

Workshop 1: Hepatology/Metabolism/Life Style

P1.01

Higher free triiodothyronine is associated with NAFLD in euthyroid subjects: the lifelines cohort study

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Background: Non-alcoholic fatty liver disease (NAFLD) is becoming the leading cause of chronic liver disease in the Western world. Thyroid hormones interact on hepatic lipid homeostasis. We determined associations of thyroid hormone parameters with NAFLD in euthyroid subjects.

Methods: The study was conducted in the LifeLines Cohort Study ($N = 167,729$), a population-based cohort study in the north of The Netherlands. Euthyroid subjects (thyroid-stimulating hormone (TSH) 0.5–4.0 mU/L, free thyroxine (FT4) 11–19.5 pmol/L and free triiodothyronine (FT3) 4.4–6.7 pmol/L) >18 years were included. Exclusion criteria were participants with missing data, excessive alcohol use, known hepatitis or cirrhosis, liver functions \geq three times the upper limit, current cancer, non-white ancestry, previous or current use of thyroid medication and current use of lipid and glucose lowering medication. A priori defined liver biochemistry, thyroid function parameters and metabolic syndrome (MetS) were studied. NAFLD was defined by using the Fatty Liver Index (FLI); FLI ≥ 60 was categorized as NAFLD. A $P < 0.01$ was considered significant.

Results: FLI ≥ 60 was found in 4274 (21.1%) of 20,289 individuals (62.1% male, median age 46 years) with increased prevalence of MetS ($P < 0.0001$). In age- and sex-adjusted analysis FLI ≥ 60 was independently associated with a higher FT3 (OR 1.34, 95% CI 1.29–1.39, per SD increment, $P < 0.0001$) and a lower FT4 (OR 0.73, 95% CI 0.70–0.75, $P < 0.0001$) but not by TSH. The strongest association was found for the FT3/FT4 ratio (OR 1.44, 95% CI 1.39–1.49, $P < 0.0001$). These associations remained similar after additional adjustment for the MetS. In subjects with enlarged waist circumference, TSH and FT4 were lower while FT3 was higher, resulting in an increased FT3/FT4 ratio ($P < 0.0001$).

Conclusion: Euthyroid subjects with suspected NAFLD are characterized by higher FT3, lower FT4 and higher FT3/FT4 ratio, probably consequent to central obesity.

P1.02

GlycA, a novel pro-inflammatory glycoprotein biomarker, and high sensitivity C-reactive protein are inversely associated with sodium intake after controlling for adiposity: PREVENT study

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Background: The extent to which dietary sodium intake may confer alterations in inflammatory status is unclear. GlycA is a novel pro-inflammatory proton nuclear magnetic resonance spectroscopy biomarker which associates with the development of cardiovascular disease and diabetes.

Objective: We determined associations of the inflammatory markers GlycA and high sensitivity C-reactive protein (hsCRP) with 24-h sodium excretion.

Design: A cross-sectional population-based study was performed among 3,935 subjects, not using anti-hypertensive medication, lipid lowering drugs or glucose lowering treatment. Urinary sodium excretion was calculated as the mean of two 24-h urine excretions. Linear regression models were used with 24-h sodium excretion as an independent variable and GlycA or Log_e hsCRP as dependent variables.

Results: Mean sodium excretion was 143.0 ± 53.4 mmol/24-h. GlycA was 343.6 ± 58.7 μ mol/L and hsCRP (geometric mean, 95% CI) was 1.20 (1.16, 1.25) mg/L, respectively. In age- and sex-adjusted analyses, GlycA and Log_e hsCRP were not significantly associated with 24-h sodium excretion (B: 1.23 (95% CI: -0.67, 3.13), $P = 0.21$ and 0.03 (95% CI: -0.004, 0.07), $P = 0.08$, respectively per 1 SD increase). After additional adjustment for body mass index (BMI), both GlycA (B: -2.76 (95% CI: -4.65, -0.86), $P = 0.004$) and Log_e hsCRP (B: -0.07 (95% CI: -0.11, -0.04), $P < 0.001$) were inversely associated with 24-h sodium excretion. These associations were similar if adjustment was performed for waist circumference instead of BMI, or if additional adjustment was performed for relevant clinical and laboratory variables, and were particularly present in men.

Conclusion: The pro-inflammatory biomarkers, GlycA and hsCRP, are inversely related to higher 24-h sodium excretion when taking into account variation in adiposity. These inverse relationships remain present after taking account of other covariates.

P1.03

The influence of anti-adalimumab antibodies on adalimumab through levels, TNF- α levels and clinical outcome

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Introduction: There is increasing evidence on the role of low through levels and the development of anti-TNF- α antibodies for the occurrence of loss of response to Infliximab therapy in patients with Crohn's Disease (CD). To date, there are limited data on the role of Adalimumab (ADA) through levels and anti-ADA antibodies (AAA) for the management of CD patients.

Aim: We assessed the role of AAA on ADA through levels, TNF- α concentrations, PCR value and clinical outcome.

Methods: In this prospective observational cohort study, performed at a single tertiary referral center, 23 [14M/9F; mean age 41 (range 21–66)] infliximab-naïve patients with CD achieving disease remission and in maintenance treatment with ADA were included in a follow-up program. Blood samples were drawn at standardized time points just before ADA injection. Trough serum concentration and antibodies against ADA were measured using an homogenous mobility shift assay. Blood samples were considered positive for AAA presence if they were ≥ 1.7 U/mL and for ADA if they levels were ≥ 5 $\mu\text{g/mL}$. Disease activity was assessed at the same points by means of biochemistry and Harvey-Bradshaw Index.

Results: We have data from 189 blood samples. AAA were observed in 42/189 (22.2%) samples, and 16/42 (38.1%) had levels of AAA ≥ 1.7 U/mL. ADA through levels were found in 183/189 (96.8%) samples, and 168/183 (91.8%) had a value of drug levels ≥ 5 $\mu\text{g/mL}$. Overall, 5/23 (21.7%) patients had AAA and 22/23 (95.6%) were positive for ADA levels. Blood samples with AAA had lower ADA through levels [7.54 (0–26.49) vs. 9.45 (0.14–23.62); $P = 0.002$] and higher TNF- α concentrations [5.9 (4.1–11.5) vs. 3.6 (0–27.2); $P = 0.0007$] than blood samples without evidence of AAA. Moreover, patients with blood samples positive for AAA reported HBI values higher compared to patients without evidence of AAA [10 (3–17) vs. 5 (2–17); $P < 0.0001$].

Conclusion: Development and presence of AAA influence ADA through levels and TNF- α concentrations in CD patients during maintenance treatment with ADA.

P1.04

TNF- α levels strongly correlated with disease activity based on HBI and CDEIS in patients with Crohn's disease in maintenance treatment with adalimumab

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Introduction: In the last few years the therapeutic paradigm of Crohn's disease (CD) has changed dramatically thanks to biological drugs. We must consider the pivotal role of tumor necrosis factor-alpha (TNF- α), a pro-inflammatory cytokine, in the pathogenesis and relapse of CD. High levels of TNF- α have been associated with the development of intestinal inflammation in CD and blocking this cytokine with anti-TNF- α molecules may result in mucosal healing. In addition several studies have shown increased TNF- α levels in the serum and in the intestinal mucosa of patients with CD. However, little is known about the

course of TNF- α levels and their relationship with disease recurrence in CD patients during maintenance treatment with Adalimumab.

Aim: We assessed TNF- α levels in patients with CD who were in maintenance treatment with ADA and correlated them with clinical and endoscopic disease activity.

Methods: In this prospective observational cohort study, performed at a single tertiary referral center, 23[14M/9F; mean age 41 (range 21–66)] infliximab-naïve patients with CD in maintenance treatment with ADA were included and followed-up. Blood samples were drawn at standardized time points, just before ADA injection. Antibodies against ADA (AAA) were measured using an homogenous mobility shift assay. Blood samples were considered positive for AAA presence if ≥ 1.7 U/mL. Disease activity was assessed at the same points by means of the Harvey-Bradshaw Index. Moreover, endoscopic activity was assessed at baseline and at the time of relapse by means of CD endoscopic index.

Results: We have data from 133 blood samples. AAA were observed in 26/133 (19.5%) samples, and 10/26 (38.5%) had a value of AAA ≥ 1.7 U/mL. TNF- α levels were present in all samples assessed (Mean 4.4, range 0–27.2). Per-patient median TNF- α levels were strongly correlated with median HBI scores ($r^2 = 0.702$, $P < 0.0001$). Moreover, TNF levels were also correlated with CDEIS ($r^2 = 0.350$, $P = 0.001$).

Conclusion: TNF- α levels strongly correlated with disease activity based on HBI and CDEIS indices in patients with CD in maintenance treatment with ADA.

P1.05

Influence of adenoviral transduction with Adv5-optHGF-RFP on phenotype of hepatic stellate cells

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Hepatic stellate cells (HSC) play an important role in liver development and differentiation of progenitor cells into hepatocytes. This becomes possible due to creation of specific microenvironment, they produce extracellular matrix components and an amount of growth factors: fibroblast growth factor 4 (FGF4), stem cell growth factor (SCF) and the key factor - hepatocytes growth factor (HGF), responsible for cells migration, proliferation and differentiation into hepatocytes. There are various ways to deliver these factors into the cells and adenoviruses a promising gene delivery vector that has a high efficiency and relative ease of construction. These advantages make this system attractive for diverse research applications. In this project we performed transduction of hepatic stellate cells (HSC) with adenoviral vector containing hepatocytes growth factor (HGF) and red fluorescent protein (RFP) as a reporter (Adv5-optHGF-RFP), which let us to visualize the transduced cells. Further changes of phenotype were studied by real-time PCR and immunocytochemical staining 3, 7, 10, 14 and 21 days of cultivation. According to our results transduction of HSC with adenovirus is the simple and effective method of growth factor gene delivery, that was confirmed by stable RFP fluorescence and high expression of HGF (1200 times higher than in control HSC culture). Transduction of HSC with Adv5-optHGF-RFP lead to their activation and increase of desmin, α -SMA expression, didn't change the morphology and proliferation of the cells (Ki-67 staining) and induced hepatogenic differentiation (appearance of α -FP). Interestingly, expression of HGF and α -

SMA, expressions of desmin and α -FP had the similar dynamics, probably, they are interrelated. On the 21st day of the experiment all expression levels gradual return to normal meanings. Adenoviral transduction of HSC is applicable for short time stimulation of genes expression and probably can be used for the study of liver regeneration by transplantation of gene-modified cells.

P1.06

Ghrelin release by carbonated beverages: the detrimental effects of soft drinks revisited

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The dangerous health risks associated with obesity makes it a very serious public health issue. Numerous studies verified a correlation between the increase in obesity and the parallel increase in soft drink consumption among world populations. The effects of one main component in soft drinks namely the carbon dioxide gas has not been studied thoroughly in any previous research. Here, we show that rats consuming gaseous beverages over a period of around 1 year gain weight at a faster rate than controls on regular degassed carbonated beverage or tap water. This is due to elevated levels of the hunger hormone ghrelin and thus greater food intake in rats drinking carbonated drinks compared to control rats. Moreover, an increase in liver lipid accumulation of rats treated with gaseous drinks is shown opposed to control rats treated with degassed beverage or tap water. In a parallel study, the levels of ghrelin hormone were increased in 20 healthy human males upon drinking carbonated beverages compared to controls. These results implicate a major role for carbon dioxide gas in soft drinks in inducing weight gain and the onset of obesity.

P1.07

Silybin-vitamin E phytosome complex counteracts lipid excess and oxidative stress in an *in vitro* model of non alcoholic steatohepatitis (NASH)

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Background and aim: Non-alcoholic fatty liver disease (NAFLD) is a major cause of liver-related morbidity and mortality. Oxidative stress, mitochondrial dysfunction and release of pro-inflammatory cytokines, such as tumor necrosis factor (TNF)- α , are major consequences of excess hepatic lipid overload and can contribute to the progression of NAFLD to the more aggressive non-alcoholic steatohepatitis (NASH). Definitive treatments for NAFLD/NASH are lacking so far. Silybin, the natural extract of the milk thistle seeds, has previously shown beneficial effects in NAFLD.

