERIALS AND METHODS: The concentration of trigger cytokines - IL-1β, IL-8, IL-17, IL-22, stress hormones - ACTH and cortisol, state of cell-antibody mediated immunity (CD3+, CD4+, CD8+, CD16+, CD22+, IRI, IgM, IgG levels) in the serum of patients were defined.

RESULTS AND DISCUSSION: We have detected the possible changes of stress-reaction mediator concentrations in the serum of patients with PA (i.e. decreasing of the status of cell immunity indices (CD3+, CD4+, CD8+ T- lymphocytes, B- lymphocytes CD22+ fraction, IRI with compensatory increasing of the levels of CD16+ T-cells, cytokines - IL-1 β, IL-8, IL-17, IL-22, stress hormone - cortisol, immunoglobulins IgM, IgG, CIC) irrespective of the disease duration which testifies the strain of their stress-realising mechanisms, even if clinical stabilization of skin and joint process is normalized.

CONCLUSIONS: Thus, the final diagnosis of PA is determined only under aggregation of anamnestic, clinical, instrumental and laboratory data and the results of additional examinations. The indices mentioned above are key mediators of stress-realizing immune-neuroendocrine system and play an ambiguous role in the development of PA, their various effects require further study.

KEYWORDS: *ACTH; cortisol; diagnostic ; immunity; psoriatic arthritis; cytokines*

EVOLUTION OF SOME INDICATORS OF SYSTEMIC IMMUNITY IN PATIENTS WITH ACNE WHILE USING LASER THERAPY

Dashko M, Syzon O, Voznyak I

Wiad Lek. *2017;70(2):196-199. (IF=0,08)*

INTRODUCTION: One of the important problems in modern dermatology is to improve treatment efficiency of acne being a common cause for cicatricial skin changes, loss of performance capability and social activity and negatively affects the psycho-emotional state of patients and their quality of life. The topicality of the disease is due to the high degree of its proliferation, chronic and recurrent course, and resistance to existing therapies. Reducing the effectiveness of skin diseases treatment, including that of acne, at present, is associated with developing resistance to drugs, which causes the use of non-drug methods in dermatology nowadays, including low-intensity laser therapy. Objective - to determine evolution of the systemic immunity indices in patients with acne with different degrees of severity in the course of a standard and comprehensive treatment by laser therapy.

MATERIALS AND METHODS: We observed 77 patients with acne aged 18-25 years; 32 of them received standard therapy, other 45 patients were additionally prescribed combined (superficial venous and external) laser therapy. We determined the indices of all patients' systemic immunity using well-known techniques.

RESULTS: It has been established, that using laser therapy in comprehensive treatment of patients with acne promotes the normalization or a tendency to normalization of the systemic immunity and phagocytosis with significant difference between the indices of the individuals who received a standard therapy alone.

KEYWORDS*: laser therapy ; systemic immunity; acne*

ALTERED GLYCAN ACCESSIBILITY ON NATIVE IMMUNOGLOBULIN G COMPLEXES IN EARLY RHEUMATOID ARTHRITIS AND ITS CHANGES DURING THERAPY

Stümer J, Biermann MHC, Knopf J, Magorivska I, Kastbom A, Svärd A, Janko C, Bilyy R et al

Clin Exp Immunol. *2017 May 16. doi: 10.1111/cei.12987. (IF=3,41)*

The goal of this study was to investigate the glycosylation profile of native immunoglobulin (Ig) G present in serum immune complexes in patients with rheumatoid arthritis (RA). To accomplish this, lectin binding assays, detecting the accessibility of glycans present on IgG-containing immune complexes by biotinylated lectins, were employed. Lectins capturing fucosyl residues (AAL), fucosylated tri-mannose N-glycan core sites (LCA), terminal sialic acid residues (SNA) and O-glycosidically linked galactose/N-acetylgalactosamine (GalNac-L) were used. Patients with recent-onset RA at baseline and after 3-year follow-up were investigated. We found that native IgG was complexed significantly more often with IgM, C1q, C3c and C-reactive protein (CRP) in RA patients, suggesting alterations of the native structure of IgG. The total accessibility of fucose residues on captured immune complexes to the respective lectin was significantly higher in patients with RA. Moreover, fucose accessibility on IgG-containing immune complexes correlated positively with the levels of antibodies to cyclic citrullinated peptides (anti-CCP). We also observed a significantly higher accessibility to sialic acid residues and galactose/GalNAc glyco-epitopes in

