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ΠΑ01

INFRASTRUCTURE FOR PRECLINICAL AND EARLY-PHASE CLINICAL DEVELOPMENT OF DRUGS, THERAPEUTICS AND BIOMEDICAL DEVICES (EATRIS-GR)

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Translational research applies scientific discoveries to improve human health and quality of life. EATRIS is a cluster of research infrastructures aiming to support biomedical researchers in advancing their findings into translational tools and further enhancing the health system. EATRIS-GR, the Network of Greek Translational Research Infrastructures is based on the collective effort of Greek Universities and Research Institutes, with considerable input from pharmaceutical/biotech enterprises and consultation with members of the EATRIS-EU Infrastructure. Participation of BRFAA in EATRIS-GR involves early-phase trials, bioequivalence studies, evaluation of new formulations of existing/established drugs, drug metabolism, pharmacokinetic studies, cytomics analysis and biochemical assays.

The infrastructure of BRFAA for preclinical and early-phase clinical development of drugs, therapeutics and biomedical devices combines State of the Art biological evaluation approaches and bioanalytical methodologies as well as experience in clinical studies with ultimate goal to provide patients with novel pharmaceutical products. The main objectives of BRFAA in EATRIS-GR are:

- To collaborate with other members of the EATRIS-GR and create a common web portal for access to translational research services at the national level.
- To enhance the reliability of services by scaling up the quality system of the Pharmacology Division from ISO 17025 to Good Laboratory Practice (GLP).
- To perform pilot studies through which the infrastructure will become prepared to provide its services to external users.
- To set the foundations for bridging EATRIS-GR with EATRIS-EU

In this presentation some representative examples of the ongoing pilot projects associated with the buildup of the network will be presented as part of our goal to inform the Pharmacology Community of the capabilities available through this network.

ΠΑ02

IN VITRO INVESTIGATION OF THE POTENTIAL ANTICANCER EFFECT OF ANTIPSYCHOTIC DRUGS

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Lung cancer is the second most frequent cancer in men and women and smoking is the major risk factor, which accounts for 75-80% of lung cancer-related deaths. Several epidemiological studies reported significantly lower cancer incidence in male schizophrenic patients following antipsychotic treatment compared to general population, although these patients are usually heavy smokers and adopt dietary habits that are largely related to carcinogenicity. Currently, the therapeutic strategies followed in lung cancer are based on schemes including more than one drug, in order to reduce the side effects of typical chemotherapeutics, such as cisplatin.

Aim: The present study investigates the potential anticancer properties of antipsychotic drugs that share a common property- they are D2-dopaminergic antagonists. The main focus was on the mechanisms involved using the A549 and H1299 Non-Small Cell Lung Cancer lines (NSCLC). Materials and Methods: For this purpose, the impact of typical and atypical antipsychotics including haloperidol, sulpiride, pimozide, clozapine and risperidone on NSCLC cell proliferation was assessed using the SRB test. The potential beneficial effect of antipsychotics on cisplatin's and paclitaxel's anticancer effect was also assessed in these cancer cell lines.

Results: Interestingly, the SRB test and flow cytometry indicated that only clozapine markedly reduced the A549 and H1299 cell population by inducing apoptosis. No similar effects were detected with the other antipsychotics, in the concentrations tested, that correspond to therapeutic doses. It should be noted that sulpiride, clozapine and risperidone markedly increased the cisplatin-induced reduction of A549 and H1299 cell populations. The antipsychotic drugs though did not affect the paclitaxel-induced reduction of A549 cells.

Conclusion: The present data suggest that several antipsychotic drugs display anticancer properties by inducing apoptotic mechanisms. These drugs, administered in sub-therapeutic doses, could enhance the anticancer effect of the regular drugs used in lung cancer, even they are used in low doses, thus ensuring a better anticancer outcome and less side effects.

ΠΑ03

CANNABIDIOL MODULATES THE MOTOR PROFILE AND NMDA RECEPTOR-RELATED ALTERATIONS INDUCED BY KETAMINE

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Introduction: Cannabidiol (CBD) is a non-addictive compound of cannabis with antipsychotic potential, while ketamine (KET), an uncompetitive NMDA receptor inhibitor, has been extensively used as a psychotomimetic. Only few studies have focused on the role of CBD on the KET-induced motor profile, while no study has investigated the impact of CBD on KET-induced alterations in NMDA receptor subunit expression and ERK phosphorylation state, in brain regions related to the neurobiology and treatment of schizophrenia.

Objectives: The purpose of this study is to evaluate the role of CBD on KET-induced hyperlocomotion and relevant glutamatergic signaling components in specific rat brain regions.

Methods: Adult male Sprague-Dawley rat locomotor activity was evaluated in the Open Field for a one-hour registration period. Protein expression of specific neurobiological indices related to glutamatergic status and neuroplasticity were assessed using western blot, in the prefrontal cortex, the nucleus accumbens, the dorsal and the ventral hippocampus.

Results: The present study demonstrated that CBD pre-administration did not reverse ketamine-induced short-lasting hyperactivity, but it prolonged it over time. CBD alone decreased motor activity at the highest dose tested (30 mg/kg) while ketamine increased motor activity at the higher doses (30, 60 mg/kg). Moreover, KET induced regionally-dependent alterations in NR1 and NR2B expression and ERK phosphorylation state that were reversed by CBD pre-administration. Specifically, in the nucleus accumbens KET per se reduced NR2B and p-ERK levels, while the CBD/KET combination increased NR2B and p-ERK levels, as compared to control.

Conclusions: It is demonstrated herein that CBD prolongs KET-induced motor stimulation and restores KET-induced effects on glutamatergic signaling and neuroplasticity-related markers. These findings shed more light on the modulatory role of CBD on KET-induced behavioral and neurobiological profiles and contribute to issues related to the antipsychotic properties of CBD in the presence of NMDA antagonists that simulate psychosis and schizophrenia.

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ΠΑ04

MITOCHONDRIAL F1FO ATP SYNTHASE AS A TARGET AGAINST MYOCARDIAL ISCHEMIC INJURY: DISCOVERY OF NOVEL HYDROLASE INHIBITORS AND IN VIVO EVALUATION OF THEIR CARDIOPROTECTIVE EFFECT

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Aim: The mitochondrial F1Fo ATP synthase is responsible for ATP production. However, during myocardial ischemia, ATP synthase reverses its activity to hydrolyze ATP leading to energetic deficit and cell death. In the present work we aimed to 1) discover and in vitro evaluate inhibitors of the hydrolytic activity of F1Fo-ATP synthase and 2) investigate the cardioprotective effect of hydrolase inhibitors on an in vivo ischemia/reperfusion (I/R) model.
Materials and Methods: Initially, we identified inhibitors of ATP hydrolysis by employing in-silico virtual screening methods on our in-house library of 2000 compounds, Pharmalab, and the National Cancer Institute (NCI) database. The best candidates were evaluated on isolated murine heart mitochondria to verify their inhibitory effect on ATP hydrolysis. Moreover, the most potent inhibitors were studied in H9C2 cardiomyoblasts to confirm their action at a cellular level and to detect toxic or non-specific effects. Primary rat cardiomyocytes (ARVCs) were challenged with the uncoupler CCCP and the time until cardiomyocyte shortening was determined upon treatment with the inhibitors. Finally, 30 adult male mice were randomized (n= 5 per group) and subjected to 30'I/120' R. The best inhibitors ID: 1117 and ID: 1119 from the in vitro assays, the established inhibitors oligomycin and BTB06584 and the corresponding vehicle solvents were administered intravenously 5 minutes before ischemia. At the end of reperfusion myocardial infarct size was determined.
Results: Seven compounds out of 53 tested in vitro showed inhibition of hydrolysis at 200 μM in isolated cardiac mitochondria and their IC50 values were determined. Three synthetic inhibitors displayed the best IC50 values (81.7 ±1.3μM, 99.8 ±1.2μM, 144.8 ±1.3μM) and were evaluated on the H9C2 cells. These compounds inhibited ATP hydrolysis at 50μM ($p<0.01$ compared to vehicle) and they extended ($p<0.001$) the time until ARVCs' shortening. Finally, the compounds 1117, 1119 and oligomycin significantly reduced the infarct size (28.3% ± 2.8 34.8% ± 3.7 and 33.4% ± 4.8 respectively against 45.4 ± 3.8% of the control group) while the selective BTB inhibitor did not exhibit cardioprotection.
Conclusion: We discovered two novel agents that act as selective inhibitors of ATP hydrolase and may be used against myocardial ischemic injury.