Methods: We tested the effects of silybin on an *in vitro* model of NASH obtained by incubating rat hepatoma FaO cells with

oleate/palmitate for 3 h to mimic liver steatosis (SS), and then with 10 ng/mL TNF- α for 24 h to mimic the progression to NASH (SH). Silybin was subsequently incubated for 24 h (50 μ mol/L as phytosome complex with vitamin E, *Realsil*®, IBI, Italy). The effects of silybin on lipid accumulation and metabolism, and on indices of oxidative stress and mitochondrial dysfunction were evaluated by absorption and fluorescence microscopy, Seahorse XF Analyzer assay, quantitative real-time PCR, Western blot, spectrophotometric and fluorimetric assays.

Results: Silybin significantly reduced lipid accumulation in NASH cells likely by promoting lipid catabolism and by inhibiting lipogenic pathways. The effects of silybin were associated with improved steatotic oxidative imbalance, as demonstrated by the reduction in free radical content, lipid peroxidation, catalase activity and nuclear factor kappa-B (NF- κ B) activation. Silybin had beneficial effects also on some mitochondrial parameters, such as the electron transport chain and oxidative phosphorylation, mtDNA defects, and mitochondria number.

Conclusions: Silybin shows a direct anti-steatotic and anti-oxidant effects in an *in vitro* model of NASH. This study further elucidates the complex cellular mechanisms of silybin protection seen in prior animal and clinical studies.

P1.08

Dietary plant polyphenols tune down hepatosteatosis and atherosclerosis in cellular models of diseases

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Background and aim: Recently, there has been an increasing interest in potential health benefits of dietary plant polyphenols (PP). Some PP could prevent/improve obesity and metabolic-related disorders such as non-alcoholic fatty liver disease (NAFLD). Olive oil contributes to human health in the Mediterranean area. Although its beneficial effects have historically been attributed to the monounsaturated oleic acid, also PP can be beneficial bioactive constituents.

Methods: Rat hepatoma (FaO) and human endothelial (HECV) cells exposed *in vitro* to oleate/palmitate mixture for 3 h mimic NAFLD and atherosclerosis, respectively. Lipid-loaded cells were treated with a polyphenolic extract from olive pomace (PEOP) or with the single PP of the extract (tyrosol, apigenin, oleuropein, p-coumaric and caffeic acid) for 24 h. Then, markers of lipid metabolism were assessed in hepatic cells, peroxisome proliferator-activated receptors (PPARs) and stearyl-CoA desaturase 1 (SCD-1), and markers of inflammation were assessed in endothelial cells, intercellular adhesion molecule-1 (ICAM-1), nuclear factor kappa-B (NF- κ B), nitric oxide (NO) release and wound-healing rate. Absorption microscopy, quantitative real-time PCR, Western blot, spectrophotometric and fluorimetric assays were employed.

Results: PEOP extract exerted a significant lipid-lowering action in lipid-loaded hepatocytes, but not in endothelial cells. Markers of fat-induced oxidative stress were significantly reduced in both cell lines. Distinct effects were observed for PP composing the PEOP extract.

Conclusions: The polyphenolic extract ameliorated hepatic lipid accumulation, through a modulation of lipid metabolism, especially by stimulation of triglyceride secretion. Moreover, PEOP was able to rescue the fat-dependent oxidative imbalance in both hepatic and endothelial cells. Potential applications of PEOP are anticipated as natural therapeutic agents tuning down both hepatosteatosis and atherosclerosis.

P1.09

Dyspeptic symptoms, gastrointestinal dysmotility and altered sympathetic function in chronic alcoholics

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Background and aim: Acute and chronic alcohol consumption may affect gastrointestinal functions but a comprehensive and simultaneous analysis on motility of different gastrointestinal tracts, dyspeptic symptoms and autonomic neuropathy in chronic alcoholics is lacking, so far. This study was aimed to explore these aspects in chronic alcoholics, also considering the effects of one year-abstinence from alcohol.

Methods: Dyspeptic symptoms (validated questionnaires), gallbladder and gastric motility (ultrasonography), oro-cecal transit time (lactulose H₂-breath test), stool form score (indirect marker of colonic transit) and autonomic neuropathy (sweat spot test for sympathetic- and Valsalva R-R ratio for parasympathetic function) were assessed in 136 chronic alcoholics and 132 healthy controls. In a subgroup of 39 patients the tests were repeated after one year abstinence.

Results: Chronic alcoholics had higher dyspeptic scores, delayed postprandial gastric emptying (i.e. increased half-emptying time) and longer oro-cecal transit time but faster gallbladder emptying with slightly accelerated colonic transit, as compared with controls. In patients, postprandial fullness was associated with impaired gastric emptying. Altered sympathetic- but not parasympathetic autonomic function was present. Upper endoscopy was positive (mild esophagitis/gastritis) in 27% of cases but all explored indices were similar in patients with normal or abnormal endoscopy.

Dyspeptic symptoms and functional alterations of gastric emptying and oro-cecal transit tests were still present after one-year abstinence, whereas gallbladder motility, stool form score and sympathetic function improved.

Conclusions: Chronic alcoholics exhibit combined presence of dyspeptic symptoms, impaired motility at different levels of the gastrointestinal tract and altered sympathetic function. Only few of these abnormalities improve after one year of abstinence from alcohol.

P1.10

Melanin produced by yeast *Pseudonadsoniella brunnea* as novel therapeutics agents in NAFLD/NASH management

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Antioxidant therapy is necessary for successful treatment of the liver injury. We have paid attention to Melanin (M) produced by yeast *Pseudonadsoniella brunnea* as novel antioxidant and anti-inflammatory agents with low toxicity. In current study we aimed to investigate the preventive effect of M on the monosodium glutamate (MSG) induced NAFLD model in rats.

The study was carried out on 45 Wistar rats. 3 groups: intact, MSG- and MSG+ M ($n = 15$ in each group). Newborn rats of MSG- and MSG+ M groups were administered with MSG (4 mg/g) at 2nd-10th days of life. Since the age of 1 month, rats of MSG-group were treated with water (0.25 mL/100 g), rats of MSG+M groups – with M (1 mg/kg) dissolved in water. Introduction had been performed intermittently (2-week courses alternated with 2-week breaks) for 3 months. In 4-month rats anthropometrical parameters and VAT mass were estimated. To assess morphological changes in liver we used NAS (NAFLD activity score). Lipid extraction from liver was performed according to Folch. The content of interleukins were measured by ELISA.

We found significantly lower total score, degree of steatosis and manifestation of lobular inflammation due to NAFLD activity score in MSG+M group compared to MSG-obesity. NASH we confirmed only in 33% of rats with MSG-obesity that was significantly higher than after M (6-7%) administration ($P = 0.033$). M administration reduce total lipids and triglycerides content in liver approximately by 22–25% ($P < 0.001$) and amount of visceral fat on 40% ($P < 0.001$) as compared to MSG-obesity group. M reduced the content of IL-1 β in rat serum and restored the level of anti-inflammatory cytokines (IL-10, TGF- β) to the control values.

Thus, the administration of M can prevent development of NAFLD/NASH in rats with MSG-induced obesity and can be considered as possible novel therapeutic agents but further studies to confirm its action needed.

P1.11

Metabolic and vascular impact of a pre-surgery ketogenic diet in morbid obese patients

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Background and aims: The "Very Low Carbohydrate Ketogenic Diet" (VLCKD), often used in pre- bariatric surgery, can be effective in the treatment of obesity. This is due to effect of reduction in lipid layer engravement and reduction in hepatic steatosis. The main purpose of this study is to investigate the anthropometric and metabolic changes after VLCKD, particularly glico-lipidic pattern, the grading of liver steatosis and flow mediated vasodilation (FMV).

Methods: Thirty-seven obese patients with BMI $45 \pm 9 \text{ kg/m}^2$ were enrolled in the study and submitted to a VLCKD ($\leq 800 \text{ kcal}$, and a daily carbohydrate intake $< 50 \text{ g}$) for approximately 3 weeks before the bariatric surgery. At the baseline and after 3 weeks the following parameters were evaluated: body mass index (BMI), blood pressure, glycemia, insulinemia, HOMA-IR, lipid pattern, GOT, GPT, γ GT; FMV, visceral fat area (VFA), and liver steatosis (these all measured by ultrasonography).

Results: Patients showed a significant reduction in BMI (45.9 to 43.8 kg/m^2 $P = 0.012$). Notably there was a decline of the visceral fat area from $258 \pm 76 \text{ cm}^2$ to $230 \pm 77 \text{ cm}^2$ ($P = 0.000$). Metabolic parameters improved with the reduction of glycemia ($P = 0.009$), insulin ($P = 0.007$), HOMA index ($P = 0.003$), triglycerides ($P = 0.003$), LDL cholesterol ($P = 0.000$); also, γ GT were reduced from 42 ± 36 to $27 \pm 15 \text{ U/L}$ ($P = 0.001$). An improvement in the grading of liver steatosis was also observed ($P = 0.001$). Nevertheless, HDL-C showed a significant reduction ($P = 0.001$) and mean FMV values did not improve (12 ± 6 to $12 \pm 5 \%$), although a significant increase in FMV values was observed in those patients with higher insulin values.

Conclusion: VLCKD demonstrated clear benefits in terms of anthropometric and metabolic parameters; the major vascular benefit seems to emerge in those with a worse metabolic pattern, but further studies are needed to investigate the role of VLCKD on endothelial vasoactivity.

P1.12

G protein-coupled receptor 30 (GPR30), a novel estrogen receptor, plays an independent role in the pathogenesis of estrogen-induced cholesterol gallstones in female mice

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Background: Gallstone prevalence is significantly higher in women than in men. The classical estrogen receptor α (ER α), but not ER β , in the liver plays a critical role in estrogen-induced gallstones in female mice. Understanding the molecular mechanisms underlying the lithogenic role of estrogen in gallstone formation became more complicated with the identification of GPR30 as a new gallstone gene *Lith18*. Our aim was to explore whether GPR30 produces an independent lithogenic action, or works in conjunction with ER α , to promote estrogen-induced gallstones in female mice.

Methods: The biliary and gallstone phenotypes were investigated by physical-chemical methods in ovariectomized (OVX) female GPR30^(-/-), ER α (-/-), and WT mice intramuscularly injected daily with the potent GPR30-selective agonist G-1 at 0 or 200 ng/day and fed a lithogenic diet for 8 weeks.

Results: GPR30 activation by G-1 enhanced cholelithogenesis by suppressing cholesterol 7 α -hydroxylase (CYP7A1), the rate-limiting enzyme for the classical pathway of bile salt synthesis. This abnormality led to biliary cholesterol hypersecretion associated with reduced bile salt secretion, causing cholesterol-supersaturated bile and accelerating cholesterol crystallization. Gallstone prevalence was 80% in OVX ER α (-/-) and WT mice treated with G-1 compared to 10% in OVX WT mice receiving no G-1. Moreover, G-1 treatment did not influence gallstone formation in OVX GPR30 (-/-) mice. G-1 treatment increased expression of the epidermal growth factor receptor (EGFR) and reduced expression of liver receptor homolog-1 (LRH-1), coupled with decreased expression of CYP7A1 in OVX ER α

(-/-) and WT, but not GPR30 (-/-) mice. *In vitro* studies using cultured primary hepatocytes isolated from ER α (-/-) and WT mice showed that when EGFR was inhibited by AG1478, a highly potent EGFR kinase inhibitor, expression of CYP7A1 was unchanged even though GPR30 was activated by G-1.

Conclusions: GPR30 plays an independent role in the pathogenesis of estrogen-induced gallstones in female mice.

P1.13

Clinical, cytological and histological profile of a large cohort of patients with thyroid nodule living in Apulia

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Background and aims: Thyroid nodules are very common in the general population. The diagnostic approach includes the ultrasound examination (US) for detecting features of suspicion of malignancy. However, only the Fine-needle aspiration biopsy (FNAB) might discriminate between benign and malignant disease for selecting patients undergoing surgery. The aim of our study was to profile a large cohort of patients with thyroid nodules according to their ultrasonographic, cytological and histological features.

Methods: In a tertiary referral center, from January 2016 to November 2016, 1066 consecutive subjects (M:F = 262:804, age $51 \text{ years} \pm 0.4 \text{ SE}$) with solitary and multiple thyroid nodules underwent clinical evaluation, US examination, US-guided FNAB, and surgery when appropriate.