native complexed IgG of patients with RA at baseline. While sialic acid accessibility increased during treatment, the accessibility of galactose/GalNAc decreased. Hence, successful treatment of RA was associated with an increase in the SNA/GalNAc-L ratio. Interestingly, the SNA/GalNAc-L ratio in particular rises after glucocorticoid treatment. In summary, this study shows the exposure of glycans in native complexed IgG of patients with early RA, revealing particular glycosylation patterns and its changes following pharmaceutical treatment.

KEYWORDS: *Aleura aurantia; GalNAc-L; Sambucus nigra; immune complex; lectin ELISA; rheumatoid arthritis*

INOSINE RELEASED FROM DYING OR DEAD CELLS STIMULATES CELL PROLIFERATION VIA ADENOSINE RECEPTORS

Chen J, Chaurio RA, Maueröder C, Kost A, Bilyy R et al.

Front Immunol. *2017 Apr 27;8:504. doi: 10.3389/fimmu.2017.00504. (IF=6,429)*

INTRODUCTION: Many antitumor therapies induce apoptotic cell death in order to cause tumor regression. Paradoxically, apoptotic cells are also known to promote wound healing, cell proliferation, and tumor cell repopulation in multicellular organisms. We aimed to characterize the nature of the regenerative signals concentrated in the micromilieu of dead and dying cells.

METHODS: Cultures of viable melanoma B16F10 cells, mouse fibroblasts, and primary human fibroblast-like synoviocytes (FLS) in the presence of dead and dying cells, their supernatants (SNs), or purified agonists and antagonists were used to evaluate the stimulation of proliferation. Viable cell quantification was performed by either flow cytometry of harvested cells or by crystal violet staining of adherent cells. High-performance liquid chromatography and liquid chromatography coupled with mass spectrometry of cell SNs were deployed to identify the nature of growth-promoting factors. Coimplantation of living cells in the presence of SNs collected from dead and dying cells and specific agonists was used to evaluate tumor growth in vivo.

RESULTS: The stimulation of proliferation of few surviving cells by bystander dead cells was confirmed for melanoma cells, mouse fibroblasts, and primary FLS. We found that small soluble molecules present in the protein-free fraction of SNs of dead and dying cells were responsible for the promotion of proliferation. The nucleoside inosine released by dead and dying cells acting via adenosine receptors was identified as putative inducer of proliferation of surviving tumor cells after irradiation and heat treatment.

CONCLUSION: Inosine released by dead and dying cells mediates tumor cell proliferation via purinergic receptors. Therapeutic strategies surmounting this pathway may help to reduce the rate of recurrence after radio- and chemotherapy.

KEYWORDS: *adenosine receptor; apoptosis; inosine; melanoma; necrosis; proliferation; repopulation*

A BLIND SPOT ON THE GLOBAL MENTAL HEALTH MAP: A SCOPING REVIEW OF 25 YEARS' DEVELOPMENT OF MENTAL HEALTH CARE FOR PEOPLE WITH SEVERE MENTAL ILLNESSES IN CENTRAL AND EASTERN EUROPE

Winkler P, Krupchanka D, Roberts T, Plevachuk O et al

Lancet Psychiatry. *2017 May 8. pii: S2215-0366(17)30135-9. doi: 10.1016/ S2215-0366(17)30135-9. (IF=1,98)*

Just over 25 years have passed since the major sociopolitical changes in central and eastern Europe; our aim was to map and analyse the development of mental health-care practice for people with severe mental illnesses in this region since then. A scoping review was complemented by an expert survey in 24 countries. Mental health-care practice in the region differs greatly across as well as within individual countries. National policies often exist but reforms remain mostly in the realm of aspiration. Services are predominantly based in psychiatric hospitals. Decision making on resource allocation is not transparent, and full economic evaluations of complex interventions and rigorous epidemiological studies are lacking. Stigma seems to be higher than in other European countries, but consideration of human rights and user involvement are increasing. The region has seen respectable development, which happened because of grassroots initiatives supported by international organisations, rather than by systematic implementation of government policies.