ΠΑ05

DEVELOPMENT OF STATE-OF-THE-ART BIOANALYTICAL METHODOLOGIES AND IN VIVO PROTOCOLS FOR THE EVALUATION OF MICROPARTICLE BASED FORMULATIONS OF EXENATIDE

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Background: Exenatide is a peptidic drug (39 amino acids) used in type II diabetes. It is a glucagon-like peptide-1 receptor agonist with multiple antidiabetic effects, including glucose-dependent stimulation of insulin secretion and suppression of glucagon secretion. During the development phase of polymer based microparticle formulations of exenatide as well as of other peptidic pharmaceutics and biopharmaceutics, scientists often face formulation issues that require evaluation in appropriate in vivo models. Thus, monitoring of the low circulating levels of peptidic drugs as well as the efficient development of clinically relevant PK/PD protocols in animals would allow the effective evaluation of crucial characteristics of these formulations.

Aims: The aims of the described study were to develop:

1) A highly sensitive bioanalytical methodology based on high pressure liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the quantification of exenatide in biological fluids

2) Appropriate animal models (mouse, rat) that would enable understanding of crucial PK/PD properties of complex formulations

Methods: Since the analysis of the intact peptide was challenging in terms of sensitivity and binding of the analyte to surfaces, it was decided to pursue an alternative approach by forming smaller tryptic fragments. The latter resulted in a significant enhancement in the sensitivity of the analysis. As exenatide mimics the action of incretin and stimulates insulin secretion and reduces the amount of glucose, we also set up methods to determine insulin (using LC-MS/MS) and glucose levels (using a glucose meter) as pharmacodynamic parameters.

Results: Different formulations of exenatide (polymer-based particles or simple aqueous formulations) were administered in rats or mice in short (24 h) and long (42-day) protocols. Plasma samples from the studies were analyzed providing crucial information about the slow release of exenatide from particle formulations as well as its pharmacodynamic effect after immediate or extended release.

Conclusion: This study provides a valuable paradigm on how to set up preclinical PK/PD approaches for the evaluation of novel formulations of peptidic drugs.

ΠΑ06

CIRCULATING MICRORNAS AS REGULATORS OF DIRECT ORAL ANTICOAGULANT RESPONSE IN ATRIAL FIBRILLATION: CLINICAL STUDY DESIGN

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State-of-the-art: Atrial fibrillation (AF) is the most common arrhythmia. It is treated with oral anticoagulants (OACs), coumarinic (COAs), and direct (DOACs). Differences in circulating microRNA (miRNA) expression have been associated with AF development, whereas their expression is restored after cardiac ablation. Additionally, several coagulation factors are regulated by miRNAs. The expression profile of miRNAs in DOAC-treated AF patients has not been studied.

Aims: The primary aims of the clinical study are to 1. study the differential expression of miRNAs in naïve AF patients starting DOAC therapy and compare it between and within patients, 2. explore differences in miRNA expression in DOAC-treated patients presenting with adverse events and 3. build and explore the DOAC miRNA-mRNA-genes regulatory network, with the grand aims of identifying novel (epi)genetic biomarkers that can be further studied as for their association with DOAC response. The ultimate goal is to propose novel circulating biomarkers for DOAC therapy monitoring.

Clinical study design: We are conducting a clinical trial combining discovery research and bioinformatic analysis in patient samples in a clinical setting. At least 66 AF naïve patients eligible for DOAC therapy -22 in each of dabigatran, rivaroxaban and apixaban- and 22 age-matched controls will be prospectively enrolled. miRNAs will be isolated and high throughput analysis of approximately 50 carefully selected human miRNAs will be performed for patients and controls at baseline, after 1 week (early response) and 4 weeks (late response). MiRNA-mRNA-genes regulatory network will be constructed and analyzed to describe the pathways that are altered during DOAC therapy. Selected genes from the derived pathways will be furthered analyzed as for their methylation profile.

Ambitions of the present research: We will deliver new knowledge on the changes of miRNA expression during DOAC therapy that will advance the clinical approach of AF treatment and improve safety of DOAC administration. The associations possibly found can constitute the basis for identifying prognostic biomarkers and building algorithms for DOAC effect on patients. We further expect to identify pathways of DOAC therapeutics that will add to our knowledge of the mechanism(s) of action of these compounds.

ΠΑ07

COMPARATIVE EFFECTIVENESS OF GLUCAGON-LIKE PEPTIDE-1 RECEPTOR AGONISTS VERSUS DIPEPTIDYL PEPTIDASE-4 INHIBITORS ON NONINVASIVE INDICES OF HEPATIC STEATOSIS AND FIBROSIS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Aim: Nonalcoholic fatty liver disease (NAFLD) is highly prevalent in patients with type 2 diabetes mellitus (T2DM). There is currently no approved treatment for NAFLD. The main aim of this study was to evaluate the effect of glucagon-like peptide-1 receptor agonists (GLP-1 RA) vs. dipeptidyl peptidase-4 inhibitor (DPP-4i) treatment on noninvasive indices of hepatic steatosis and fibrosis in patients with T2DM followed-up for 6-18 months.

Materials and Methods: In this retrospective comparative study, three noninvasive indices of hepatic steatosis [hepatic steatosis index (HSI), NAFLD ridge score, and triglycerides (TG) to high-density lipoprotein cholesterol (HDL-C) ratio] and five of fibrosis [aspartate aminotransferase (AST)-to-platelet (PLT) ratio index (APRI), Fibrosis-4 index (FIB-4), NAFLD fibrosis score (NFS), body mass index (BMI)-age-alanine aminotransferase (ALT)-TG (BAAT) and BMI AST/ALT Ratio Diabetes (BARD)] were calculated before and after (6 to 18 months) the addition of a DPP-4i (n=152) or a GLP-1 RA (n=37) in patients with T2DM.

Results: Regarding steatosis indices, NAFLD ridge score was significantly decreased in the GLP-1 RA group (baseline: 0.9±0.34, follow-up: 0.67±0.24; p=0.001), but not in the DPP-4i group (p=0.25); the difference for group*time interaction was significant (p=0.02). HSI showed a trend between groups, being different at baseline and follow-up (p<0.001) with no significant difference in group*time interaction. Indices of fibrosis were not essentially changed within or between groups.

Conclusions: NAFLD ridge score was decreased after the addition of GLP-1 RA in patients with T2DM. This study warrants further prospective clinical trials to clarify the comparative effectiveness of GLP-1 RA vs. DPP-4i on hepatic histology.

ΠΑ08

DEVELOPMENT OF 3D INTESTINAL ORGANOIDS FROM HUMAN EMBRYONIC STEM CELLS; A NOVEL IN VITRO MODEL FOR INTESTINAL INFLAMMATION AND FIBROSIS

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Aim: Organoids are self-renewing, 3D structures, consisting of different cell types, with histology and physiology features very close to the physiology of the studied organ. Specifically, human Intestinal Organoids (HIOs) develop epithelial crypts consisting of all subtypes of intestinal epithelial cells and are surrounded by mesenchymal cells. Our aim was first to develop 3D HIOs from human embryonic stem cells (ESCs) and then to examine the expression of pro-fibrotic and mesenchymal factors during their maturation process. Additionally, we investigated the time-dependent effect of the innate cytokines, IL-1α and TNF-α on the expression of pro-fibrotic/-inflammatory mediators in HIOs.

Methods: The human ESC line (H1) was cultured and then differentiated towards HIOs using commercially available kit. HIOs and their main differentiation stages were characterized by immunofluorescence. HIOs were passaged every 8-10 days and total RNA was collected. In order to examine their maturation process, we compared the mRNA expression of pro-fibrotic and mesenchymal markers from passages 6-13. In order to examine their functionality, HIOs were stimulated with 5ng/ml IL-1α and 50ng/ml TNF-α for 12, 24 and 48 hours, total RNA was collected and the pro-fibrotic/-inflammatory mRNA expression was examined. mRNA transcripts for α-SMA, Tissue Factor (TF), CD90, IL-8, collagen type I, III and fibronectin were measured by reverse transcription quantitative PCR.