Results: According to sonographic patterns, 18/1066 showed high suspicion of malignancy, 633/1066 intermediate suspicion, 396/1066 with low suspicion, 19/1066 benign. According to ATA 2015 guidelines, 373/1066 subjects (35%) (18 with high suspicion, 280 with intermediate, and 75 with low) performed FNAB. According to cytological results, 22 (6%) were TIR1; 209 (56%) TIR2; 104 (28%) TIR3 (TIR3A = 62, TIR3B = 42); 14 (4%) TIR4; 24 (6%) TIR5. Based on clinical, US and FNAB features, 127 subjects underwent surgery. Thyroid cancer was found in 56% of surgical patients. According to the overall subjects, prevalence of cancer was 12%.

Conclusion: US-guided FNAB has a high accuracy in detecting suspicion patterns and influences clinical decisions in managing patients. However, the high prevalence of nodules with undetermined significance (TIR3) means that it is necessary to use additional techniques in order to avoid unnecessary surgeries.

P1.14

Therapy with synbiotic "OPEFERA" prevents the development of experimental obesity

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The search of new non-toxic drugs for preventing the development of obesity is the most important challenge of modern science. The question about impact of probiotics and prebiotics on fat metabolism and obesity is being actively debated in the scientific literature. So the aim of the study was to investigate

the effect of synbiotic "OPEFERA" (SO) on development of experimental obesity.

The study was carried out on 36 white rats, that were divided into 6 groups (I-III – males, IV-VI – females). I and IV groups were intact control (4-month old). Newborn rats of groups II and III s.c. in volume 8 $\mu\text{L/g}$ were administered a saline or monosodium glutamate (MSG) (4 mg/g) at 2–10 days of life. Since the age of 1 month, rats of III and V group had been injected with water, rats III and VI groups - SO (World Medicine) in a dose of 1.94×10^9 KOE+2.9 mg/inulin. Introduction had been performed with 2-week course for 3 months.

In male rats, there were more pronounced changes - body weight and visceral fat exceeded benchmarks in 3 and 5 ($P \leq 0.001$) times, respectively. Body weight and visceral fat of female rats in group III was higher by 125% and 338% ($P \leq 0.001$), respectively. It was established that under condition of obesity caused by the introduction of MSG, the level of adiponectin in serum decreased in male rats by 59% and 23% ($P \leq 0.05$) in females compared with intact rats. The use of SO therapy led to recovery of adiponectin level: its concentration in serum grew in 1.9 times in males and in 1.4 times ($P \leq 0.05$) in females which were treated with probiotic compared with rats injected with placebo.

Thus, the introduction of SO increased adiponectin levels in animals injected with MSG, that shows the effectiveness of probiotic therapy for the prevention of obesity.

P1.15

Genetically modified with HGF and FGF-4 genes hepatic stellate cells enhances transplanted cells repopulation in rat liver after acute liver damage

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Cell therapies, which are based on using regional stem cells for disease treatment, are under active development. In recent years hepatic stellate cells (HSC) are considered to be hepatic stem cells. Furthermore, genetic modification of cells is considered as an approach that could increase the therapeutic potential of transplanted cells.

We aimed to study the role of genetically modified HSCs (gm-HSCs) on liver regeneration after transplantation into the rats after partial hepatectomy (PH) – classical model of acute liver damage. Genetic modification of HSC was carried out by using adenoviral vector, which contained hepatocyte and fibroblast growth factors (HGF, FGF-4) and red fluorescent protein (RFP). Gm-HSCs were injected into rat portal vein during the PH (experimental group, EG). Control group (CG) of animals received the same cells without PH. The animals were sacrificed on 1, 3, 5, 7, 14, 28 days after the HSCs transplantation. Paraffin slices were stained by immunohistochemistry with antibodies to RFP, HGF, FGF-4, desmin – HSC marker and α -SMA – myofibroblast marker.

RFP, HGF and FGF-4 expression were detected in both groups even at first days after transplantation: small oval-shaped and hepatocyte-like cells were observed near the portal tracts. Maximal number of such cells was found on the 5th day after transplantation in both groups, but in the EG average number of cells was higher. Coincidentally, the intensity and quickness of hepatocyte repopulation in liver were higher in the EG. Number of

desmin+cells increased more in the EG. In both groups α -SMA+cells were not detected. It is interesting that the number of RFP+cells was much more than we observed earlier after native HSCs transplantation.

We conclude that gm-HSCs save their viability after transplantation into the rat after PH, migrate, integrate into the damaged liver parenchyma and increase hepatocyte repopulation without the risk of liver fibrosis.

P1.16

A case of rare pancreatic lesions: groove pancreatitis

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Groove pancreatitis is a rare cause of chronic pancreatitis that affects the groove area (i.e., the anatomic space between the head of pancreas, duodenum and common bile duct). The lesion can mimic or coexist with pancreatic carcinoma, and is still a diagnostic challenge for clinicians. We describe a case of a 43 year old man with recurrent epigastric pain and weight loss (7 kg) in the last 6 months. The medical history included hiatal hernia and gastroesophageal reflux disease. No alcohol abuse was recorded (daily alcohol assumption 15 g). Biochemical tests were normal. A prior abdominal ultrasonography suggested a mass of the pancreatic head; abdominal CT revealed dishomogeneity and increased volume and a 3 cm hypodense area of the pancreatic head. PET TC showed moderate increase of glucose metabolism at the head of the pancreas associated with hypermetabolic lymphadenopathy. The abdominal MR revealed cystic structures in the space between duodenum and pancreas, and duodenal wall thickening. An endoscopic ultrasonography with biopsy was performed, and histology diagnosed a picture of chronic pancreatitis. A final diagnosis of groove pancreatitis was made and the patient was treated conservatively. Conclusion: groove pancreatitis should be considered in the differential diagnosis of patients presenting with pancreatic head lesions. The diagnosis requires a complete workup to correctly manage this rare condition.

P1.17

A novel cellular model to study the combined actions of fructose and fatty acids in inducing a steatohepatitis condition

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Background and aim: Fructose is widely employed as sugar additive in processed foods and beverages. Excess dietary fructose is acknowledged as a leading cause of dyslipidemia, insulin resistance, obesity, liver steatosis. Clinical and animal studies point to the close relationship between excessive fructose consumption and non-alcoholic fatty liver disease (NAFLD). We

developed a cellular liver model to study the combined effects of fructose and fatty acids (FAs) on lipid metabolism and to further dissect subtle and complex pathogenetic mechanisms at the heart of the deleterious effect of excessive fructose consumption. **Methods:** Hepatoma FaO cells were exposed to 5.5 mM fructose for 72 h and then to 0.75 mM oleate/palmitate for 3 h. We verified cell viability (resazurin and sulforhodamine B-SRB assays), lipid accumulation (intracellular triacylglycerol quantification and neutral lipid staining), and cellular apoptosis (caspase 3-like activity). The effects of fructose and FAs either alone or combined on lipid metabolism, and on indices of oxidative stress were also evaluated (spectrophotometric assays and quantitative real-time PCR).

Results: Fructose/FA combination exacerbated the reduced cell viability associated to the fructose treatment and increased cellular apoptosis. Also the expression of NAFLD markers such as the adipose differentiation-related protein (ADRP), the peroxisome proliferator-activated receptor γ (PPAR γ), the I κ B kinase β interacting protein (IkBip) and the interleukin-1 β (IL-1 β) was increased when cells were exposed to Fructose/FA combination. Similar effects of Fructose/FA combination were observed in terms of oxidative stress.

Conclusions: Liver steatosis *in vitro* is greatly worsened by the combination of fructose and FAs and this approach steers the process in the direction of the inflammatory steatohepatitis. Our cellular model allows to study the potentially deleterious steatogenic effects of dietary fructose and provides the basis to test the protective effect of endogenous and exogenous compounds.

P1.18

Crosstalk between hyperglycemia and inflammation in acute pancreatitis

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Background: Pancreatic inflammation results in destruction of pancreatic islet with loss of β -cells. However, the pathogenesis of hyperglycemia in acute pancreatitis (AP) is still unknown.

Purpose the investigation is determining the level of glucose in the blood serum of AP patients with and its relationship with markers of inflammation.

Materials and methods: We examined 66 patients with acute pancreatitis. According to the international classification in 10 patients we diagnosed the mild AP, in 24 patients the moderate AP, and in 32 patients the severe AP. We determined the glucose level, indicators of inflammation.

Results: In acute pancreatitis patients was shown a direct correlation between hyperglycemic state in the blood serum with concentration of interleukin 2 ($F = 8.5386$, $P = 0.00480$), interleukin 6 ($F = 7.2821$, $P = 0.00890$), tumor necrosis factor α ($F = 6.8345$, $P = 0.01114$), C reactive protein ($F = 5.5902$, $P = 0.02111$). The level of glucose in the blood serum significantly increased with increasing concentrations of interleukin 2 ($R = 0.247179$, $P = 0.045405$).

Conclusions: Acute pancreatitis is characterized by local and systemic inflammation, which leads to metabolic dysfunction. In the pathogenesis of hyperglycemia plays an important role increased synthesis of proinflammatory cytokines and C reactive protein.

P1.19

Pharmacological correction by novel molecule agents prevents development of experimental obesity in rats induced of monosodium glutamate

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Today the prevalence of obesity continues to increase that provides challenge to scientists. Obesity is strongly associated with systemic inflammation and with oxidative stress. Remains a demand for distinct, safe, and effective treatments for obesity, with the current therapies often associated with side effects. Free fatty acid receptors are now regarded as targets for novel drugs for the treatment of metabolic syndromes because they are activated by FFAs.

We included newborn 18 male rats and divided to 3 groups of 6 animals each. Rats of group 1 - newborns rats of control group were administered with saline subcutaneously (s.c.) in the volume of 8 mL/g at 2nd, 4th, 6th, 8th and 10th postnatal days. Rats of group 2 and 3 - newborns rats of MSG-group and MSG + Z56822977 group received a solution of MSG (4.0 mg/g of body weight) s.c. at 2nd, 4th, 6th, 8th and 10th postnatal days. MSG + Z56822977 group received aqueous solution of Z56822977 in dose 25 mg/kg at volume 1 mL/kg per os (p.o.) during 7 days. MSG-group respectively received 1 mL/kg of water (p.o.). In 4-month rats anthropometrical parameters and VAT mass were estimated.

In 4-month rats we diagnosed the changes of the anthropometrical parameters (body weight, BMI, Lee index) and significant increase of fat mass (subcutaneous, gonadal, visceral adipose tissue) that suggest development of visceral obesity. The introduction of Z56822977 prevents MSG-induced obesity in rats obesity that was confirmed by reduction of index Lee and body mass index. It was established that Z56822977 decreased index Lee by 7.2% ($P < 0.01$) and body mass index by 17.7% ($P < 0.01$) compared with MSG-group rats. The introduction of Z56822977 decreased the subcutaneous and visceral adipose tissue by 49.1% ($P < 0.01$) and 55% ($P < 0.01$) compared with MSG-group. Thus, the introduction of Z56822977 prevents MSG-induced obesity.

P1.20

Melatonin prevents the systemic and cerebral alterations induced by high-fructose intake

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Background: Since excessive fructose consumption is an important factor for the development of diseases such as obesity, metabolic syndrome, diabetes and even neurodegenerative diseases, in this study we evaluated blood biochemical parameters together with the expression of some neurodegenerative markers in brains from Syrian hamsters fed with a high-fructose diet. In addition, we also analyzed the possible beneficial effects of a treatment with melatonin.

Material and methods: Sixteen male Syrian hamsters, 6-week-old, were divided into four groups that were fed for 10 weeks with normal diet; normal diet + melatonin (daily dose of 500 μ g/kg body weight); high-fructose diet and high-fructose

diet + melatonin. We measured blood biochemical parameters (glucose, insulin, LDL cholesterol, HDL cholesterol and uric acid) and brain protein levels of α -synuclein and tau phosphorylation by immunoblotting.