MEASUREMENT OF BLOOD CALPROTECTIN (MRP-8/MRP-14) LEVELS IN PATIENTS WITH JUVENILE IDIOPATHIC ARTHRITIS

Bojko J

Reumatologia. *2017;55(1):10-14. doi: 10.5114/reum.2017.66682. (IF=0,12)*

OBJECTIVES: The aim of the investigation was to compare blood calprotectin (MRP8/14, S100A 8/9) levels in patients with systemic-onset, polyarticular, RF-negative and oligoarticular subtypes of juvenile idiopathic arthritis (JIA), and to explore links between blood calprotectin levels and clinical and laboratory markers of JIA activity.

MATERIAL AND METHODS: Measurement of calprotectin in blood serum was performed in 160 patients with JIA followed up at Lviv Regional Council Public Institution «Western-Ukrainian Specialised Children›s Medical Centre». Seventeen patients with systemic-onset JIA (sJIA) and 49 patients with other JIA subtypes (RF-negative polyarthritis and oligoarthritis) in the active phase of the disease were included in this study. Determination of calprotectin levels in blood serum was performed using EK-MRP8/14 Buhlmann Calprotectin reagents (Buhlmann, Switzerland) by the ELISA method.

RESULTS: The results of the investigations showed that blood calprotectin levels were higher in patients with systemic-onset subtype of the disease (median 13,800 ng/ml), and differed significantly from levels in healthy children (median 1,800 ng/ml, p = 0.00002), levels in patients with articular subtypes of JIA (median 2,700 ng/ml, p = 0.000008), and patients with RF-negative polyarthritis (median 3,800 ng/ml, p = 0.003226) and oligoarthritis (median 2,500 ng/ml, p = 0.000009). The highest blood calprotectin levels were found in patients with newly diagnosed sJIA, the median being 32,500 ng/ml (range: 13,800-177,000 ng/ml). Direct correlations were found

between blood calprotectin and JADAS 27 activity score (p = 0.000009), ESR (p = 0.000079) and CRP (p = 0.000058).

CONCLUSIONS: Blood calprotectin level is one of the measures that can be used to confirm the diagnosis of sJIA and to monitor the disease activity and therapy effectiveness.

KEYWORDS: *MRP-8/MRP-14; biomarkers; blood calprotectin; juvenile idiopathic arthritis*

ANTI-HISTONE H1 IGGS POSSESS PROLIFERATIVE ACTIVITY TOWARDS HUMAN T-LEUKAEMIA CEM CELLS

Kit Y, Magorivska I, Bilyi R, Myronovskij S, Stoika R

Exp Oncol. *2017 Mar;39(1):36-41. (IF=1,24)*

The aim of this study was to characterize the proliferative activity of the anti-histone H1 IgGs towards human T-leukaemia CEM cells.

MATERIALS AND METHODS: Anti-histone H1 IgGs were purified from blood serum of systemic lupus erythematosus patients by precipitation of serum proteins with 50% ammonium sulfate followed by a sequential affinity chromatography on Protein G-Sepharose and histone H1-Sepharose columns. To avoid contamination with other proteins, anti-histone H1 IgGs were subjected to strongly acidic pH 2.0 during gel filtration through HPLC column. The effects of the anti-histone H1 IgGs on cell viability and cell cycle were tested by MTS-assay and flow cytometry, correspondingly. The cross-reactivity of the anti-histone H1 antibodies towards heterogenetic and cellular antigens was evaluated by Western-blot analysis.