Results: HIOs were successfully developed as they were stained positive for all tested markers throughout their developmental process. Regarding their maturation process, though all pro-fibrotic and mesenchymal markers were strongly expressed at passage 6, they were found significantly downregulated in the following passages. HIOs (p6) were stimulated with IL-1α/TNF-α and at 12h of stimulation the α-SMA, Fibronectin, Collagen Type I, III, CD90 and IL-8 expression was significantly increased and then decreased at 24h and 48h. TF expression, although unaltered at 12h and 24h, was found upregulated at 48h of stimulation.

Conclusion: We have successfully developed HIOs from human ESCs and highlighted that undergo changes throughout the passages, as their mesenchyme part seems to gradually disappear. We also proved that HIOs are functional and a promising 3D in vitro model for studying intestinal inflammation and fibrosis, as they respond to pro-inflammatory stimuli.

ΠΑ09

THE EFFECT OF PROBIOTICS IN THE PRO-FIBROTIC PHENOTYPE OF SUBEPITHELIAL COLONIC MYOFIBROBLASTS

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Aim: Probiotics seem to play a positive role in Inflammatory Bowel Disease (IBD) and recent literature suggest that they might support mucosal healing. Myofibroblasts have a pivotal role in mucosal healing and fibrosis. Our aim was to investigate whether different types of probiotics can affect the migration capacity and the fibrotic phenotype of SEMFs.

Methods: Primary SEMFs were isolated from endoscopically obtained colonic biopsies from healthy individuals. Healthy SEMFs were stimulated with TGF-b (5 ng/ml), IFN-γ (150 U/ml), 102, 104 and 108BU/ml of Lactolevure (Uni-Pharma) and Lactobacillus plantarum for 24h in order to assess their effect on migration rate through the wound healing assay. Healthy SEMFs were stimulated with 102, 104, 108 BU/ml of Lactolevure for 6 hours and total RNA was collected. mRNA transcripts for collagen type I, III, fibronectin, tissue factor (TF) and a-SMA were measured by reverse transcription quantitative PCR.

Results: Adding the probiotic mix Lactolevure to SEMFs in all three concentrations 102, 104 and 108BU/ml resulted in the overall downregulation of collagen protein expression (102BU/ml: 3,057-fold, 2,586-2,894, $p<0,05$) and downregulation of the mRNA expression of collagen type I (104BU/ml: 2,717-fold, 0,05231-0,05731, $p<0,05$; 108BU/ml: 2,265-fold, 0,02628-0,09979, $p<0,05$) and collagen type III (104BU/ml: 2,378-fold, 0,1272-0,1317, $p<0,05$; 108BU/ml: 2,717-fold, 0,01924-0,1363, $p<0,05$), as well as fibronectin and a-smooth muscle actin. On the other hand, when stimulated with 102 BU/ml of Lactolevure, there was an elevated production of the TF mRNA compared to unstimulated SEMFs (102: 2,717-fold, 10,74-11,12, $p<0,05$). Finally, it was shown that both Lactolevure and Lactobacillus plantarum resulted in decreased migration rate of SEMFs.

Conclusion: Our results show that probiotics have an important impact on fibrosis, since they decrease the production of profibrotic factors, and lead to reduced rate of SEMFs migration, and therefore, could be further studied as a possible anti-fibrotic therapy.

ΑΝΑΡΤΗΜΕΝΕΣ ΑΝΑΚΟΙΝΩΣΕΙΣ

AA01

IN VITRO INVESTIGATION OF POTENTIAL ANTIOXIDANT AND NEUROPROTECTIVE EFFECTS OF MOLECULES ISOLATED FROM OLEA EUROPEA AND CROCUS SATIVUS

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Alzheimer's disease acknowledged as a progressive neurodegenerative disorder and is the leading cause of dementia in late adult life. It is characterized by intracellular neurofibrillary tangles and extracellular amyloidal protein deposits contributing to senile plaques. The traditional therapeutic approaches based on drugs do not provide cure or significant elimination of the symptoms and often involve serious side effects. Therefore, the scientific interest focuses on medicinal plants for new molecules or herbal extracts with potential therapeutic applications in dementia.

Aim: The present study investigates the potential neuroprotective and antioxidant effects of several molecules isolated from olea europa and crocus sativus.

Materials and Methods: For this purpose, SH-SY5Y neuroblastoma cells were cultured in a mixture of Dulbecco's modified Eagle's medium (DMEM GlutaMAX) and Ham's F12 NutrientMix (1:1) for 24h, followed by a replacement of the medium without containing FBS but only Retinoic Acid (RA, 5μM), thus allowing the differentiation of the cells. Differentiated SH-SH5Y cells were treated with several concentrations of oleuropein, oleocanthale, oleocein, the total phenolic fraction of the Olea leaves, crocetin, trans-crocin 1, cis-trans-crocin 2, cis-trans-crocin 3, cis-trans-crocin 4 and picrocrocin. In order to investigate the potential neuroprotective effects of these compounds, the differentiated cells were treated with the Amyloid precursor protein (APP) and their effects were assessed using the MTS assay. The potential antioxidant effects of the above compounds were assessed in differentiated cells exposed to H2O2 (750μM).

Results: It was found that oleuropein and cis-trans-crocin 4 have mild neuroprotective and antioxidant effects on differentiated SH-SY5Y neuroblastoma cells.

Conclusions: The findings of this study suggest that oleuropein and cis-trans-crocin 4 could be beneficial in preventing or delaying the progression of neurodegenerative disorders, such as Alzheimer's disease. Further studies though, should be designed employing in vitro and in vivo models and behavioral tests to thorough investigate this hypothesis.

AA02

EFFECT OF VINE LEAF EXTRACTS ON MOLECULAR TARGETS OF VENOUS INSUFFICIENCY

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Chronic venous insufficiency (CVI) is characterized by increased venous pressure and valve damage which leads to blood stagnation. Reactive oxygen species, inflammatory cytokines and matrix metalloproteases (especially MMP2) all contribute to disease initiation and progression. Currently, the EMA has approved the use of herbal medicinal products from Vitis vinifera folium for the treatment of CVI and haemorrhoids, based on its well-established and traditional use. Herein, we investigated the effect of vine leaf extracts, rich in flavonoids and other bioactive molecules, on molecular targets associated with CVI.

EA.hy926, lung fibroblasts DLF, monocytes U937 were used to assess the effect of aqueous and organic solvent extracts, that were prepared from different varieties of vines and from different parts of the vine plant (leaves, stones, roots, trunk). Extracts were characterized by mass-spectroscopy for phytochemical content. Cells were treated for 30 mins with extracts following treatment with the inflammatory stimulator TNF-α (100ng/ml). U937 cells were stimulated with TNF-α (100ng/ml) and LPS (25μg/ml). Twenty-four hours following TNF-α exposure, the conditioned medium was collected and analysed for IL-6, TGF-β1, MMP2 and sVCAM. NOX-derived ROS production was measured using a lucigenin chemiluminescence assay.

At a concentration of 5μg/ml most extracts increased or did not alter TNFα-induced IL-6 in EA.hy926, U937 and DLF cells. A leaf ethanolic and a grape resin-prepared extract attenuated TFG-β1 from endothelial cells, while a leaf ethanolic and a leaf aqueous extract reduced TGF-β1 release from fibroblasts. Leaf extracts caused no change in ROS production (as assessed by NOX activity) in endothelial cells and fibroblasts. Leaf isopropanol and ethanol extracts inhibited NOX activity in U937. Most leaf extracts reduced MMP2 in endothelial cells but had no effect in sICAM and sVCAM.

The current data show that vine leaf extracts were effective in reducing selected inflammation-associated molecules that are upregulated in CVI. Among them, TGF-β1 and MMP2 seems to be the most prominent target.

This study was funded by the RESEARCH - CREATE - INNOVATE Action and was co-financed by the European Regional Development Fund (ERDF) of the European Union and national resources through the operational program Competitiveness, Entrepreneurship & Innovation (project code: T1EΔK-04103.

AA03

EVALUATION OF ANALGESIC POTENTIAL OF CANNABIDIOL AND CANNABIDIOLIC ACID IN RATS

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Background and Objectives: Studies have shown that cannabinoids exert analgesic properties. However, few studies have investigated the analgesic effect of cannabidiolic acid (CBDA) and cannabidiol (CBD). This study focuses on the possible analgesic effect of CBDA and CBD using the hot plate paradigm. Moreover, the effects of CBDA and CBD on motor activity were assessed, in order to detect any interference with analgesic properties.

Methods: Hot plate test was performed 30, 60 and 120 min after acute administration of VEH or CBDA and CBD. Additionally, the chronic treatment of CBD on analgesic potential was also evaluated. Motor activity following the administration of both cannabinoids was studied using the open field test.