Results: Animals fed with excessive fructose showed higher levels of blood glucose, LDL and uric acid without appreciating significant differences in insulin and HDL levels than those fed with normal diet. In addition, although α -synuclein expression was similar among brains from animals with both types of diets, we found an accumulation of tau phosphorylation in animals fed with high-fructose. Melatonin was able to reduce LDL, uric acid and glucose to levels found in hamsters fed with normal diet by increasing insulin concentrations and it was also able to reduce tau phosphorylation.

Conclusion: This study suggests a risk of high fructose consumption for the development of insulin resistance and early neurodegenerative changes. Melatonin is able to counteract harmful effects of this kind of diet and protect the organism at brain and systemic levels.

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P1.21

Melanin toxic effects on the embryos

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Melanins' nanoparticles, which are produced by *Pseudomonas aeruginosa*, have antimicrobial, antioxidant and anti-inflammatory properties. It was shown that Melanin obtains preventive and therapeutic effects on the development of erosive/ulcerative lesions in the gastric mucosa. We confirmed that gels/creams based on Melanin demonstrate wound healing properties. Main idea is to create new dermatropic drug. One of the first step is to conduct preclinical investigations.

Aim: To investigate the embryotoxicity of Melanin.

Materials and methods: The study was carried out on white nonlinear rats. To study the influence of Melanin on early embryo development (EED) before implantation 3 group were created: 1 – control, 2 – therapeutic dose (TD) (0.3 mg/kg diluted in tap water 100 μ l/100 g, *per os*), 3 – 10 times - therapeutic dose (10*TD) (3 mg/kg diluted in same way) in all these group preparation was injected 1st – 6th day of pregnancy. Rats were treated 6th – 16th of pregnancy with the same 3 dose group, as shown above, to investigate changes in embryo-fetal development (EFD). To detect estrous cycle phases, to registrat first day of pregnancy the vaginal smears were used. Females were sacrificed on 20th day of gestation and different mortalities, internal organs, skeleton were studied.

Results: Administration of Melanin at TD both, during EED and EFD study, caused no changes at any kind of mortality, organ and skeleton development compare to control group. It was shown that 10*TD injection from 1st till 6th day of pregnancy decreased mortality before implantation and total mortality compare to control. After EFD study was concluded that 10*TD decreased mortality after implantation and total mortality compare to control.

Conclusion: Melanin does not possess toxic effects on the embryos while its administration at different periods of gestation and even at some dose may decrease mortality of embryos.

P1.22

Metabolic targets of potential clinical significance in liver cancer

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Introduction: Metabolic alteration is a crucial cancer hallmark, which enables their sustained survival and proliferation. In liver cancer – most of which are hepatocellular carcinoma (HCC) – the extent, relevance and mediators of altered metabolism are largely unknown. We aimed to identify metabolic gene alterations that characterize HCC.

Methods: We studied the expression pattern of metabolic genes in clinical HCC microarray using bioinformatics resources, including Gene Set Enrichment Analysis software (GSEA), Database for Annotation, Visualization and Integrated Discovery (DAVID), and platforms such as cBioPortal and OncoPrint. Furthermore, we performed gene functional annotation to predict metabolic pathways mostly altered in HCC. The expression of selected genes, as well as the consequence of inhibiting metabolic pathways, was subsequently analyzed in HCC cell lines using quantitative PCR and functional assays respectively.

Results: We found that the topmost downregulated metabolic genes include organic anion transporter of bilirubin (*SLCO1B3*); cytochrome P450 3A4 (*CYP3A4*), fructose 1,6 bisphosphatase (*FBP1*) and liver isoform of glutaminase 2 (*GLS2*), while those in the upregulated category include retinal reductase (*AKR1B10*), transketolase (*TKT*), squalene epoxidase (*SQLE*), ATP citrate lyase (*ACLY*), thymidylate synthetase (*TYMS*) and muscle isoform of pyruvate kinase (*PKM2*). Pathway analysis revealed metabolic alterations that consistently feature in HCC, notably the downregulation of genes in xenobiotics/drug metabolism, gluconeogenesis and fatty acid metabolism, and the upregulation of genes in high energy-yielding pathways such as glycolysis, pentose phosphate pathway and nucleotide biosynthesis.

Conclusion: Our study has revealed metabolic gene alterations and specific targets for further investigation as potential clinical biomarkers or therapeutic targets in HCC.

P1.23

Gestational diabetes as long-term negative predictive factor for the descendants – experimental model

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Aim: We designed an animal model using pregnant Wistar rats to prove whether maternal diabetes has long-term implications on descendants prognosis. Diabetes was defined as combination of obesity and impaired glucose tolerance, generating multiple metabolic disorders. Material and method: We used 30 rats weighing between 200–250 grams to whom we induced obesity was by high-fat, high-calorie food intake. During gestation half of them received normal diet as controls, while the others continued the fat diet. Serum glucose and lipids, lipid peroxidation and antioxidant levels (malonyldialdehyde, thiols, glutathione

from maternal serum and placental homogenates) and placental histopathology were assessed. The female descendants of these rats were also bred and their pregnancies and offspring were analyzed.

Results: Glycemia and serum lipids values (cholesterol, total lipids, LDL lipids, triglycerides) were significantly higher in the group with fat diet versus control. These results positively correlated with high peroxidation levels measured by malonyldialdehyde and total thiols and low antioxidant levels of glutathione from maternal serum and placental homogenates. Placental microscopic analysis showed dysplastic epithelial and mesoderm cells in the yolk sac, a higher density of inflammatory cells and congested vessels with thrombotic areas and glycogen trophoblast deposits in the fat group.

Discussions: The descendants of the obesogenic diet rats weighed significantly more at birth and 72% became obese as adults compared to 41% in the control group. Diabetes was studied as risk factor in the female offspring exposed to high fat diet as second generation of rats and confirmed the fact that their descendants showed the same metabolic alterations.

Conclusions: In conclusion our experiment proves that in utero exposure to maternal diabetes is a risk factor for programming fetal carbohydrate and lipid metabolism disorders and adverse metabolic phenotypic in adult life. Gestational diabetes is a negative long-term prognostic factor, affecting subsequent generations.

P1.24

Severe lysosomal ACID lipase deficiency in a greek child homozygous for a novel splicing mutation of the lipa gene

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Lysosomal Acid Lipase (LAL) is a key enzyme which hydrolyzes cholesteryl esters and triglycerides from apoB containing lipoproteins internalized via receptor-mediated endocytosis into the cells, including hepatocytes. LAL-deficiency is associated with a large variability of the phenotypic expression, from neonatal lethal form due to complete absence of LAL activity, to forms detectable in infancy, adolescence or adult life with variable residual enzymatic activity. Clinical characteristics are hepatic steatosis, evolving in fibrosis and micronodular cirrhosis, hyperlipidemia and premature atherosclerosis. Here we report the clinical and genetic features of a novel case of severe LAL deficiency which allows a minimal residual activity, at least in the liver. The patient was a Greek boy with hepatomegaly and elevated transaminases at one year of age. At three years of age a liver biopsy showed panlobular steatosis and F-2 fibrosis. At six years elastography documented F-3 grade of fibrosis (kPa 12.5). Over the last years the biochemical blood parameters were: cholesterol 6.98 ± 0.73, LDL chol. 5.54 ± 0.83, HDL chol. 0.88 ± 0.10, triglycerides 1.28 ± 0.34 mmol/L; apoA-I 101.7 ± 8.4, apoB 160.0 ± 10.9 mg/dl; AST 70.5 ± 11.9, ALT 87.5 ± 29.7, GGT 17.2 ± 1.3 IU/L. LAL activity in peripheral blood leukocytes resulted 0.0 nmol/mg protein/min in the proband, 16 nmol/mg protein/min in his father, 10.2 nmol/mg protein/min in his mother and 33 nmol/mg protein/min in

his 4-year old healthy brother (18.7-40.0 nmol/mg protein/min in controls). The sequencing of the *LIPA* gene showed an homozygous A > C substitution in the third nucleotide of intron 3 (c.229 + 3C/C), his parents were heterozygous (c.229 + 3A/C) and the brother homozygous for the wild type nucleotide (c.229 + 3A/A). The *in silico* analysis tools predicted that this intronic variant affects the donor splice site and probably causes the retention of intron 3. However, in view of the phenotypic expression, although severe, we can suppose that the mutation allows a minimal quote of normally spliced mRNA.

P1.25

Phenotypic expression and genotype analysis of eleven patients with cholesteryl ester storage disease and identification of a novel lipa gene variant

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Background: Cholesteryl ester storage disease (CESD) is a recessive disorder due to a defect of Lysosomal Acid Lipase (LAL) that hydrolyses cholesteryl esters and triglycerides derived from internalized apoB containing lipoproteins. The disease is characterized by a multi-organ involvement including liver, spleen, intestine and cardiovascular system.

Objective: Aim of the study was the characterization of clinical and molecular features of 11 (10 unrelated) previously unreported CESD patients.

Methods: Data collected included clinical and laboratory investigations, liver imaging, liver biopsy, the response to lipid-lowering medications or liver transplantation and *LIPA* gene analysis.

Results: CESD was suspected in infancy (4.9 ± 3.5 years) for the presence of: (i) hepatomegaly; (ii) elevated serum transaminases; (iii) hypercholesterolemia, and was confirmed by liver biopsy/imaging and LAL assay. Mean follow up period was 7.6 years. Patients treated with statins with or without ezetimibe showed 31% reduction of plasma LDL-cholesterol. This treatment was not associated with a tangible effect on the progression of liver damage. The common c.894G > A variant was identified either in homozygosity (*n* = 4) or compound heterozygosity (*n* = 4). We identified four previously reported missense variants: p.(Thr288Ile) in homozygosity and p.(His295-Tyr), p.(Gly342Arg), and p.(Leu294Ser) in compound

heterozygosity. Two patients were carriers of p.(Asp345Asn), a novel variant affecting the LAL catalytic triad. The p.(Thr288Ile), p.(His295Tyr) and p.(Asp345Asn) have been found so far only in Italian patients from restricted geographical areas.

Conclusions: This study provides additional data on the clinical and molecular features of CESD patients and describes a novel variant affecting LAL catalytic site.

P1.26

Organoleptic profile of two almond varieties in subjects living in Apulia: a clue to Mediterranean diet?

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Background and aim: Almond is the edible seed of the *Prunus amygdalus* tree. As nuts, almonds in southern Italy are a popular component of the "rural" Mediterranean diet and are enriched in fat (~55%, mainly [39-4%] monounsaturated fatty acids MUFA), vit. E, Mg, Cu, P, fibers, riboflavin, proteins, phenols and polyphenols. The beneficial effects of almonds on human health include the modulation of risk factors linked to diabetes and cardiovascular disease. Almond consumption is associated with lower serum levels of cholesterol and triglycerides due to content in polyunsaturated fatty acids, and with enhanced resistance of LDL against oxidation due to bioavailable vit. E. We tested if the organoleptic perception of almonds was influenced by varieties, age, and body weight.

Methods: We studied 13 lean (BMI < 24.9 kg/cm², age 18–65 yrs), 8 obese (BMI ≥ 30 kg/cm²) adults and 10 lean elderly (> 65 yrs) subjects ingesting 2 varieties in a random and blind fashion: the imported Californian (*Carmel*) and the local Toritto (*Filippo Cea*) almonds (N = 5 of each = 5 g ≈ 2.5 g fat). Fasting subjects scored for olfactory, taste, chewing and visual perceptions on a 0-10 cm Visual Analogue Scale (VAS). Before each test the mouth was rinsed with 150 mL water. Drinking or smoking was prohibited 2 hr before the test.

Results: Lean adult subjects perceived significantly differently (0.001 < P < 0.04). Toritto almond yielded significantly lower VAS scores than Californian almond for olfactory (3.7 ± 0.5 vs. 5.0 ± 0.6 cm), taste (5.1 ± 0.3 vs. 7.2 ± 0.5 cm), chewing (5.9 ± 0.4 vs. 7.0 ± 0.6 cm), visual (5.2 ± 0.6 vs. 8.0 ± 0.5 cm) perceptions, without gender-related differences. Obese and elderly subjects had similar perceptions; furthermore, perception was not influenced by age and body weight.

Conclusion: The imported Californian and local Toritto almond varieties provide distinct gustative perceptions especially in lean subjects. The lack of distinct organoleptic perception by obese and elderly subjects might disclose ongoing pathophysiological conditions.