RESULTS: It was found that incubation of CEM cells with the HPLC-purified anti-histone H1 IgGs resulted in significant stimulation of cell growth by 46% after 48 h of incubation. These IgGs possess an antigenic poly-specificity to positively charged heterogenetic antigens and different cellular antigens. FITC-labeled and biotinylated anti-histone H1 IgGs are internalized by CEM cells and preferentially accumulated in the cytoplasm.

CONCLUSION: The anti-histone H1 IgGs are shown to internalize human T-leukemia CEM and stimulate their proliferation. These IgGs are polyspecific toward cellular antigens.

FIBROMUSCULAR DYSPLASIA OF THE CORONARY ARTERIES: A CASE REPORT AND REVIEW OF THE LITERATURE

Kuzyk J, Boiko O, Stetsko T

Turk Patoloji Derg. *2017 Feb 4. doi: 10.5146/tjpath.2015.01341. (IF=0,58)*

Fibromuscular dysplasia is a nonatherosclerotic and non-inflammatory vascular disease with primary lesion of renal and internal carotid arteries. We present a neonatal case of fibromuscular dysplasia who died on the second day of life. The newborn suffered from fibromuscular dysplasia of the coronary arteries and a congenital heart defect. The interesting feature of this case was the formation of aneurysms of the coronary arteries with pulmonary atresia. This case demonstrates a casuistically rare form of association between fibromuscular dysplasia of the coronary arteries and pulmonary artery atresia.

PHYSIOCHEMICAL TUNING OF POTENT ESCHERICHIA COLI ANTI-ADHESIVES BY MICROENCAPSULATION AND METHYLENE HOMOLOGATION

Alvarez Dorta D, Chalopin T, Sivignon A, de Ruyck J, Dumych TI, Bilyy RO et al.

ChemMedChem. *2017 Jun 21;12(12):986-998. doi: 10.1002/cmdc.201700061. (IF=3,225)*

Thiazolylaminomannosides (TazMan) are FimH antagonists with anti-adhesive potential against adherent-invasive Escherichia coli (AIEC) promoting gut inflammation in patients with Crohn's disease. The lead TazMan is highly potent in vitro, but shows limited in vivo efficiency, probably due to low pH stability and water solubility. We recently developed a second generation of stable TazMan, but the anti-adhesive effect was lower than the first. Herein we report a co-crystal structure of the lead TazMan in FimH, revealing that the anomeric NH group and the second thiazole moiety provide a positive hydrogen bonding interaction with a trapped water molecule, and π-stacking with Tyr48 of FimH, respectively. Consequently, we developed NeoTazMan homologated with a methylene group for low-pH and mannosidase stability with a conserved NH group and bearing various heterocyclic aglycones. Microencapsulation of the lead NeoTazMan in γ-cyclodextrin dramatically improved water solubility without disrupting the affinity for FimH or the anti-adhesive effect against AIEC isolated from patients with Crohn's disease.

KEYWORDS: C-mannosides; Crohn's disease; FimH antagonists; anti-adhesive therapy; microencapsulation

RAPID GENERATION OF HYDROGEN PEROXIDE CONTRIBUTES TO THE COMPLEX CELL DEATH INDUCTION BY THE ANGUCYCLINE ANTIBIOTIC LANDOMYCIN E

Panchuk RR, Lehka LV, Terenzi A, Matselyukh BP, Kril IJ, Stoika RS et al.