Results: CBDA did not affect motor activity while CBD decreased motor activity following the highest dose used. The lowest dose of acute CBD administration induced analgesia, an effect which was also observed following low doses of CBDA. Interestingly, chronic CBD treatment induced analgesia-like effect.

Conclusions: CBDA and CBD induced mild analgesic effects following acute administration. Chronic CBD treatment also induced analgesic effect as assessed by the Hot Plate paradigm.

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AA04

THE CEREBELLAR VERMIS REGULATES ETHANOL INTAKE AND PREFERENCE ALONG WITH DOPAMINE TRANSPORTER PROTEIN EXPRESSION LEVELS IN LIMBIC BRAIN AREAS

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Aim: It has long been known that cerebellar outputs affect the dopaminergic system in a topographic manner, that lack of a functional cerebellar cortex leads to topographic changes in dopamine receptor and transporter levels, and that the cerebellar vermis is involved in limbic functions. Here, we sought to investigate the regulatory role of the cerebellar vermis on the manifestation of a limbic function, ethanol intake and preference, and on dopamine transporter protein expression.

Materials and Methods: Single-housed adult male Sprague-Dawley rats were stereotaxically injected with kainic acid or saline in the cortex of the cerebellar vermis. Three weeks post-surgery the rats were presented two bottles in their home cages, one containing tap water and the other containing increasing concentrations of ethanol. Ethanol intake and preference, food intake, and body weights were regularly measured for a three-week period. Six weeks post-surgery the rats were euthanized, with ethanol onboard, and limbic brain areas were harvested for the evaluation of dopamine transporter protein expression levels.

Results: Lesion in the cerebellar vermis led to a significant increase in ethanol intake and preference in all ethanol concentrations tested. Dopamine transporter protein expression levels increased after ethanol intake in sham operated rats, an effect that was not observed in lesioned animals.

Conclusions: The cerebellar vermis is involved in the regulation of ethanol intake and preference along with dopamine transporter protein expression levels in limbic brain areas.

AA05

DEVELOPMENT OF A MATHEMATICAL MODEL THAT MAY PREDICT THE ACTIVITY OF POTENTIAL ANTICANCER AGENTS IN A PANCREATIC DUCTAL ADENOCARCINOMA (PDAC) XENOGRAFT

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Aim: Mathematical models are considered important tools in studying and understanding cancer biology and in the improvement of anticancer therapies. Herein, we present an advanced non-linear mathematical model that can predict accurately the effect of an anticancer agent on the growth of a solid tumor.

Materials and Methods: Advanced non-linear mathematical optimization techniques and human-to-mouse experimental data were used to develop a tumor growth inhibition (TGI) estimation model. Human pancreatic cancer xenografts developed by injecting subcutaneous NOD/SCID mice with AsPC1 cancer cells were used to validate the model. Gemcitabine injected intraperitoneally at a dose of 100mg/kg once a week used as the model drug.

Results: Using this mathematical model, we could accurately predict the tumor mass in this human-to-mouse pancreatic ductal adenocarcinoma (PDAC) xenograft under gemcitabine treatment up to five time periods (points) ahead of the last treatment.

Conclusion: The ability of the identified TGI dynamic model to perform satisfactory short-term predictions of the tumor growth in a PDAC in vivo model for up to five time periods ahead was investigated, evaluated, and validated for the first time. Such a prediction model could not only assist the pre-clinical testing of putative anticancer agents for the treatment of PDAC and other cancers, but also the early modification of a chemotherapy schedule towards increased efficacy in the context of precision medicine.

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AA06

STUDY OF THE RELATIONSHIP BETWEEN SIGMA RECEPTORS LEVELS AND SOME COMMON SIGMA LIGANDS ACTIVITY IN CANCER USING HUMAN CANCER CELL LINES OF THE NCI-60 CELL LINE PANEL

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Introduction: The aim of this study is to investigate the expression levels of σ receptors in several human cancer cell lines representing different human cancer types and to address the in vitro anticancer activity of some commonly used σ1 and σ2 ligands against those cell lines in order to a) compare their activity under the same experimental conditions and b) study the relationship between σ receptors expression levels and σ ligands activity.

Materials and Methods: The expression levels of σ1 receptor, PGRMC1 and TMEM97 were studied simultaneously in 23 established human cancer cell lines, by western blot analysis. The antiproliferative activity of various selective σ ligands (siramesine, PB28 dihydrochloride, rimcazole, SM-21 maleate, and BD-1047 dihydrobromide) was studied in vitro by sulforhodamine B assay (SRB). Also, the NCI COMPARE algorithm was used for the estimation of the mechanism of action of the selective σ ligands.

Results: Sigma-1 receptor, PGRMC1 and TMEM97 are heterogeneously expressed in the cell lines studied without showing any selectivity in a particular type of cancer. In addition, it seems that the compounds tested here, even though they show in vitro anticancer activity, exert this effect mainly through mechanisms that do not involve the σ receptors. Among the tested σ ligands, σ2 agonist, siramesine, exhibits the best anticancer activity and appears to be the most promising compound. Also, among the tested types of cancer, pancreatic cancer appears to be the more sensitive against siramesine's antiproliferative activity.

Conclusion: Here, we provide evidence that sigma receptors do not mediate the antiproliferative efficacy of a panel of σ ligands. Amongst all sigma ligands tested herein, the σ2 receptor ligand, siramesine, shows the most potent anticancer activity underlying the need to be further evaluated in vitro and in vivo as a potential anticancer compound.

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AA07

SIRAMESINE, A SIGMA-2 RECEPTOR AGONIST INDUCES CELL DEATH VIA INDUCTION OF BOTH APOPTOSIS AND AUTOPHAGY IN A PATIENT DERIVED PDAC MODEL IN VITRO AND IN VIVO

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Introduction: The aim of this study was to investigate the anticancer activity of siramesine on human pancreatic ductal adenocarcinoma. In this context, we studied, siramesine's in vitro and in vivo efficacy in animal cancer models derived from patients with pancreatic cancer (PDX) either as monotherapy or in combination with gemcitabine.

Materials and Methods: First, the expression levels of σ receptors were studied in two primary patient derived ex vivo pancreatic cancer cell populations, isolated in our laboratory, by western blot analysis. In vitro evaluation of siramesine against patient derived tumor cells followed. Toxicity evaluation of siramesine in NOD/SCID mice and in zebrafish model including embryo developmental effect and overall mortality observations was further performed. Finally, the in vivo efficacy of siramesine was tested in animal cancer models derived from patients with pancreatic cancer (PDX) that were developed in our laboratory.

Results and Discussion: In vitro studies showed that siramesine could kill tumor cells via both autophagy and apoptosis induction. Studies of siramesine, in tumor xenografts derived from patients (PDX) with pancreatic cancer, confirmed a promising anticancer activity. Also, our experimental data show that siramesine can be combined with gemcitabine resulting in an improved therapeutic outcome in the PDX tested. The major disadvantage of siramesine appears to be its dose dependent toxicity, which can be overcome, using controlled release drug nanoparticles such as liposomes, as the corresponding study has shown.

Conclusion: Sigma-2 ligand, siramesine, is a promising anticancer compound for the treatment of pancreatic cancer either as monotherapy or acting as a sensitizer to standard chemotherapies such as gemcitabine. Further studies of siramesine are needed to optimize its anticancer efficacy and elucidate its underlying mechanism of action.

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AA08

DEVELOPMENT OF A SIMPLE AND INEXPENSIVE METHOD FOR THE STUDY OF GEMCITABINE LEVELS IN RAT SERUM USING AN IMPROVED REVERSED – PHASE HPLC ASSAY COUPLED TO UV DETECTION

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Aim: The aim of the current study was to develop and validate a simple and inexpensive method for separating and quantifying gemcitabine (gem) and its main metabolite 2´,2´-difluorodeoxyuridine (dFDU) in serum.

Materials and Methods: Wistar male rats with an average weight of 300g allocated into three groups (n= 3/group) used for this study. One group was used as control while two groups received a single dose of Gem at 100 mg/kg either intraperitoneally (i.p.) or subcutaneously (s.c.). Blood samples were collected via tail vein at 30min, 1, 2 and 24h post gem administration. 1,7-Dimethyluric Acid (1,7 U) was used as internal standard (IS). Samples and Standard analysis was performed using a Sperisorb S5ODS2 (4,6mm x 25cm) column and a mobile phase consisted of 3 % v/v methanol and 97 % v/v PBS (pH=6,6) which was delivered isocratically at a flow rate of 1.0ml/min. The detection wavelength was 267nm and the column temperature was 40ºC.