P1.27

A novel formulation of *Bifidobacterium longum* BB536, *Lactobacillus rhamnosus* HN001, and B6 vitamin improves symptoms and Quality of Life in patients with Roma IV gas-related symptoms. A preliminary open study

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Background: Functional abdominal bloating (FAB) is a highly prevalent problem, and often associated with other gastrointestinal symptoms, including abdominal discomfort. Symptoms can greatly affect the overall quality of life (QoL) of patients and repeated consultations may contribute to increased costs of medical care. We tested the short-term efficacy of a novel probiotic in a group of patients with FAB.

Methods: 85 lean patients (63F:22M, age 35 yrs ± SEM1.2, BMI = 23.6 ± 0.3SE kg/m²) with at least 6 mo. of FAB (Rome IV criteria) and abdominal discomfort entered the 3 mo. study. Organic diseases (i.e. IBD, celiac disease), lactose/fructose intolerance and small intestinal bacterial overgrowth were excluded by appropriate clinical, biochemical and instrumental assessment. At baseline, and after 90 days patients were specifically evaluated for symptom severity (VAS, visual analogue scale 0-100 mm) and VAS questionnaire for deterioration of QoL. The intervention included maintaining a standard "mediterranean diet" with controlled intake of Fermentable Oligo- Di- Mono-saccharides and Polyols (FODMAPs), charcoal (1 cp x2/day at meals) with or without a probiotic containing *B. Longum* BB536, *L. Rhamnosus* HN001, and vit. B6 (Zircambi®, Alfa Wassermann, Italy).

Results: At baseline, VAS-FAB, VAS-discomfort, and VAS-QoL were 86 ± 0.9 mm, 22.4 ± 2.2 mm, and 73.5 ± 0.8 mm, respectively. Treatment with probiotic/charcoal, compared to charcoal alone was associated with a significant (P < 0.00001) greater decrease of FAB (-72.2 ± 2.2% vs. -40.5 ± 1.2%), abdominal discomfort (-79.4 ± 2.9% vs. -46.8 ± 1.7%), and higher improvement of QoL (68.3 ± 2.4% vs. 35.6 ± 1.8%)

Conclusions: The occurrence of functional abdominal symptoms contribute to unnecessary use of medical resources and increase costs. A short-term trial with the probiotic *B. Longum* BB536, *L. Rhamnosus* HN001, and vit. B6 is worth trying because decreases symptom severity and improves QoL. Further controlled studies are on the way to investigate the efficacy of this approach in IBS populations.

P1.28

A case of Guillain-Barré syndrome with acute pancreatitis. A role for Internal Medicine in a complex clinical scenario

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A 61 yrs old woman was admitted to neurosurgery for head and neck injury with C1 fracture; because of loss of swallowing reflex, she was put on total parenteral nutrition (TPN) program and 25 days after she was transferred to the Internal Medicine ward with fever (39°C), septic status, acute kidney failure, dehydration and hypernatremia (170 mEq/L), acute pancreatitis (likely TPN-dependent), and flaccid quadriplegia. A chest X-ray revealed bronchopneumonia (likely by aspiration) while

cranial and vertebral MRI excluded cerebral involvement or medullar compression. A CRI.MY.NE (Critical Illness Myopathy & Neuropathy) or a Guillain-Barré syndrome (GBS) were suspected, but the lumbar puncture confirmed the GBS (elevated CSF total protein without pleocytosis). Antibiotic, supportive therapy and rehydration were promptly started and immune globulins were administered (i.v. 0.4 g/kg/day x 7 days). After 5 days the general conditions improved, with recovery of some voluntary movements and muscle-tendon reflexes, complete recovery of the swallowing reflex. Pancreatic markers (serum lipase and amylase) normalized and oral re-feeding was started. Following neurorehabilitation, the patient had partial recovery of autonomy, new septic complications, without walking ability. **Conclusion:** The likely sequence in this case was accidental C1 fracture, fever and flaccid paralysis. The Guillain-Barré syndrome should be suspected and differentiated from CRI.MY.NE. A late diagnosis of Guillain-Barré syndrome often leads to several associated complications (i.e. acute pancreatitis) and poor clinical outcome.

P1.29

Heterogeneous management of patients with H. pylori infection in a geographical area with high clarithromycin resistance yield uncertain and unpredictable eradication rates. Results from a southern Italian study and emerging role for the novel bismuth-based quadruple treatment

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Background: Helicobacter pylori (HP) eradication is currently recommended in all diagnosed subjects but eradication rates are strongly affected by the ongoing antibiotic resistance. We aimed to describe a "real life" scenario about management of a heterogeneous group of patients with HP-related problems, and to report on therapeutic outcomes of chosen regimens, including the bismuth-based quadruple therapy (Pylera[®]), recently marketed in Italy.

Methods: 2,224 subjects were studied at two centers in a geographical area with clarithromycin resistance > 15% (Apulia region). Analyses included reason for referral, diagnostic procedures (¹³C-urea breath test -UBT- or upper endoscopy) and outcomes of eradication regimens.

Results: Over 80% of patients (more women) were referred by family physicians, with 60% as naïve subjects. The percentage of asymptomatic subjects was significantly higher when referred for noninvasive UBT than EGDS (30% vs. 2%, $P < 0.0001$); 6% and 8% of referred subjects had H. pylori-unrelated symptoms or underwent UBT only for familial history of H. pylori infection. The overall infection rate was 32.5%, and similar in patients asymptomatic (31.1%) or with HP-related symptoms/clinical conditions (34.3%). In the 987 HP+ve patients undergoing therapy, the overall eradication rate (ER) was 80.2% but a great

variability in therapeutic regimens was noticed outside of the referral centers with ERs ranking 59.6% (unconventional), 70.7% (7d-triple), 73.2% (undefined), 89% (10d-sequential) and 96.9% (10d-Pylera[®] across 1st-5th line regimens). Overall, minor side effects were recorded in 5% of patients on eradication therapies.

Conclusions: In a "real life" scenario, the use of heterogeneous approaches in terms of patient's referral, diagnostic techniques and personal choices, puts patients with H. Pylori infection at risk of poor eradication outcomes and unnecessary procedures. Among all employed treatments, sequential and bismuth-based quadruple treatments yield the highest ERs even in a geographical area with high clarithromycin resistance.

P1.30

Environmental / lifestyle factors and endometriosis - a comprehensive point of view

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Background: Endometriosis, defined as estrogen-dependent lesions containing endometrial glands and stroma located outside the uterus, is a chronic, often painful gynecological condition that affects 6 to 10 % of reproductive aged women.

Aim: This work aims to describe the state-of-the-art regarding the interrelation of lifestyle factors and endometriosis and make a comprehensive point of view.

Material and method: The database PubMed was used. The terms used most frequently in the search were: "endometriosis", "lifestyle factors", "environmental" and "quality of life". The search was limited to the last 5 years.

Discussions: Epidemiological data regarding endometriosis are still limited. The few studies that have been undertaken suggest that lifestyle and dietary factors may be associated with susceptibility to developing endometriosis. The results found that a diet rich in fruit and vegetables and poor in meat products was protective against developing endometriosis. Additionally, women with few or no children and low body mass index were at a higher risk for developing endometriosis. Alcohol consumption was cited as a risk factor for endometriosis, although not all studies are concordant. Other authors suggested that exposure to synthetic compounds such as dioxin and other polychlorinated biphenyls could lead to the development of endometriosis due to their effects as endocrine disruptors. Human data on dioxin exposure and endometriosis risk is scant and sometimes contradictory. Despite dioxin and bisphenol increased serum levels observed in endometriosis patients, a conclusive association between environmental toxicant exposure and increased risk for developing endometriosis has yet to be established.

Conclusions: Given the variety and conflicting notions pertaining to the origin and development of endometriosis, it becomes clear why endometriosis is often referred to as the "disease of theories".

P1.31

Noninvasive characterization of metabolic liver steatosis/steatohepatitis across multicenter studies

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Background-Aims: Nonalcoholic fatty liver disease (NAFLD), a worrisome health problem worldwide, is often a fellow traveller with the metabolic syndrome and a marker of increased cardiovascular risk. The necro-inflammatory form (nonalcoholic steatohepatitis, NASH) puts 5-8% of patients at risk of cryptogenic cirrhosis within 5 yrs. The ultimate diagnosis of NASH relies on liver biopsy, but noninvasive tests are searched for patients requiring further consultation. Fibromax® (www.bio.predictive.it) informs about liver fatty infiltration, inflammation, and fibrosis. The performance of ultrasonography [US], fatty liver index [FLI] and Fibromax® was evaluated in NAFLD involving family practice.

Methods: 259 patients (165M, 94F, age 51 ± SD10 yrs) with clinical, ultrasonographic features of NAFLD were enrolled. Measurements: BMI, waist circumference, semiquantitative score of liver steatosis (US), blood analyses (serum insulin, AST, ALT, total cholesterol, triglycerides, glucose, haptoglobin, bilirubin, GGT, A1-apolipoprotein, and alpha2-macroglobulin) to calculate FLI algorithm and Fibromax scores as Steatotest (0-3), NASHTest (absent, borderline, present), and Fibrotest (0-4).

Results: Patients had mild (16.2%), moderate (69.9%), or severe (13.9%) liver steatosis (60.2% hypertransaminasemia). Prevalence of overweight, obesity, diabetes, hypertension, and dyslipidemia was 42.7%, 46.5% (4.2% severe obesity), 24.7%, 40.9%, and 56.4%, respectively. Lean patients (10.8%) had normotransaminasemia in 2/3 cases; 12.3% of patients had risk for NASH (sensitivity 50% specificity of 94.7%). Fibromax identified 34 patients (13.1%) with likely advanced fibrosis and found that over 28% of patients with moderate (ultrasonographic) steatosis were likely carrying severe steatosis. Steatotest score was significantly associated with BMI, waist circumference, ALT, triglycerides, FLI. Fibrotest correlated with ALT. FLI identified 73.4% of patients likely carrying fatty liver.

Conclusions: NAFLD should be systematically searched and characterized in all dysmetabolic patients with cardiovascular risk. Asymptomatic subjects at risk need screening for NAFLD. Fibromax is a promising noninvasive diagnostic tool, specifically in family medicine for identifying those patients at risk for NAFLD requiring targeted follow-up.

P1.32

Omega 3 versus Omega 6 fatty acids supplementation in obese pregnant rats – understanding the discrepant fetal prognosis

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Aim of the study. Considering that maternal obesity generates negative metabolic fetal and adult life prognosis, we searched possible reprogramming methods using dietary supplements. **Material and method.** We imagined an animal model using 30 pregnant Wistar rats, overweight by high-fat high calorie alimentation using gavage. Knowing that gestational obesity induces inflammation and lipid metabolism alteration causing accelerated adipogenesis, we submitted them to interventional therapies comparing their consequences. During gestation, 10 rats started dietary changes involving normal food intake, 10 received Omega 3 fatty acids (docosahexaenoic acid and eicosapentaenoic acid) 1 mL/kg and the other 10 received Omega 6 fatty acids 1 mL/kg. **Results.** We measured serum glucose and lipid profile, lipid peroxidation and antioxidant levels (malonyldialdehyde, thiols, glutathione from maternal serum and placental homogenates). Surprisingly, Omega 3 and 6 fatty acid supplementation generated completely different maternal-fetal consequences. Glycemia and serum lipids values (cholesterol, total lipids, LDL lipids, triglycerides) were significantly higher in the Omega 6 versus Omega 3 group, the normal diet group revealing results in between. This positively correlated with the high peroxidation markers - malonyldialdehyde and total thiols and low antioxidant molecules - glutathione from maternal serum and placental homogenates in the Omega 6 group, and reverse results in the Omega 3 group. **Discussions.** Although we expected similar results both for mother and fetus from dietary changes using fatty acids supplementation, our experimental results were conflicting. According to our study, Omega 3 fatty acids have a beneficial metabolic maternal-fetal result when used in obese mothers, while Omega 6 supplements proved to generate an adverse outcome. **Conclusion.** Although the fetal reprogramming process has already been demonstrated, the choice of dietary and lifestyle changes for gestational obesity still remains a controversy. Using polyunsaturated fatty acids in order to improve fetal prognosis has different results according to the supplements used.