Free Radic Biol Med. *2017 May;106:134-147. doi: 10.1016/j.freeradbiomed.2017.02.024. (IF=5,606)*

Landomycin E (LE) is an angucycline antibiotic produced by Streptomyces globisporus. Previously, we have shown a broad anticancer activity of LE which is, in contrast to the structurally related and clinically used anthracycline doxorubicin (Dx), only mildly affected by multidrug resistance-mediated drug efflux. In the present study, cellular and molecular mechanisms underlying the anticancer activity of landomycin E towards Jurkat T-cell leukemia cells were dissected focusing on the involvement of radical oxygen species (ROS). LE-induced apoptosis distinctly differed in several aspects from the one induced by Dx. Rapid generation of both extracellular and cell-derived hydrogen peroxide already at one hour drug exposure was observed in case of LE but not found before 24h for Dx. In contrast, Dx but not LE induced production of superoxide radicals. Mitochondrial damage, as revealed by JC-1 staining, was weakly enhanced already at 3h LE treatment and increased significantly with time. Accordingly, activation of the intrinsic apoptosis pathway initiator caspase-9 was not detectable before 12h exposure. In contrast, cleavage of the down-stream caspase substrate PARP-1 was clearly induced already at the three hour time point. Out of all caspases tested, only activation of effector caspase-7 was induced at this early time points paralleling the LE-induced oxidative burst. Accordingly, this massive cleavage of caspase-7 at early time points was inhibitable by the radical scavenger N-acetylcysteine (NAC). Additionally, only simultaneous inhibition of multiple caspases reduced LE-induced apoptosis.

Specific scavengers of both H_2O_2 and $OH^{\bullet}$effectively decreased LE-induced ROS production, but only partially inhibited LE-induced apoptosis. In contrast, NAC efficiently blocked both parameters. Summarizing, rapid H_2O_2 generation and a complex caspase activation pattern contribute to the antileukemic effects of LE. As superoxide generation is considered as the main cardiotoxic mechanism of Dx, LE might represent a better tolerable drug candidate for further (pre)clinical development.

KEYWORDS: *Anticancer drugs; Apoptosis; Hydrogen peroxide; Landomycin E; Multi-drug resistance; N-acetylcysteine; Reactive oxygen species; Superoxide radicals*

VITAMIN K STATUS IN CYSTIC FIBROSIS PATIENTS WITH LIVER CIRRHOSIS

Krzyżanowska P, Drzymała-Czyż S, Pogorzelski A, Skorupa W, Bober L, Rohovyk N et al.

Dig Liver Dis. *2017 Jun;49(6):672-675. doi: 10.1016/j.dld.2017.01.155. (IF=2,719)*

The available data on the influence of liver cirrhosis on vitamin K status in CF patients is scarce. Therefore, the aims of the present study were to assess the prevalence of vitamin K deficiency in cirrhotic CF subjects and to determine whether it correlates with liver cirrhosis. The study group comprised of 27 CF patients with and 63 without liver cirrhosis. Vitamin K status was assessed using prothrombin induced by vitamin K absence (PIVKA-II) and the percentage of undercarboxylated osteocalcin (u-OC). PIVKA-II concentrations were higher in cirrhotic than in non-cirrhotic CF patients (median [1st-3rd quartile]: 3.2ng/ml [1.0-10.0] vs. 1.3ng/ml [0.2-2.6], p=0.0029). However, the differences in u-OC percentages between the studied groups did not reach the level of significance (49.4% [7.0-73.8] vs. 8.0% [2.6-59.1], p=0.0501). Based on multiple linear regression analysis the dose of vitamin K and F508del mutation were potentially defined as determinants of vitamin K deficiency. Liver cirrhosis was not documented to be an independent risk factor. In CF patients with liver cirrhosis vitamin K deficiency is not only more frequent, but also more severe. However, not liver cirrhosis, but the presence of a F508del CFTR mutation constitutes an independent risk factor for vitamin K deficiency.

KEYWORDS: *Fat-soluble vitamin; Hepatology diseases; Prothrombin inducted by vitamin K absence-II; Undercarboxylated osteocalcin*

SAFETY, EFFICACY AND PHARMACOKINETICS OF RVIII-SINGLECHAIN IN CHILDREN WITH SEVERE HEMOPHILIA A: RESULTS OF A MULTICENTER CLINICAL TRIAL

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J Thromb Haemost. *2017 Apr;15(4):636-644. doi: 10.1111/jth.13647. (IF=5,287)*

Essentials rVIII-SingleChain is a novel recombinant factor VIII with covalently bonded heavy and light chains. Efficacy, safety and pharmacokinetics were studied in pediatric patients with severe hemophilia A. Across all prophylaxis regimens, the median annualized spontaneous bleeding rate was 0.00. rVIII-SingleChain showed excellent hemostatic efficacy and a favorable safety profile.