Results: Calibration curve was linear at concentrations between 1-500μM (R^2=0,997). Coefficient of variation was ≤6.52% and Bias ≤-7.77%. Quantitation and detection limit or the method were at 1μM and 0,17μM, respectively. Retention times for Gem, IS and dFDU were 8,74 (±0,19), 9,98 (±0,3) and 12,34 (±0,11) min, respectively. A subsequent pharmacokinetic study applying this method, revealed substantial differences between the two modes of administration with i.p. resulting at higher serum concentrations at the same time points (figure 1).

Conclusion: We developed a simple and inexpensive method as compared to already existed methods for the measurement of gem in rat serum that seems to be useful for the monitoring of Gem and dFDU levels in rat serum, and possibly, in human serum (ongoing studies). Subsequent PK analysis using this method demonstrated significant differences between i.p. and s.c. administration with the i.p. to be superior in terms of gem serum concentration.

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AA09

CHANGES IN TISSUE 3-MERCAPTOPYRUVATE SULFURTRANSFERASE IN A MOUSE MODEL OF METABOLIC SYNDROME

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Aim: Hydrogen sulfide (H2S) is an endogenously produced signaling molecule, synthesized by cystathionine γ-lyase (CSE), cystathionine β-synthase (CBS) and 3-mercaptopyruvate sulfurtransferase (3-MST). H2S exerts many physiological functions, but also contributes to the pathogenesis and/or severity of cardiometabolic diseases. Metabolic syndrome (MS) is a complex disease and a risk factor for cardiovascular disease and diabetes mellitus. In the context of this project, we have studied the expression of H2S-producing enzymes in a model of metabolic syndrome. Our goal was to determine possible changes in the levels of H2S-producing enzymes and to use H2S-based pharmacological interventions to treat the manifestations of MS.

Methods: Our mouse model of MS is based in a combination of high fat diet-induced obesity and hyperglycemia, and a pharmacological model of hypertension based in administration of a nitric oxide synthase inhibitor, NG-nitro-L-arginine methyl ester (L-NAME) Weekly changes in body weight, fasting glucose, glucose tolerance test (GTT) and insulin tolerance test (ITT) were measured. In addition, we determined the elevation of systolic and diastolic blood pressure following L-NAME administration, using the tail cuff method. Subsequently, tissues were isolated and the expression of H2S producing enzymes was measured by western blot analysis.

Results: In cardiovascular tissues, while 3MST expression was decreased in aorta of animals with MS, no change was observed in the heart. We also found reduced expression of the enzyme in the kidney, which is also observed in the group of animals that were only obese (no L-NAME treatment). In the liver, 3MST expression was elevated in MS group and in adipose tissue we observed reduced expression of 3MST in all groups of obese animals, regardless of the coexistence of hypertension. The expression of CSE and CBS enzymes was not significantly affected under any conditions.

Conclusion: Our results indicate that 3MST may play an important role in pathophysiology of MS, as changes in its expression have been observed in most tissues studied.

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AA10

TARGETED MS-BASED STRATEGIES FOR THE QUANTIFICATION OF WELL-ESTABLISHED RECEPTOR TARGETS IMPLICATED IN BREAST AND PROSTATE CANCER

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The main goal of this study was the development of novel proteomic methodologies for the detection and absolute quantification of well-known receptor targets, such as G protein-coupled receptors (GPCRs). GPCRs play a central role in cell signaling and their implication in a variety of human cancers, makes them attractive targets for patient stratification and targeted therapies. In our effort to quantify low abundance GPCRs whose detection by antibody-based assays is challenging we have developed and applied Targeted LC-MS/MS-based approaches, such as Multiple Reaction Monitoring (MRM) and Parallel reaction monitoring (PRM), using representative BC and CaP cell lines. We chose the Gonadotropin Releasing Hormone Receptor (GnRHR) and the Epidermal Growth Factor Receptor (EGFR), established therapeutic targets known to be overexpressed in BC and CaP.

Our strategy for the quantification of GnRHR/EGFR in cancer cells was based on the detection and measurement of the expression levels of synthetic proteotypic peptides. We managed to develop and validate an LC-MRM method for the detection and quantification of the mixture of the reference synthetic peptides of GnRHR/EGFR, with the use of a Triple Quadrupole MS system. In order to further improve the sensitivity and the reproducibility of our methodology, we proceeded to the LC-PRM technique with the use of a high-resolution Q Exactive Hybrid Quadrupole-Orbitrap MS.

The LC-PRM method using a high-resolution MS system goes beyond LC-MRM in that it covers a wider dynamic concentration range with higher mass accuracy (ppm- to sub-ppm levels) and it can monitor all precursor to product transitions. As a result, the LC-PRM technique reduces the number of co-eluting peaks which generate interferences and leads to better sensitivity and reproducibility. Finally, we succeeded to determine accurately EGFR and GnRHR expression levels in specific BC and CaP cell lines.

Our Targeted LC-PRM strategy is anticipated to be of great importance in clinical practice since we could assess and monitor the expression levels of key receptor targets in cancer cells that could enable efficient and targeted pharmacologic interventions for cancer patients. The targeted proteomics approach can be applied to additional proteins of clinical importance that cannot be detected with antibody-based assays.

AA11

PHARMACOLOGICAL CHARACTERIZATION OF A NOVEL OLIGOPEPTIDE TARGETING THE N-REGION OF CRF1R

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Aim: Corticotropin releasing factor is a key molecule for the maintenance of homeostasis by interacting with type 1 receptor (CRF1R) and regulating important physiological and pathophysiological pathways. Malfunctioning of the CRF/CRF1R unit is associated with anxiety and depression. Although non-peptide CRF1R-selective antagonists have been shown to exert anxiolytic and antidepressant effects on experimental animals, none of them is in clinical use today. In an effort to develop novel CRF1R antagonists we designed the (R)-LMI, a small tripeptide analogue of CRF, based on the crystal structure of the N-extracellular domain (N-domain) of CRF1R/CRF complex. The aim of this study is to pharmacologically characterize the (R)-LMI.

Materials and Methods: In order to pharmacologically evaluate the (R)-LMI we performed cAMP accumulation assays in HEK 293 cells, which were stimulated by 10nM CRF with or without increasing concentrations of (R)-LMI. In addition we determined the increased basal cAMP levels in HEK 293 cells expressing the CRF1R or a constitutively active N-truncated chimera (N-chimera) of CRF1R, before and after addition of 1000 nM (R)-LMI or antalarmin, a small non-peptide CRF1R antagonist. We also determined the production of interleukin (CXCL1) in adipocytes and the proliferation rate of RAW 264.7 cells before and after their incubation with 10 nM CRF and/or 1000 nM (R)-LMI.

Results: (R)-LMI inhibited CRF stimulated cAMP accumulation in a dose-response manner, with an antagonistic potency (IC50) of 10 nM. Moreover, (R) - LMI significantly decreased the CRF-stimulated proliferation rate of RAW 264.7 cells and the production of CXCL1 from adipocytes. In contrast to antalarmin, the (R)-LMI did not decrease the constitutively increased levels of cAMP in cells expressing the N-chimera.

Conclusion: (R)-LMI is a CRF1R antagonist inhibiting CRF biological effects. The tripeptide acts by binding to the N-terminal extracellular segment of CRF1R, because it was unable to exert its actions in the N-chimera, which lacks this portion of receptor. (R)-LMI could set the basis for synthesis of a new series of CRF1R antagonists with enhanced pharmacodynamic and pharmacokinetic properties.

AA12

NOVEL NON-PEPTIDE ANALOGUES TARGETING THE TYPE 1 RECEPTOR OF THE CORTICOTROPIN RELEASING FACTOR

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Aim: Corticotropin releasing factor is a key molecule for the maintenance of homeostasis by interacting with type 1 receptor (CRF1R) and regulating important physiological and pathophysiological pathways. Malfunctioning of the CRF/CRF1R unit is associated with anxiety and depression. Although non-peptide CRF1R-selective antagonists have been shown to exert anxiolytic and antidepressant effects on experimental animals, none of them is in clinical use today. In an effort to develop novel CRF1R antagonists we designed and synthesized a series of non-peptide CRF pyrimidine derivatives. The aim of this study is to pharmacologically characterize them.