Workshop 2: Cardiology

P2.01

Intr plaque expression of C-reactive protein predicts cardiovascular events in patients with severe atherosclerotic carotid artery stenosis

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Background and aims: Serum C-reactive protein (CRP) levels are widely used for the assessment of intermediate cardiovascular (CV) risk in addition to “classical” risk factors. In light of this, we investigated whether systemic or intraplaque CRP levels might predict major adverse cardiovascular events (MACEs) in patients with severe carotid stenosis over a 18-month follow-up period.

Methods: CRP levels have been assessed in the serum and within different portions (upstream and downstream) of carotid atheromata of 217 patients undergoing endarterectomy due to an extra cranial, high-grade stenosis (> 70% luminal narrowing) of the internal carotid artery. The study has been conducted at a single center (IRCCS Azienda Ospedaliera Universitaria San Martino-IST Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy) from March 2008 to June 2011. The associations between CRP and intraplaque lipids, collagen, neutrophils, smooth muscle cells (SMCs), as well as M1 and M2 macrophages have been determined.

Results: Serum CRP levels did not correlate with any investigated intraplaque inflammatory biomarker. In upstream portions, CRP content showed a positive correlation with intraplaque neutrophils, total macrophages, and M1 macrophages and a negative correlation with SMC content. In downstream portions, intraplaque CRP correlated with M1 and M2 macrophages. According to the cut-off point (CRP > 2.9%) identified by a receiver operator characteristic analysis in upstream portions, Kaplan-Meier analysis demonstrated that patients with high CRP levels showed a greater rate of MACEs (Log Rank Test: $P = 0.0042$). The risk of MACEs increased along with the increase in CRP levels in upstream plaques (HR 6.22 [95% CI

1.49–26.05]; $P = 0.012$), independently of age, male gender, serum CRP, and statin use (HR 8.57 [95% CI 1.89–38.77]; $P = 0.005$).

Conclusion: In patients with severe carotid artery stenosis, high CRP levels within upstream portions of plaques positively correlate with intraplaque inflammatory cells and predict MACEs over a 18-month follow-up period.

P2.02

Anti-RANKL treatment in a mouse model of acute myocardial infarction

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Background: Selective anti-inflammatory treatments reducing reperfusion injury might be associated with immunosuppression or inflammatory rebounds. In order to identify better-tolerated therapeutic strategies, we investigated if the neutralizing approach of the cytokine receptor activator of nuclear factor kappa-B ligand [RANKL] might impact on neutrophil-mediated cardiac reperfusion injury and repair.

Materials and methods: Myocardial ischemia (60 min) and reperfusion injury (up to 24 h) was surgically induced in adult C57Bl/6 mice.

Results: In hearts and serum, RANKL was early upregulated during reperfusion. A “one-shot” injection with neutralizing anti-RANKL IgG during ischemia ameliorated myocardial infarct size and function, but not adverse remodeling (determined by Magnetic Resonance Imaging [MRI]) as compared to Vehicle or control IgG. These beneficial effects were accompanied in vivo by reduction in cardiac neutrophil infiltration, reactive oxygen species (ROS) and MMP-9 release. Anti-RANKL IgG treatment suppressed sudden peak of neutrophil granule products in mouse serum early after reperfusion onset. In vitro, RANK mRNA expression was detected in isolated mouse neutrophils. Co-incubation with neutralizing anti-RANKL IgG abrogated RANKL-induced mouse neutrophil degranulation and migration, suggesting a critical role of RANKL in neutrophil-mediated injury. Conversely, anti-RANKL IgG did not affect salvage pathways in cardiac cells (i.e. ERK p42/p44, Akt and STAT-3) or macrophage cardiac infiltration. Finally, treatment with anti-RANKL IgG showed no effect on B and T lymphocyte polarization (in serum, spleen and infarcted myocardium) and circulating chemokines as compared with Vehicle or control IgG. **Conclusions:** Acute treatment with an antibody neutralizing RANKL improved cardiac infarct size and function by potentially impacting on neutrophil-mediated injury and repair.

P2.03

High expression of vitamin D receptor (VDR) within human carotid plaques correlates with lower risk of MACEs

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Background: In the last decades, several studies investigated the role of Vitamin D as a cardiovascular risk factor. In observational studies, Vitamin D deficiency has been correlated with hypertension, dyslipidemia, insulin resistance and obesity. Recently, these associations were widely debated and controversy remains.

Material and methods: In this prospective observational study, we analysed 168 human carotid plaques from patients undergoing elective endarterectomy for carotid stenosis. Our primary end point was to determine whether intraplaque VDR expression at the time of surgery predicted major adverse cardiovascular events (MACEs) during 18-month follow-up. Both the upstream and downstream portions of the plaque were immunostained to quantify VDR, lipids, collagen and macrophage subsets. *In vitro*, human primary monocytes were differentiated into M1 and M2 macrophages and incubated with different concentrations of 1,25 (OH)₂D₃.

Results: Intraplaque VDR expression positively correlated with total and M1 macrophage content. Downstream portions of the plaques having higher VDR expression were characterized by increase M1 macrophage infiltration. According to Kaplan-Maier analysis, the rate of MACEs was greater in patients with low downstream VDR expression (8.2% vs 1.3% $P = 0.005$). The beneficial effect of high VDR expression on MACEs risk was then confirmed by Cox proportional hazard regression analysis (adjusted HR 0.78 [95% CI 0.62–0.98]; $P = 0.032$). *In vitro* experiments showed a prevalent VDR expression in M1 as compared to M2 subset. Furthermore, co-incubation of M1 macrophages with 1,25(OH)₂D₃, increased VDR expression and suppressed toll-like receptor 4 (TLR4) expression.

Conclusions: Results from the clinical cohort suggested a protective role of VDR expression within human atherosclerotic plaques. *In vitro*, the activation of the local vitamin D system might trigger anti-inflammatory pathways on M1 macrophages. Further studies are needed to clarify the potential role of VDR expression as biomarkers of vulnerable plaque or even as potential therapeutic target in human atherosclerosis.

P2.04

Role of NT proBNP in preventing left ventricular dysfunction in diabetic patients

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Background: To prove the importance of NTproBNP in guiding treatment to prevent left ventricular dysfunction at diabetic patients.

Materials and methods: We included 174 patients who were presented at hospital during two years, with diabetes. We excluded patients who are already diagnosed with heart failure or who had current symptoms of heart failure. We randomized patients into 2 equal groups: a control group and an intervention group. NTproBNP value was determined for all patients. In the intervention group patients were treated according to the NTproBNP. Patients with NTproBNP < 125 pg/dl (38 patients) received standard treatment for their symptoms. Patients with NTproBNP > 125 pg/dl (49 patients) were the ones on which we intervened to prevent heart failure. They were investigated by cardiac ultrasound and other tests and they received specific treatment. Patients in the control group received standard treatment regardless of the NTproBNP value.

Results: After two years, the end points were: left ventricular dysfunction, and the rate of hospitalizations for cardiovascular pathology. After two years 28 (27.5%) patients were diagnosed with left ventricular systolic dysfunction, compared to eighteen (20.6%) in the intervention group. Also, and rate of admissions for cardiovascular pathology was higher in the control group 34 (39%) versus 27 (31%) in the intervention group.

Conclusions: Patients in the intervention group, in which the value of NTproBNP was used in choosing therapeutic management, had lower rate of incidence of left ventricular dysfunction or cardiovascular events than patients in the control group. And more, medical intervention guided by NTproBNP can prevent or delay left ventricular dysfunction.

P2.05

Brain natriuretic peptide in morbid obesity

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Background and aims: Morbid obesity is associated with cardiovascular comorbidity, however a "bizarre paradox" could influence this relationship: obese patients typically show low plasmatic levels of brain natriuretic peptide (BNP). The aim of our study is to investigate, in a wide morbid obese population, the relationship between BNP and obesity-related markers, along with echocardiographic and vascular parameters.

Methods: In 154 morbid obese patients (52 males, 102 females, mean BMI 44.79 kg/m²), we evaluated anthropometric parameters and glycometabolic/lipid profile, performed bioimpedance and echocardiography, and measured visceral fat area and flow-mediated dilation (FMD) by ultrasonography.

Results: Population has been divided in two groups according to median BMI levels; patients with higher BMI had significantly lower BNP ($P = 0.008$), FMD ($P = 0.014$) and HDL-C ($P = 0.001$) and showed a more impaired heart function. We found a similar trend subdividing patients according to median visceral fat area.

BNP showed a significant inverse correlation with BMI ($P < 0.001$), left ventricular mass ($P = 0.026$) and inter-ventricular septum thickness ($P = 0.007$) and a significant positive correlation with FMD ($P = 0.008$), HDL-C ($P = 0.022$), and ejection fraction ($P = 0.013$). BMI and triglycerides were independent predictors of BNP levels.

Conclusions: Morbid obese patients with higher BMI seem to have a higher BNP clearance, probably due to the greater total body fat amount. In this population BNP seems to play a key role in endothelium-dependent vasodilation and, being the natriuretic peptide inversely associated with BMI, this is another mechanism by which obesity could increase cardiovascular risk. Moreover, the correlation between BNP and cardiac impairment could represent another link between obesity and cardiovascular damage.

P2.06

Indoxyl sulphate activates cardiac fibroblasts with enhanced collagen synthesis, upregulated angiotensin-neprilysin system, and paracrine modulation of cardiomyocyte hypertrophy

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Background: Growing evidence indicates that cardiac fibroblast (Fib)-driven fibrosis is a major feature of the cardiomyopathy associated with advanced chronic kidney disease (CKD). In this setting, a role has been proposed indoxyl sulphate (IS), a tryptophan metabolite that accumulates in CKD.

Methods: We investigated the effects of 50 mM IS, a concentration found in moderate CKD, on mouse neonatal cardiac Fib. Oxidative stress was evaluated by using the CellROX assay; proliferation by BrdU incorporation and flow cytometry for CFSE; α -smooth muscle actin (aSMA), selected paracrine mediators, and the neprilysin-angiotensin (Ang) axis by RT-PCR, western blotting and/or immunocytochemistry; and collagen production by RT-PCR and picrosirius red staining. After incubating mouse neonatal ventricular cardiomyocytes (mNVCM) with the conditioned medium of control or IS-treated Fib, the expression of hypertrophy genes was analysed by RT-PCR and glycolysis by enzymatic assays. To determine the relevance of AngII in mediating IS action, cells were co-cultured with IS and the AngII type 1 receptor (AT1R) antagonist, valsartan (Val).

Results: IS triggered mild oxidative stress in Fib and enhanced proliferation, aSMA immunopositivity and collagen expression and synthesis. Moreover, it increased the levels of TNF- α and myostatin, an emerging mediator of IS action. The genes encoding angiotensinogen, Ang-converting enzyme, and AT1R were also upregulated in Fib exposed to IS, as well as the gene for neprilysin. Compared with control cells, mNVCM treated with the conditioned medium of IS-primed Fib exhibited higher levels of β -myosin heavy chain and heightened activity of glycolytic enzymes. Val counteracted only part of IS effects.

Conclusions: IS may contribute to CKD-related cardiomyopathy from the early phases of renal dysfunction, by eliciting Fib activation with collagen production, induction of a cell-autonomous

Ang system, and release of paracrine factors driving cardiomyocyte hypertrophy. Dual blockade of neprilysin and AT1R may represent a valid approach to antagonize IS action.

P2.07

Evidence for a crosstalk between ErbB2 and Notch-1 in cardiomyocytes

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Objectives: The ErbB2 and Notch-1 pathways drive cardiomyocyte (CM) proliferation in the first days after birth. Since ErbB2 has been reported to control Notch-1 in cancer, we sought to determine whether such a modulation also occurs in CM and, if so, whether it is involved in early postnatal proliferation.