SUMMARY*:* Background rVIII-SingleChain is a novel B-domain truncated recombinant factor VIII (rFVIII) comprised of covalently bonded FVIII heavy and light chains, demonstrating a high binding affinity to von Willebrand factor. Objectives This phase III study investigated the safety, efficacy and pharmacokinetics of rVIII-SingleChain in previously treated pediatric patients < 12 years of age with severe hemophilia A. Patients/Methods Patients could be assigned to prophylaxis or on-demand therapy by the investigator. For patients assigned to prophylaxis, the treatment regimen and dose were based on the bleeding phenotype. For patients receiving on-demand therapy, dosing was guided by World Federation of Hemophilia recommendations. The primary endpoint was treatment success, defined as a rating of 'excellent' or 'good' on the investigator's clinical assessment of hemostatic efficacy for all treated bleeding events. Results The study enrolled 84 patients (0 to < 6 years, n = 35; ≥ 6 to < 12 years, n = 49); 81 were assigned to prophylaxis and three to an on-demand regimen. Patients accumulated a total of 5239 exposure days (EDs), with 65 participants reaching > 50 EDs. In the 347 bleeds treated and evaluated by the investigator, hemostatic efficacy was rated as excellent or good in 96.3%. The median annualized spontaneous bleeding rate was 0.00 (Q1, Q3: 0.00, 2.20), and the median annualized bleeding rate was 3.69 (Q1, Q3: 0.00, 7.20) across all prophylaxis regimens. No participant developed an inhibitor. Conclusions rVIII-SingleChain is a novel rFVIII molecule showing excellent hemostatic efficacy and a favorable safety profile in a clinical study in children < 12 years of age with severe hemophilia A.

KEYWORDS: *clinical trial; factor VIII; hemophilia A; pediatric; pharmacokinetics; safety*

RENAL CELL CARCINOMA: APPLICABILITY OF THE APPARENT COEFFICIENT OF THE DIFFUSION-WEIGHTED ESTIMATED BY MRI FOR IMPROVING THEIR DIFFERENTIAL DIAGNOSIS, HISTOLOGIC SUBTYPING, AND DIFFERENTIATION GRADE

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Int Urol Nephrol. *2017 Feb;49(2):215-224. doi: 10.1007/s11255-016-1460-3. (IF=1,564)*

BACKGROUND: Renal cell carcinoma (RCC) represents the most common malignant epithelial neoplasm of the kidney. Accurate assessment of the renal masses, defining the histologic subtype and the grade of differentiation of the tumor, is vital to ensure an adequate case management as well as for staging and prognosis. Recently, diffusion-weighted imaging (DWI) magnetic resonance imaging (MRI) tends to be increasingly appealing for the clinicians as an imaging procedure of choice for the diagnosis and staging of the RCC, which is predetermined by several advantages over CT. The goal of the survey was to assess the applicability of the apparent diffusion coefficient (ADC) of the DWI MRI for the differential diagnostics, histologic subtyping, and defining the grade of differentiation of the RCC.

METHODS: The study enrolled 288 adult patients with renal lesions: 188 patients with solid RCC-126 patients with clear cell subtype (ccRCC), 32 patients with papillary RCC (pRCC), 30 patients with chromophobe RCC (chRCC); 27 patient with cystic form or RCC (Bosniak cyst, category IV); 32 patients with renal angiomyolipoma (AML); 25 patients with renal oncocytoma (OC); and 16 patients with the renal abscess (AB). In total, 245 lesions were pathologically verified. As a reference, 19 healthy volunteers were included into the study. All patients underwent MRI of the kidneys, involving DWI with subsequent evaluation of the ADC.