Materials and Methods: In order to pharmacologically evaluate the synthesized compounds (1-6) at first we tested their ability to inhibit the specific binding of [125I]-Tyr0sauvagine to membranes from HEK 293 cells stably expressing the CRF1R at a single concentration of 300nM. The best compound (compound 6) was further pharmacologically characterized by determining its binding affinity (log Ki) in competition experiments performed under equilibrium conditions.

Results: In the screening experiment, compound 6 was able to inhibit more than 50% of [125I]-Tyr0sauvagine specific binding while the remaining five analogues inhibited the specific binding less than 50%. Pharmacological evaluation of the best compound (compound 6) was found to bind to CRF1R at a dose dependent manner displaying a binding affinity (Log ki ± SE value) of -6.82 ± 0.36 (or 151 nM)

Conclusion: A series of pyrimidine analogues have been designed and synthesized. Pharmacological evaluation of these analogues suggested that analogue 6 bound to CRF1R in a dose response manner and with a relatively high affinity. This study shed more light on the structure-activity-relationships of this series of compounds and that further research in this direction may lead to development of better drug candidates as CRF1R antagonists.

AA13

EXPLORING THERAPEUTIC EFFECTS OF MITOCHONDRIAL-TARGETED ANTIOXIDANTS

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Aim: Millions of new cases of cancer are recorded every year and the incidence rate is expected to increase. It is therefore crucial to identify novel therapeutic agents with anti-proliferative properties for devising optimized therapeutics. Here, we present a novel, optimized version of the antioxidant hydroxytyrosol that is targeted selectively to mitochondria.

Materials and Methods: We treated human HEK-293 cells, a transformed, high malignant and well established cell line in cancer biology, with mitochondria-targeted hydroxytyrosol. We then investigated treatment effects on cell survival and oxidative stress readouts with biochemical and immunobased approaches.

Results: Our work showed that the mitochondrial-targeted hydroxytyrosol dramatically inhibited cell growth of HEK-293 cells in a dose- and time-dependent manner. The mitochondrial targeted hydroxytyrosol is significantly more efficient in inhibiting cell proliferation than hydroxytyrosol. The growth arrest of cells was accompanied by a decrease of a-enolase expression levels, a main enzyme of glycolysis. We also report changes in oxidative stress-related markers.

Conclusions: These results suggest anti-proliferative properties of mitochondria-targeted antioxidants and highlight the therapeutic potential of mitochondrial targeting approaches in cancer.

AA14

THIN FILM FABRICATION VIA LASER-INDUCED FORWARD TRANSFER, AIMED AS A DRUG DELIVERY TOOL

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The use of different 3D printing technologies for pharmaceutical manufacturing provides new opportunities for personalized medicine and on-demand tailored drug products, such as implants and other dosage forms. In this work we present our recent achievements in developing a manufacturing process aiming in printed personalized dosage forms of liquid-phase active substances (i.e. paclitaxel) and the optimization of the printing process onto glass slide substrates via the Laser Induced Forward Transfer technique (LIFT). Key laser printing parameters for reproducible deposition have been investigated, while the deposited paclitaxel films have been studied by means of Mass Spectrometry (MS)-based analytical technique. In the context of investigating the efficacy of LIFT printing, the active pharmaceutical ingredient (API) quantification of the printed paclitaxel films was confirmed using High-Performance Liquid Chromatography tandem Mass Spectrometry (HPLC-MS/MS) analysis. Initial experiments of APIs' laser printing have showcased good feasibility of this technique, highlighting LIFT as a promising method for pharmaceutical applications.

AA15

EFFECTS OF NITRITE AND HYDROGEN SULFIDE ON LIPID ACCUMULATION IN ADIPOCYTE-LIKE CELLS

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Aim: Gasotransmitters are a family of endogenously produced gaseous signaling molecules that share common features with respect to their production, mode of action and function. The family consists of nitric oxide (NO), hydrogen sulfide (H2S) and carbon monoxide (CO). In the cardiovascular system, NO and H2S trigger vasorelaxation, promote angiogenesis, inhibit atherosclerosis and exert cardioprotection. H2S is produced from L-cysteine through three distinct enzymes, while NO is generated from L-arginine by NO synthase. An alternative pathway that generates NO from nitrite also exists. Reduced NO and H2S activity/levels have been observed in multiple disease animal models including hypertension, atherosclerosis and diabetes. The impact of NO and H2S on metabolism and obesity are not as well understood. The aim of the present study was to determine the ability of nitrite and H2S donors to limit lipid accumulation in adipocyte-like cells.

Methods: 3T3-L1 cells were differentiated following incubation in medium containing isobutylmethylxanthine (0,5mM), insulin (10μg/ml) and dexamethosone (1μM) for 7 days. They were then treated with vehicle, GYY4137 (100μM-5mM), NaNO2 (0.1-100 μM) and erucin (3-300 μM) for 48hr. At the end of the incubation period lipid accumulation in adipocyte-like cells was determined using Oil Red O staining.

Results: NaNO2 inhibited lipid accumulation in a concentration-dependent manner that reached significance at 100 μM. Similarly, the naturally-occurring H2S donor erucin attenuated Oil Red O staining at concentrations over 30 μM, while the slow releasing donor GYY-4137 had no effect. H2S can also exist in the form of polysulfides. Treatment of cells with sodium trisulfide (Na2S3) had no effect on lipid accumulation up to a concentration of 100μM.

Conclusion: Our findings indicate that NO2- and H2S reduce lipid content in adipocyte-like cells. Future experiments will investigate the in vivo effects of these pharmacological agents in high fat diet models and the synergistic actions of nitrite and H2S pathways is reducing lipid accumulation and obesity.

Funding: This study was funded by the RESEARCH - CREATE - INNOVATE Action and was co-financed by the European Regional Development Fund (ERDF) of the European Union and national resources through the operational program Competitiveness, Entrepreneurship & Innovation (project code: T2EΔK-00843)

AA16

LINCS1000 BIOINFORMATIC SOFTWARE AS A DRUG REPOSITIONING TOOL FOR SYSTEMIC LUPUS ERYTHEMATOSUS: HEAT SHOCK PROTEIN-90 (HSP-90) AS A NEW POTENTIAL DRUG TARGET FOR THE TREATMENT OF SLE

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Aim: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with complex pathogenesis, including activation of monocytes. Current therapies used, are the disease-modifying antirheumatic drugs (DMARDs), corticosteroids and the non-steroid anti-inflammatory drugs (NSAIDs), as well as, more targeted therapies, such as monoclonal antibodies. Even though biological agents are very efficient in reducing the progression of the disease, nevertheless are associated with several other drawbacks, including inadequate clinical response over time, need of parenteral administration, as well as increased risk of several infections, due to the progressively diminished immune response. There is a clear need for per os administered, well-tolerated, inexpensive drugs to treat SLE.

Materials-Methods: We performed RNA seq in peripheral blood monocytes derived from 15 active SLE patients, as well as age and sex matched healthy controls. We further analyzed differentially expressed genes, by the use of the bioinformatic software Lincs 1000.This program enables the user to identify patented small molecules from the Lincs database, that can mimic or reverse a specific gene expression profile. As an input signature, we used significantly differentially expressed genes (DEGs) (Fold Change≥ 1.5, p value≤0.01), from RNA seq data from peripheral blood monocytes derived from 2 different groups of SLE patients. The first group included 7 SLE patients (6 females and 1male) with disease activity index (SLEDAI) higher than 8 (active disease), as well as, characterized by high IFN-α serum levels. The second group included 8 SLE patients (6 females and 2 males) with SLEDAI higher than 10 (active disease).

Results: We identified two compounds, that could reverse the gene expression profile of monocytes derived from lupus patients, while having the same protein target, the heat shock protein 90 (HSP90). HSP90 has been linked with SLE pathogenesis, since it is associated with the delivery of TLR7/9 receptors from the endoplasmic reticulum to early endosomes for ligand (nucleic-acid) recognition and therefore to IFN-α production in plasmacytoid dendritic cells from SLE patients or lupus-prone MRL/lpr mice.

Conclusions: Application of Lincs1000 can provide an unbiased approach into drug discovery in SLE, providing biologically meaningful drug targets such as Heat Shock Protein-90 (HSP-90).