Methods: Neonatal cardiac cells were harvested from C57BL/6 pups and cultured with or without: i) CP-724714, a selective inhibitor of ErbB2 phosphorylation; ii) neuregulin-1 (NRG-1), an ErbB4 ligand which stimulates ErbB2 signaling via ErbB4/ErbB2 heterodimers; iii) NRG-1 preceded by pre-treatment with CP-724714, DAPT or MK0752 - DAPT and MK0752 block the cleavage of Notch-1 to the biologically active intracellular fragment, NICD1, in response to juxtacrine ligands. Experiments were started 24 h after seeding cells. Expression of total and phosphorylated ErbB2, Notch-1, NICD1 and the main intracellular mediators of ErbB2 signaling was evaluated by western blotting and/or immunofluorescence. Levels of the Notch-1 target genes, *Hey1*, *Hey2* and *Hes1*, were assessed by qRT-PCR. CM proliferation was examined by counting cells co-stained for troponin T and Ki67 in immunofluorescence.

Results: ErbB2 and Notch-1 were mainly identified in troponin T-positive CM. Likely due to a stromal component secreting NRG-1 and expressing Notch-1 ligands, untreated cells displayed some ErbB2 phosphorylation and NICD1 accumulation. The amount of NICD1 was decreased by DAPT or MK0752, but also by CP-724714. Exposure to CP-724714 was also associated with downregulation of Notch-1 target genes. As expected, NRG-1 stimulated the phosphorylation of ErbB2 and Erk1/2, the accumulation of β -catenin, and CM proliferation. Furthermore, it increased NICD1 levels. Both DAPT and MK0752 significantly reduced NRG-1 induced proliferation of CM.

Conclusions: Our preliminary results suggest that ErbB2 regulates Notch-1 activation in CM. On the other hand, Notch-1 contributes to ErbB2 mitogenic effect on neonatal CM. Further work is necessary to characterize this ErbB2/Notch-1 crosstalk.

P2.08

Lipoprotein lipase and transforming growth factor gene polymorphism in patients with atherosclerosis of different severity

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Introduction: The ultimate cause of atherosclerosis has still not been elucidated. Lipid metabolism and inflammation are known

to play an essential role in atherogenesis. Rare genotype in *lipoprotein lipase* (*LPL*) single nucleotide polymorphism (SNP) rs328 and *transforming growth factor* (*TGF*) SNP rs1800469 may associate with predisposition to atherosclerosis. However their impact in atherosclerosis progression remain unclear.

Methods: Total 319 patients aged from 44 to 73 years from central Volga region with angiographically verified atherosclerosis were observed. All patients were divided into groups according to severity of atherosclerotic process based on angiographic data. The first group included patients with severe atherosclerosis with any occlusion and/or two or more arterial stenosis bigger than 70% (191 patient). The second group consisted of patients without any occlusions and with no more than one bad stenosis bigger than 70% (128 patient). *LPL* rs328 and *TGF* rs1800469 genotype was determined in all patients.

Results: Presence of minor -G allele in *LPL* rs328 was much more common in cases with severe atherosclerosis (OR = 1,7 95% C.I. = (0.86-3.4), $P < 0.05$). No significant diversity of *TGF* rs1800469 genotype in different cohorts was found.

Conclusion: Our study revealed no association of *TGF* rs1800469 genotype with atherosclerosis severity. In the same time *LPL* genotype influence on atherosclerotic process was found. However, our analysis revealed that carriers of minor allele are predisposed to highly more severe course of the disease that is contradictory to basic statement about protective role of rare *LPL* SNP's variant. Patients sorting specificity could explain this data. We suppose that such effect may reveal only on final stages of atherosclerotic process, which is found in comparison of patients with different atherosclerosis severity.. The influence of *LPL* single nucleotide polymorphism rs328 on severity of atherosclerosis warrants further investigation.

P2.09

Alamandine inhibits neutrophil-mediated activities in mouse atherosclerosis

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Backgrounds and aims: Considering the critical role of neutrophils as determinants of atherosclerotic plaque vulnerability, their inhibition has been considered as a potential therapeutic strategy. Here, we investigated whether treatment with the recently discovered agonist of the Mas-related G-coupled receptor type D (MrgD) alamandine would be effective in suppressing neutrophil degranulation *in vivo* and *in vitro*.

Material and methods: Fifteen-week-old ApoE^{-/-} mice were fed with a Western-type diet for an additional 11 weeks. After the first 2 weeks, a 'cast' device was implanted to increase the blood shear stress in the common carotid artery. During the last 4 weeks before euthanasia, mice were randomly assigned to subcutaneously receive vehicle (NaCl) or alamandine (24 µg/kg/h) by mini osmotic pump. Neutrophils for *in vitro*

experiments were obtained from 11-week-old ApoE^{-/-} mice after intraperitoneal injection of thioglycollate.

Results: Treatment with alamandine had no effects on chemokine expression or carotid shear stress. Accordingly, alamandine failed to inhibit the recruitment of neutrophils and macrophages within atherosclerotic plaques. Conversely, alamandine administration was demonstrated to suppress serum levels of neutrophil enzymes, such as MMP-9 and MPO. A significant reduction of MMP-9 content was also observed within aortic root plaques, alongside with a suppression of MMP-9 mRNA. Trough PCR analysis, the alamandine receptor MrgD has been identified in mouse primary neutrophils, whereas pretreatment with alamandine was shown to dose-dependently abrogate PMA-induced neutrophil degranulation of both MMP-9 and MPO *in vitro*.

Conclusions: Overall, our results suggest that alamandine is able to inhibit MMP-9 levels in an atherosclerotic milieu. On the other, hand, this effect seems to not contribute to improve histological parameters of plaque stabilization. Further studies are needed to clarify the therapeutic potential of a treatment with MrgD agonists in atherosclerosis.

P2.10

Feasibility of multi-electrode contact measurement as predictor of long-term success with circular pulmonary vein electroporation ablation

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Background: Electrode-tissue contact is an important predictor for the success of pulmonary vein (PV) isolation. With multi-electrode circular ablation catheters, measuring electrode-tissue contact force for each individual electrode is technically challenging.

Objective: In this porcine study, we assessed the feasibility of a novel electrical measurement of electrode-tissue contact for predicting long-term success of circular PV electroporation ablation.

Methods: Individual electrode interface impedance (EII) of a circular decapolar catheter was measured by applying a small current between pairs of neighboring electrodes while measuring the voltage between each electrode and a skin patch. In 10 pigs, one PV was ablated solely based on interface impedance (EII group), the other PV was ablated using local electrogram information (EP group) with the operator blinded for interface impedance. IRE ablations were performed at 200J. After 3 months, recurrence of conduction was assessed.

Results: A total of 43 IRE applications were delivered in 19 PVs. Acutely, no reconnections were observed in either group. After 3 months, 0 vs. 3 ($P = 0.21$) PVs showed recurrence of conduction in the EII and EP groups respectively.

Conclusion: Data of this study suggest that interface impedance predicts effective IRE applications. For the long-term success of electrical PV isolation with circular IRE applications, measurement of electrode interface impedance was at least as good as the classical electrophysiological approach for

determining the need for additional IRE applications. Data of this study also suggest that circular IRE is an adequate ablation technique for electrical PV isolation.

P2.11

Anti-apolipoprotein A-1 auto-antibodies are associated with immunodeficiency and systemic inflammation in HIV patients

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Objectives: To determine the existence of autoantibodies against apolipoprotein A-1 (anti-apoA-1 IgG) in HIV patients and to explore their association with biological features of HIV severity such as cytopenia, detectable viremia (< 40 copy/mL), and different inflammatory biomarkers involved in atherogenesis. Being biologically active molecules, we also evaluated their impact on CD4⁺ lymphocytes survival *in vitro*.

Methods and results: Anti-apoA-1 IgG plasma levels were assessed by ELISA in 237 HIV patients in primary prevention naive from statin therapy. High anti-apoA-1 IgG levels was retrieved in 58% ($n = 138$) of patients and were associated with lower thrombocytes, leucocytes, and CD4⁺ counts, but higher viremia, and higher levels of systemic inflammation. These associations were further confirmed by significant Spearman correlations. Logistic regression analyses indicated that high anti-apoA-1 IgG levels were associated with a 10-fold increased risk of displaying CD4⁺ levels below 200 cells/mm³, independently of viremia and treatment (adjusted Odds ratio [OR]:9.72, 95% Confidence interval [95% CI]: 1.22–77.32; $P = 0.03$), and a 6-fold increased risk of having a detectable viremia, independently of treatment (OR:6.36; 95%CI: 1.97–20.51; $P = 0.001$). *In vitro*, anti-apoA-1 IgG induced a dose and time-dependent CD4⁺ apoptosis that was increased by exposure to HIV RNA, but insensitive to L-type calcium channel, toll-like receptor-2-4 or CD14 inhibition.

Conclusions: In HIV, anti-apoA-1 IgG levels are raised and associated with low thrombocytes and CD4⁺ counts, but high viremia and a pro-inflammatory systemic profile. Experimental evidences showed that *per se* anti-apoA-1 IgG can promote CD4⁺ lymphocyte apoptosis through novel and yet undefined pathways. The clinical implications of these findings require further investigations.

P2.12

Plasma palmitoylethanolamide as potential predictor of coronary dysfunction

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Background and aims: Among endocannabinoid-related mediators, oleoyl-ethanolamide (OEA) and palmitoyl-ethanolamide (PEA), have been recognized to be endogenous PPAR α agonists with lipolytic and anti-inflammatory action, without direct agonist activity on the cannabinoid CB₁ and CB₂ receptors. Here, we assessed the potential association of the circulating endocannabinoids PEA and OEA with the coronary function in a cohort of patients with different body mass index (BMI) (normal, overweight, obese, and morbidly obese [MOB]).

Methods: Myocardial perfusion and endothelium-related myocardial blood flow (MBF) responses to cold pressor test (CPT) and during pharmacological vasodilation with dipyridamole were measured with ¹³N-ammonia positron emission tomography/computed tomography in 107 patients without cardiovascular disease. OEA and PEA were extracted from human plasma by liquid-liquid extraction, separated by liquid chromatography and quantified by mass spectrometry. Serum levels of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 (VCAM-1) were measured by colorimetric enzyme-linked immunosorbent assay.

Results: Circulating levels of PEA and VCAM-1 were increased in MOB as compared to normal weight subjects. Circulating levels of OEA and PEA were independently associated with BMI ($\beta = 0.350$ and $\beta = 0.268$, respectively; $P < 0.05$ for both), but not with adhesion molecule expression. Furthermore, PEA was inversely and significantly correlated with MBF during hyperemia and Δ MBF to CPT in the overall cohort and particularly in MOB patients. According with ROC curve analysis, PEA showed a significant accuracy in predicting worsened coronary function, as assessed by MBF during hyperemia (AUC 0.695; $P = 0.002$) or Δ MBF to CPT (AUC 0.642; $P = 0.018$). Specifically, PEA values ≤ 3.176 ng/mL significantly predicted the best MBF during hyperemia, also in adjusted analysis (OR 0.26 [0.08-0.85]; $P = 0.026$).

Conclusion: Those results indicate PEA as a potential circulating biomarker of coronary dysfunction in both MOB patients and general population. However, alteration in PEA levels has to be considered in an integrated way which considers different lipid classes.

P2.13

Efficacy and safety of renal denervation in a single-center cohort of patients with pharmacological resistant hypertension: features and clinical outcome

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Background: Hypertension is one of the most important cardiovascular risk factors. Despite widespread antihypertensive drugs, a considerable amount of patients do not achieve the recommended blood pressure target values of < 140/90 mmHg even if an adequate pharmacological therapy has been established. The results of various studies on the hypotensive effect of renal denervation (RDN) in patients with antihypertensive therapy have led to controversial results. A retrospective analysis with a follow-up of 6, 12 and 24 months to investigate the efficacy and safety of catheter-based renal artery ablation was performed at the University Hospital Zurich in 41 patients after RDN.

Methods: In addition to medical antihypertensive therapy, RDN using three different ablation systems (Symplicity™-RDN-System, EnligHTN™-Multi-Electrode-Renal-Denervation-System and Vessix™-Renal-Denervation-System) was carried out. The primary endpoint was the reduction of ambulatory blood pressure monitor at 6, 12 and 24 months after RD. The primary safety endpoint was the changes in plasma creatinine levels after 12 and 24 months.

Results: The mean systolic blood pressure reduction after 6, 12 and 24 months was 28.0 ± 4.1 mmHg and 31.3 ± 4.2 mmHg and 35.4 ± 3.5 mmHg. There were no complications after the RDN nor any relevant adverse vascular, renal or cardiovascular events were observed.