RESULTS: There was a reliable difference ($p < 0.05$) in mean ADC values between the normal renal parenchyma (NRP), solid RCC of different histologic subtypes and grades, cystic RCC, and

benign renal lesions. The mean ADC values obtained in the result of the study were ($\times 10^{-3}$ mm^2/s): 2.47 ± 0.12 in NRP, 1.63 ± 0.29 in all solid RCCs, 1.82 ± 0.22 in solid ccRCC (1.92 ± 0.11-Fuhrman grade I, 1.84 ± 0.14-Fuhrman grade II, 1.79 ± 0.10-Fuhrman grade III, 1.72 ± 0.06-Fuhrman grade IV), 1.61 ± 0.07 in pRCC, 1.46 ± 0.09 in chRCC, 2.68 ± 0.11 in cystic RCC, 2.13 ± 0.08 in AML, 2.26 ± 0.06 in OC, and 3.30 ± 0.07 in AB.

CONCLUSION: The data received in our study demonstrate a substantial restriction of diffusion of hydrogen molecules in tissues of ccRCC in comparison with the healthy renal parenchyma preconditioned by the greater density of tumor. A statistically significant difference in mean ADC values of ccRCC with different grades of nuclear pleomorphism by Fuhrman was observed: Low-grade tumors showed higher mean ADC values compared to high-grade tumors. The modality of the MRI DWI along with ADC measurement allows to reliably differentiate between the solid RCC of main histologic subtypes and grades, cystic RCC, and the benign renal lesions.

KEYWORDS: *Apparent diffusion coefficient; Diffusion-weighted imaging; Magnetic resonance imaging; Renal cell carcinoma*

BEVACIZUMAB PLUS PACLITAXEL VERSUS PLACEBO PLUS PACLITAXEL AS FIRST-LINE THERAPY FOR HER2-NEGATIVE METASTATIC BREAST CANCER (MERIDIAN): A DOUBLE-BLIND PLACEBO-CONTROLLED RANDOMISED PHASE III TRIAL WITH PROSPECTIVE BIOMARKER EVALUATION

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Eur J Cancer. *2017 Jan;70:146-155. doi: 10.1016/j.ejca.2016.09.024. (IF=6,029)*

AIM: MERiDiAN evaluated plasma vascular endothelial growth factor-A (pVEGF-A) prospectively as a predictive biomarker for bevacizumab efficacy in metastatic breast cancer (mBC).

METHODS: In this double-blind placebo-controlled randomised phase III trial, eligible patients had HER2-negative mBC previously untreated with chemotherapy. pVEGF-A was measured before randomisation to paclitaxel 90 mg/m^2 on days 1, 8 and 15 with either placebo or bevacizumab 10 mg/kg on days 1 and 15, repeated every 4 weeks until disease progression, unacceptable toxicity or consent withdrawal. Stratification factors were baseline pVEGF-A, prior adjuvant chemotherapy, hormone receptor status and geographic region. Co-primary end-points were investigator-assessed progression-free survival (PFS) in the intent-to-treat and pVEGF-A_{high} populations.

RESULTS: Of 481 patients randomised (242 placebo-paclitaxel; 239 bevacizumab-paclitaxel), 471 received study treatment. The stratified PFS hazard ratio was 0.68 (99% confidence interval, 0.51-0.91; log-rank p = 0.0007) in the intent-to-treat population (median 8.8 months with placebo-paclitaxel versus 11.0 months with bevacizumab-paclitaxel) and 0.64 (96% confidence interval, 0.47-0.88; log-rank p = 0.0038) in the pVEGF-A_{high} subgroup. The PFS treatment-by-VEGF-A interaction p value (secondary end-point) was 0.4619. Bevacizumab was associated with increased incidences of bleeding (all grades: 45% versus 27% with placebo), neutropenia (all grades: 39% versus 29%; grade ≥3: 25% versus 13%) and hypertension (all grades: 31% versus 13%; grade ≥3: 11% versus 4%).