AA17

ASSOCIATION OF THE COMMON ANKK1 AND TH GENE POLYMORPHISMS WITH RESPONSE TO ANTIPSYCHOTIC TREATMENT IN SCHIZOPHRENIA AND OTHER PSYCHOTIC DISORDERS

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Aim: The TaqIA single nucleotide polymorphism (SNP, rs1800497), located in the gene that codes for the putative kinase ANKK1 (ANKK1), near the termination codon of the D2dopamine receptor gene (DRD2), is the most studied genetic variation in a broad range of psychiatric disorders and addictions. The single nucleotide polymorphism (SNP, rs10770141), located in the proximal promoter of the TH gene, was associated with intellectual ability, personality traits and neuropsychiatric disorders. The aim of our study was to examine the association of the SNPs rs1800497 and rs10770141 with response to antipsychotic treatment of psychotic patients, in a naturalistic setting in Greece.

Methods: One hundred six patients suffering from schizophrenia and other psychotic disorders were included in the study. Dosages were normalized to chlorpromazine equivalents. A four-week follow-up period was considered for evaluating treatment response. Treatment efficacy was assessed using the Positive and Negative Scale. Clinical factors such as gender, age, age of onset and diagnosis were also recorded. The genotyping of the polymorphisms was achieved with PCR-RFLP and the data analysis was accomplished using SPSS.Genotype distributions and allele frequencies of each SNP were tested for association with the Positive and Negative Scale (PANSS base, PANSS bpos, PANSS bneg και PANSS bgen), its difference after a four-week follow-up period (PANNS pctdiff_30, PANSS dpospct7, PANSS dnegpct7 και PANSS dgenpct16) and the average daily administration of antipsychotic drugs, normalized to chlorpromazine equivalents (CPZeq).

Results: We have detected a trend of the rs10770141 TT genotype to associate with more intense general psychopathology symptoms (PANSSbase, general psychopatholgy).

Conclusion: Our finding highlights the potential importance of TH gene polymorphisms in the intensity of symptoms and it could bring an insight to elucidate the pathogenic mechanisms of schizophrenia.

AA18

STUDY OF NOVEL IMIDAZOPYRIDINE DERIVATIVES AS PUTATIVE PI3K INHIBITORS, EFFECTS ON THE AKT/MTORC1 SIGNALING AND CELL PROLIFERATION

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Objective: Pharmacological characterization of novel imidazopyridine analogues as PI3K inhibitors, in comparison to known inhibitors, emphasizing on their inhibitory effect on the Akt/PI3K/mTORC1 signaling and proliferation in cancer cell lines.

Methods: Standard chemical synthesis and procedures were used for synthesis of five aryl substituted imidazopyridine analogues with the code names 163, 110, 137, 109 and 124. Two human cancer cell lines were used, A549 and PC3. Pan-PI3K inhibitor Pictilisib and isoform-specific inhibitors A66 and TGX-221 were used as standard PI3K inhibitors. Sulforhodamine B Assay was used to quantify cell proliferation. Protein extracts were prepared for western blotting with antibodies against pAkt, pS6 and total Akt and S6. Apoptotic cell death was analyzed by FACS, with the Annexin-PI assay. Cell Microscopy was used to detect effects on cellular morphology.

Results: SRB Assay on both cell lines indicated partial reduction of proliferation (10-40%) with 124 being more potent. Pictilisib and A66 reduced growth rate to a greater extent (70-80%). Potential inhibitors as well as known PI3K inhibitors caused minor induction of apoptosis. Pictilisib and TGX-221, but not A66, caused changes in morphology in both cell lines with a progressive prominent appearance of presumably autophagic vacuoles. Tested compounds moderately reduced the phosphorylation of Akt and S6 at Ser473 and Ser235/236, with compounds 124 and 109 being more potent. Known inhibitors totally inhibited these phosphorylation's in A549 cells, while A66 showed a paradoxical increase of pAktSer473.

Conclusions: Our novel imidazopyridine analogues partially reduced cell growth in both cell lines tested. In addition, they modestly inhibited pAktSer473 and pS6235/236 upon short and long-term treatments showing a positive effect on signaling PI3K/Akt/mTORC1. PC3 were more sensitive to the tested compounds. Such differences might be due to the genetic background, as PC3 is a PTEN-null cell line All compounds caused only minor induction of apoptosis with compound 110 being the most potent. The appearance of autophagic vacuoles was detected for Pictilisib and TGX-221 but not for other compounds tested. Conclusively, tested compounds reduced the cell growth and dampen PI3K/Akt/mTORC1 signaling on both cell lines making them potential PI3K inhibitors.

AA19

RED BLOOD CELL-CONDITIONED MEDIUM FROM PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE CONTAIN INCREASED MCP1 AND INDUCE TNF-α RELEASE IN RAW 264.7 MACROPHAGE CELLS

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Aim: Despite their known role as active mediators in immunometabolic and inflammatory interactions, the putative array of cytokines and bioactive lipids released by erythrocytes has not been explored in the context of NAFLD. Our aim has been to investigate whether erythrocytes from NAFLD patients: I) Release different patterns of cytokines compared to healthy ones, ii) Induce pro-inflammatory phenotypes of RAW 264.7 macrophages, iii) Signal through bioactive lipids to macrophages.

Materials and Methods: 10 patients (4 men/6 women) aged 57,9 +/- 15,2 years with ultrasonography-validated hepatosteatosis, and 10 healthy controls (4 men/6 women) aged 39,3 +/- 15,4 years participated in our study.

Whole blood was centrifuged at 200 g for 10 minutes at 4oC. Plasma and buffy coat were removed. This step was repeated 4 times.

Erythrocytes (5X107/ml) or RAW 264.7 macrophages (passage 12-15, 2X105 cells/well in 6 well plate) were incubated in RPMI 1640, supplemented with 10% FBS, 1% streptomycin/penicillin, at 5% CO2, 37°C, for 24 hours. Patient- and healthy control-derived Red Blood Cell-Conditioned Medium (P-RBC-CM and H-RBC-CM) was collected with centrifugation at 200 g for 10 minutes.

RAW 264.7 macrophages were exposed to RBC-CM for 24 hours at 80% confluency.

IFNα, IL-1β, IL-12P40, IL-17A, TNF-α, CCL2, CCL4, CCL5, CXCL8 were studied using MILLIPLEX technology (Millipore, USA). VPC 23019, an antagonist for S1P receptors1,3 and AM966, an antagonist for LPA receptor1, (CAYMAN, chemicals, USA) were used at concentration of 10μM and 25mM respectively, after diluting with DMSO. DMSO, VPC23019 and AM966 had no effect on the cytokine profile released by RAW 264.7 macrophages.

Results: P-RBC-CM contain increased MCP1 ($p<0.05$), and induce TNFα release by RAW 264.7 ($p<0.05$) in comparison to H-RBC-CM.

Conclusion: Erythrocytes possibly contribute to NAFLD, releasing MCP1 and inducing the expression of TNF-α in macrophages, without the action of LPAR1 and S1PR1,3.

AA20

PRELIMINARY CHARACTERIZATION OF NOVEL QUINOLINE DERIVATIVES AND THEIR COMPLEXES WITH COPPER AS MULTIFUNCTIONAL COMPOUNDS FOR ALZHEIMER'S DISEASE

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There is urgent need for development of novel compounds for treatment of Alzheimer's disease (AD) due to limitations of acetylcholinesterase inhibitors and lack of clinical efficiency of several recently studied novel drugs and antibodies. Metal ions play a major role in all three main hypotheses of AD (amyloid, metal ion, oxidative stress hypotheses) and multifunctional drugs containing metal chelators represent a promising alternative therapeutic approach. This study investigated the neuroprotective effect of eight newly synthesized and characterized quinoline-copper complexes against Aβ peptide- and H_2O_2-toxicity and their possibility of interacting with acetylcholinesterase. Four novel copper chelators Hbpq, H_2dqpyca, H_2bqch and H_2bqen were synthesized using 8-aminoquinoline and 8-hydroxyquinoline as starting compounds. The structures of copper complexes and stability and possible interactions with culture medium components were studied by X-ray crystallography, IR spectroscopy and EPR spectroscopy. Non-differentiated and retinoic acid-differentiated SH-SY5Y cells and the MTS assay were used for assessing toxicity of compounds and complexes and neuroprotection against Aβ peptide- and H_2O_2-insults. X-ray and IR experiments showed that novel quinoline derivatives bind copper via oxygen and nitrogen donors albeit with different geometries. [Cu(bpq)Cl] copper is five-coordinated and has a distorted square-pyramidal geometry while [$Cu_2(\mu_2$dqpyca$)_2$] is a binuclear compound and each copper has an octahedral geometry. In [Cu(H_2bqch)Cl_2] and [Cu(H_2bqen)Cl_2] complexes, copper is six-coordinated with an octahedral geometry. EPR experiments suggested that metal chelators do not interact with culture medium components but some complexes such as [$Cu_2(\mu_2$dqpyca$)_2$] may be partially unstable in cell culture medium. Compounds Hbpq and [Cu(bpq)Cl] showed significant toxicity at 1-10μM and excluded from further study. All other compounds were non-toxic at concentrations ≤5μM. H_2dqpyca exhibited a significant neuroprotective effect against both Aβ peptide/H_2O_2-toxicity, while complex [Cu(H_2bqch)Cl_2] protected cells from H_2O_2-toxicity in a dose-dependent manner with an EC_{50} value of 80nM. Molecular docking simulations, using the acetylcholinesterase-rivastigmine crystal structure as a template, suggested an increased likelihood of interactions of H_2dqpyca and H_2bqch with acetylcholinesterase active site. In conclusion, we have identified two novel promising quinoline compounds, H_2dqpyca and H_2bqch, that should be developed further as multifunctional lead compounds aiming at acetylcholinesterase and the amyloid, metal ion, oxidative stress hypotheses of AD.

AA21

ANALYSIS OF AKT/GSK3/MTORC1 SIGNALLING PROFILES IN PERIPHERAL BLOOD CELLS FROM DRUG-NAÏVE FIRST-EPISODE-OF-PSYCHOSIS PATIENTS

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Introduction: Schizophrenia is a chronic psychiatric disease characterized by changes in many genes interacting with a host of exogenous factors. An ongoing research goal is focused on biomarkers for categorization and prediction of disease progression and antipsychotic drug responses. In addition to classical biomarkers, studies approaching schizophrenia at the systems biology level have highlighted the role of signaling pathways. A central signaling pathway associated with schizophrenia is the Akt/GSK3/mTORC1 pathway. However, despite genetic and biological validation in chronic schizophrenia patient panels and preclinical animal models, whether Akt/GSK3/mTORC1 signalling is deregulated at an early clinical stage as in drug-naïve schizophrenia patients at the time of diagnosis is unknown.

Aim: In the present report, we have conducted a pilot study investigating Akt/GSK3/mTORC1 pathway activity in peripheral blood mononuclear cells (PBMCs) of patients with first psychotic episode (FEP).

Materials and Methods: Thirty patients were recruited using standard criteria, blood samples were obtained and PBMCs were isolated. Blood samples from healthy volunteers were processed in parallel. PBMCs protein extracts were prepared and processed for western blotting with antibodies against phosphorylated Akt, GSK3 and S6.

Results: Our results indicate dysregulation of Akt, GSK3 and mTORC1 signalling activities in PBMCs from FEP patients compared to healthy controls. Mostly evident at the level of mTORC1 and ribosomal S6K, as phospho-S6 is significantly decreased in FEP patients ($p<0.05$, Mann-Whitney test).

Conclusions: These preliminary findings corroborate post-mortem studies showing decreased mTORC1 activity in chronic schizophrenia patients and further suggest that peripheral mTORC1 signalling is decreased in drug naïve FEP patients.

AA22

ROLE OF ISOTRETINOIN ON THE APPEARANCE AND PROGRESSION OF PSYCHIATRIC DISORDERS

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Aim: Isotretinoin (IT) is the only treatment that affects all the major etiological factors involved in acne. It achieves this remarkable efficiency by influencing cell cycle evolution, cell differentiation, cell survival and apoptosis. However, there is evidence from case reports that isotretinoin, and other retinoids, may be associated with the development of depression and major psychiatric disorders. In this direction, we present a study of mechanistic and pilot studies analysis on the potent effects of isotretinoin towards the direction of severe psychiatric disorders.

Methods: Literature research and review of articles relevant with the topic, in digital databases: PubMed, Scopus, Science Direct, using “Isotretinoin”, “Mental disorders”, “Major depressive disorders”, “Bipolar disorder”, “Schizophrenia” as keywords.

Results: Isotretinoin acts in the hypothalamus, where the synthesis of retinoic acid and its receptors have been found, such as those involved in the synthesis of corticosteroid hormone. In the mechanistic part it appears to be involved and the dopamine and serotonin system with IT to be involved in the reduction of their levels in the synaptic space.

There have been 4,992 reported cases of various psychiatric side effects due to consumption of IT during the period 1982 to 2004 in the United States. It has been found that 11% of patients report feeling depressed after 4 months of isotretinoin treatment with both low and high dose of IT. However, there is some controversy towards the above mentioned findings that support that there is no real cause-and-effect relationship between IT and depression, since acne causes anxiety and depression, while treating acne with isotretinoin is a way to treat depression through improved appearance and positive behaviors.

Conclusion: The data described in this report tend to demonstrate the association between isotretinoin use and psychopathology. Interestingly, isotretinoin is the only non-psychotropic drug on the FDA's list of the top 10 depression-related drugs. The obvious benefit of isotretinoin in the treatment of acne should determine its continuation. Patients and their relatives should be informed and encouraged to report any depressive symptoms immediately. Therefore, health professionals should be vigilant for possible psychiatric side effects after treatment with isotretinoin, especially in vulnerable populations.

AA23

ONCOSTATIN M AND ITS RECEPTORS ARE EXPRESSED IN HUMAN SUBEPITHELIAL MYOFIBROBLASTS AND POSSIBLY IMPLICATED IN THE PATHOGENESIS OF INFLAMMATORY BOWEL DISEASES

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Aim: Oncostatin M (OSM) has been implicated in the pathogenesis of Inflammatory Bowel Disease (IBD), as both OSM and its receptor, are upregulated in inflamed colonic regions of IBD patients. Moreover, high OSM expression has been associated with failure to respond to anti-TNF. Our aims were to investigate whether OSM and its receptors, OSMR, and gp-130 are expressed in primary subepithelial myofibroblasts (SEMFs) from healthy individuals and patients with Crohn's Disease (CD) or Ulcerative Colitis (UC), to examine whether this expression is regulated by the innate cytokines, IL-1α and TNF-α and to study the effect of OSM on the pro-fibrotic phenotype of SEMFs.

Methods: Primary SEMFs were isolated from endoscopically obtained colonic biopsies from healthy individuals, CD and UC patients. Total RNA was extracted from isolated SEMFs. Healthy SEMFs were stimulated with 5ng/ml IL-1α and/or 50ng/ml TNF-α for 6 hours and total RNA was collected. Healthy SEMFs were stimulated with 5ng/ml IL-1α and 50ng/ml TNF-α for 24h, then with 100ng/ml OSM for another 6h and total RNA was collected. mRNA transcripts for OSM, OSMR, LIFR, gp-130, collagen type I, III and fibronectin were measured by reverse transcription quantitative PCR.

Results: Unstimulated SEMFs had a basal expression of both OSM and its receptor subunits. Compared to controls, CD SEMFs overexpressed OSM (4.81-fold, 2.1-7.08 $p<0.05$), but not its receptor, whereas UC SEMFs overexpressed both subunits of its receptor (OSMR: 2.89-fold, 2.04-3.68, $p<0.05$; gp130: 2.99-fold, 1.72-3.81, $p<0.05$) and not OSM. Regarding IL-1α or TNF-α stimulations, they induced a statistically significant upregulation of OSM (IL-1α+TNF-α: 13.42-fold, 11.32-17.16; $p<0.0001$), OSMR (IL-1α+TNF-α: 2.06-fold, 1.83-2.34, $p<0.0001$), and gp-130 (IL-1α+TNF-α: 1.59-fold, 1.31-2.51; $p<0.01$). Finally, healthy SEMFs overexpressed collagen type I (4.6-fold, 2.86-7.04; $p<0.05$), III (3.48-fold, 2.21-6.38; $p<0.05$) and fibronectin (2.75-fold, 1.99-5.22; $p<0.05$) when OSM was added in the medium, after the stimulation of IL-1α/TNF-α.

Conclusion: Our results show that OSM and OSMR are expressed on primary human SEMFs and are upregulated in pathological situations and following pro-inflammatory stimuli. OSM can induce the expression of pro-fibrotic factors in SEMFs. These data further support a potential role of this system of inflammatory mediators in the pathogenesis of intestinal inflammation and fibrosis.