CONCLUSION: The significant PFS improvement with bevacizumab is consistent with previous placebo-controlled first-line trials in mBC. Results do not support using baseline pVEGF-A to identify patients benefitting most from bevacizumab.

CLINICAL TRIALS REGISTRATION: ClinicalTrials.gov NCT01663727.

KEYWORDS: *Bevacizumab; Biomarker; Double-blind; Metastatic breast cancer; Predictive; Prospective; VEGF-A; Weekly paclitaxel*

SPECTROCHIM ACTA A MOL BIOMOL SPECTROSC. 2017 JAN 5;170:184-90. DOI: 10.1016/J.SAA.2016.07.019. (IF=2,536)

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Fluorometric enzymatic assay of l-arginine

The enzymes of l-arginine (further - Arg) metabolism are promising tools for elaboration of selective methods for quantitative Arg analysis. In our study we propose an enzymatic method for Arg assay based on fluorometric monitoring of ammonia, a final product of Arg splitting by human liver arginase I (further - arginase), isolated from the recombinant yeast strain, and commercial urease. The selective analysis of ammonia (at 415nm under excitation at 360nm) is based on reaction with o-phthalaldehyde (OPA) in the presence of sulfite in alkali medium: these conditions permit to avoid the reaction of OPA with any amino acid. A linearity range of the fluorometric arginase-urease-OPA method is from 100nM to 6μM with a limit of detection of 34nM Arg. The method was used for the quantitative determination of Arg in the pooled sample of blood serum. The obtained results proved to be in a good correlation with the reference enzymatic method and literature data. The proposed arginase-urease-OPA method being sensitive, economical, selective and suitable for both routine and micro-volume formats, can be used in clinical diagnostics for the simultaneous determination of Arg as well as urea and ammonia in serum samples.

KEYWORDS: *Enzymatic assay; Recombinant arginase I; Sulfite; Urease; l-arginine; o-Phthalaldehyde*

EUROPEAN SCREENING FOR ALPHA1 -ANTITRYPSIN DEFICIENCY IN SUBJECTS WITH LUNG DISEASE

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Clin Respir J. *2017 Jan;11(1):90-97. doi: 10.1111/crj.12310. (IF=2,356)*

BACKGROUND AND AIMS: Alpha$_1$ -antitrypsin deficiency (AATD) predisposes individuals to early-onset emphysema. Despite its prevalence, especially among patients with chronic obstructive pulmonary disease, AATD is still underdiagnosed. The aim of this study is to identify individuals with lung disease and severe AATD in central-eastern Europe.

METHODS: Subjects with respiratory symptoms that could be indicative of AATD provided blood samples as dried blood spot. The alpha$_1$ -antitrypsin (AAT) concentration was determined by nephelometry and, if lower than 1.70 mg/dL in dried blood spot (equivalent to 1.04 g/L in serum),

polymerase chain reaction was used to detect the PiS and PiZ alleles. Isoelectric focusing was used for confirmation of doubtful genotype results.

RESULTS: From 13 countries, 11 648 subjects were included. Genotyping of 1404 samples with AAT levels <1.70 mg/dL revealed 71 (5.06%) PiS, 151 (10.8%) PiZ, 1 (0.071%) PiSS, 8 (0.57%) PiSZ and 32 (2.28%) PiZZ. Phenotyping of 1363 samples negative for the S and Z alleles or with PiS and PiZ genotype showed two (0.147%) PiZ(rare) and two (0.147%) Pi(null)(null). The countries with the highest rate of severe AATD were Croatia, Russia and Slovakia. By regions, the Baltic countries area showed the highest rate of both PiZ and severe AATD (2.45% and 1.20%, respectively) while the lowest rates were observed in the Balkan Peninsula (0.48% and 0.31%, respectively).

CONCLUSION: This study confirms the need for targeted testing of symptomatic patients and provides AATD genotype data from countries for which only some estimates of prevalence were available until now.

KEYWORDS: *COPD; alpha1-antitrypsin; dried blood spot; epidemiology; screening*

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