Conclusions: The data presented in this single center registry confirm that the RDN is an efficient and safe treatment option for hypertension. Whether this new therapy option leads in the long term to a reduction of the cardiovascular mortality, will definitely show through future studies.

P2.14

Sirtuin 5 deletion confers cerebral protection by attenuating BBB disruption in mice following middle cerebral artery occlusion

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Introduction: Sirtuin 5 (SIRT5) is a metabolic regulator inducing post-translational modifications of proteins. Multiple (patho-) physiological functions of SIRT5 are currently emerging. Herein, we sought to investigate the role of SIRT5 in ischemia reperfusion-induced brain injury using a murine model of stroke, an in vitro model of human brain microvascular endothelial cells (HBMVECs) as well as peripheral blood monocytes (PBMs) from ischemic stroke patients.

Methods: SIRT5 knockout (SIRT5^{-/-}) and wild type (WT) mice were subjected to transient middle cerebral artery occlusion

(MCAO) for 45 min followed by 48 h of reperfusion. Infarction, neurological deficits, IgG extravasation and tight junction expression were examined 48 h after MCAO. Finally, in order to test the relevance in human cells, HBMVECs were transfected with either SIRT5 siRNA or scrambled and exposed to 4 h hypoxia followed by 4 h reoxygenation. In addition, SIRT5 expression was determined in PBMs of ischemic stroke patients and compared to age-, sex- and risk factor-matched control subjects.

Results: SIRT5^{-/-} displayed smaller infarct volumes compared to WT mice after 48 h of reperfusion (19.6 ± 11.02 vs 40.93 ± 9.96 % $P = 0.0005$). The decrease in infarction was functionally relevance since SIRT5^{-/-} also displayed improved neurological deficits at 48 h compared to WT mice. Blood brain barrier (BBB) permeability was also decreased in SIRT5^{-/-} as well as the degradation of the tight junction occludin. Likewise, knockdown of SIRT5 decreases brain endothelial permeability and increases occludin expression in HBMVECs. However, this effect was prevented in the presence of the PI3K inhibitor, wortmannin. Finally, SIRT5 isoform 1 gene expression is PBMs of patients with ischemic stroke and correlates with stroke severity. **Conclusions:** Deletion of SIRT5 improves stroke outcome including stroke size and neurological deficits by attenuating blood brain barrier disruption and occludin degradation in a murine model of stroke.

P2.15

Validation of a novel method for electrode-tissue measurements with multi-electrode catheters

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Introduction: Adequate electrode-tissue contact is important for the effectiveness of pulmonary vein ablation. In a previous study we have shown that the electrode-interface resistance (IR) is a good electrical indicator for measuring electrode-tissue contact with circular multi-electrode catheter. With IR, a drive current is applied between two neighbouring electrodes while the voltage is measured between the target electrode and a skin patch. Stimulation thresholds are influenced by quality of electrode-tissue contact. In this study we sought for other IR characteristics that influence stimulation thresholds. Movement of the catheter caused by breathing and heart contraction led to fluctuations in IR values. Large fluctuations could influence stimulation thresholds.

Methods: In four pigs a 7F circular octopolar catheter, was positioned inside the left atrium at several locations. Stimulation thresholds were determined for each electrode separately while simultaneously measuring IR for each electrode. Standard deviations of the IR values were compared with stimulation thresholds.

Results: The average IR value for no-contact was 81.2 ± 2.6 Ω. Impedance increase and the stimulation threshold correlated significantly, $\rho = -0.47$ ($P < 0.001$). There was no significant correlation between the standard deviation of the IR values and the stimulation threshold, $\rho = 0.17$ ($P > 0.05$).

Conclusion: While the correlation between IR and stimulation threshold was significant, fluctuation of the IR values did not

influence this correlation. Sites without capture showed a significantly lower impedance increase than sites with capture, indicating IR values are influenced by quality of the contact. In combination with previous work we can conclude IR measurements show good potential for quantification of electrode-tissue contact.

P2.16

The visceral adiposity index predicts cardiovascular events both in coronary artery disease patients with and in coronary artery disease patients without diabetes

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Aim: The visceral adiposity index (VAI) is a validated tool for the evaluation of visceral adiposity, using waist circumference, serum triglycerides, age and gender to diagnose this metabolic abnormality. It has recently been associated with cardiovascular risk in primary care patients. No data are available on the association of the VAI with mortality in patients with established CAD.

Methods: We therefore calculated the VAI in 1472 consecutive patients with angiographically proven stable CAD according to the Amato formula. T2DM was defined according to the ADA definition. The incidence of vascular events was recorded over 10 years.

Results: At baseline, the VAI was significantly higher in CAD patients with T2DM than in those without diabetes (362 ± 330 vs. 247 ± 224 ; $P < 0.001$). Prospectively, 539 vascular events occurred; the event rate were significantly higher in patients with T2DM than in those who did not have diabetes (44.8% vs. 33.7%; $P < 0.001$). The VAI significantly predicted cardiovascular events in CAD patients with T2DM (standardized adjusted hazard ratio (HR) 1.16 [1.01-1.33]; $P = 0.037$) as well as in those without T2DM (HR 1.14 [1.02- 1.27]; $P = 0.018$).

Conclusion: We conclude that the VAI predicts cardiovascular events both in CAD patients with and in CAD patients without diabetes.

P2.17

Evaluation of the mitochondrial transition pore opening in experiment and clinic as an early diagnostic tool for severity of myocardial ischemic injury

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Mitochondrial permeability transition pores (MPTP) are well-considered target for prevention of reperfusion damage. We developed the procedure for evaluation of "opened"/"closed"

state of MPTP by measuring the levels of mitochondrial factor (MF) which is the mixture of substances of purine nucleotides origin released from inner mitochondrial space into solutes outflow from isolated heart and patients' blood.

Coronary outflow samples were collected from the Langendorff isolated rat hearts before and after ischemia or ischemic preconditioning. Blood samples were collected in healthy individuals and patients before and during surgery of myocardial revascularization, then centrifuged at 3000 rpm for 15 min. Plasma was separated and proteins precipitated with 50% trichloroacetic acid. Next, the samples were centrifuged again, and optical density (OD) was measured in UV. The levels of MF were calculated as difference in OD before and after ischemia/reperfusion. Standard blood biochemical tests (creatinine phosphokinase, lactate dehydrogenase, MB-creatinine phosphokinase, aspartate aminotransferase) were performed.

Ischemia/reperfusion of the isolated heart induced considerable increase of MF in solutes collected at the 1st min of reperfusion. That was greatly prevented by IPC as well as by cyclosporine A or sanglifehrin A pretreatment. Furthermore, mitochondria isolated from ICP hearts showed decreased sensitivity to MPTP inductor – calcium ions indicating increased resistance to ischemia/reperfusion damage. In clinic, MF levels were significantly lower in healthy individuals aged 18-34 comparing to the older ones (35-58y.o.). Patients with coronary artery disease (60.5 ± 12.9 y.o.) showed double increase in MF comparing to healthy mates. 5 min of reperfusion of the heart at surgery with cardioplegia induced increase of MF levels ($P < 0.01$), however, surgery without cardioplegia decreased the MF levels ($P < 0.001$). MF levels had strong correlation with blood enzymes levels.

Evaluation of MF levels in outflow solution or blood samples seems to be very promising for early diagnostics of ischemic damages in experimental and clinical practice.

P2.18

Rapid restoration of cardiac function in catecholamine-induced cardiomyopathy after pheochromocytoma resection

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Background: Pheochromocytoma is a rare catecholamine secreting tumour with heterogeneous clinical presentation. We report how complete restoration of cardiac function and morphology was very rapid after surgical excision of the tumour

Methods and results: A 45-year-old man was admitted to our ward for dyspnoea and fatigue in daily activity, episodes of palpitations, headache and irritability, sweating, anxiety and weight loss, began 3 months before. He was a smoker, his medical history was positive for arterial hypertension. Physical examination was negative except for high blood pressure values. Electrocardiogram showed sinus rhythm and signs of left ventricular hypertrophy. Blood tests showed hyperglycaemia with high glycated haemoglobin (54 mmol/mol), elevated NT-proBNP (246 pg/mL). Transthoracic echocardiography showed left ventricular dilatation and eccentric hypertrophy with an ejection fraction of 40%. Abdominal ultrasound revealed an incidental mass above the right adrenal gland. A catecholamine-releasing tumor was suspected: 24-h urinary levels of total catecholamine and normetanephrine were high. Abdominal

magnetic resonance was performed and confirmed the presence of a heterogeneously enhancing mass in the right adrenal gland. The patient was scheduled for laparoscopic resection of the adrenal mass, whose histological analysis was compatible with pheochromocytoma. At the six-months follow-up visit the patient reported a great improvement of his clinical conditions: blood pressure was well-controlled and electrocardiogram showed a significant reduction in abnormalities. Transthoracic echocardiography showed a complete regression of left ventricular hypertrophy and improvement of left ventricle size and function (ejection fraction 58%). These features suggested the diagnosis of "Catecholamine-induced cardiomyopathy" due to pheochromocytoma.

Conclusions: In the presence of a dilated and hypokinetic heart in an hypertensive patient, a catecholamine-secreting tumour has to be suspected, since surgical treatment can lead to complete regression of the cardiomyopathy. Peer-reviewed guidelines are still needed to guide the investigation and management of suspected catecholamine-induced left ventricular dysfunction.

P2.19

Prevalence of pulmonary hypertension in the echocardiographic laboratory

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Background and aim: Pulmonary hypertension is defined as an increased mean pulmonary arterial pressure ≥ 25 mmHg at rest assessed by right heart catheterization. It can be caused by several clinical conditions, and the different etiologies require distinctive management, specific therapies and have different prognosis.

Transthoracic echocardiography is commonly used for initial assessment of pulmonary hypertension, moreover it plays an important role in the identification of the underlying aetiology. Pulmonary arterial systolic pressure (PASP), estimated by tricuspid regurgitation's flow velocity, considering right atrium dimension and diameter and collapse with inspiration of inferior vena cava, is one of the most common parameters employed. Estimated PASP has high sensitivity, specificity and negative predictive value.

Nowadays, few data about pulmonary hypertension prevalence among patients who undergo transthoracic echocardiography are available. The aim of this retrospective study is to evaluate the prevalence of elevated PASP, defined as an estimated PASP ≥ 40 .

Methods: We enrolled patients referred to our echocardiographic laboratory for transthoracic echocardiography, from January 2011 to January 2014. Patients who had elevation in PASP underwent to clinical, laboratory and instrumental investigations to be further categorized in each aetiological group.

Results and conclusions: We evaluated 3684 patients: 2695 (73%) of these had normal values of PAPS, 332 (9%) had increased one. In the selected patients with pulmonary hypertension, Pulmonary Arterial Hypertension prevalence (Group 1) was 4.7% instead PH secondary to left heart diseases (Group 2) was 83.6%. Group 3, 4 and 5 are less frequent (9.5%, 1.6%, and 0.6%, respectively). Concerning the general characteristics of the groups, patients with Group 1 pulmonary hypertension were younger (mean age 57.1 ± 15.1 years) and mostly female (83.3%).

In our echocardiographic laboratory, pulmonary prevalence was 9% according to other reports in the literature.

These results confirm that Group 1 pulmonary hypertension is a rare disease that affects mainly young women.

P2.20

Fusobacterium nucleatum as a rare cause of acute pericarditis and pericardial effusion

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In March 2015, a 42 years-old woman was admitted to our ward for fever, cough and pleuritic pain, started few weeks before. Upon admission, the patient had fever, sinus tachycardia, hypotension (90/60 mmHg), tachypnea and 96% O₂-saturation in room air. Her medical history was negative. On physical examination, heart sounds were parafonic and ECG revealed sinus tachycardia and ST-alterations in multiple leads.

Blood tests showed normochromic normocytic anemia, neutrophilic granulocytosis, elevated values of troponin and C reactive protein. A CT-scan revealed bilateral pulmonary consolidations, pleural effusion and a large pericardial effusion. In few hours the clinical picture worsened, since the patient developed marked hypotension and increase in transaminases, so we performed an urgent trans-thoracic echocardiogram that confirmed a severe posterior pericardial effusion (6x10 cm) with initial signs of pericardial tamponade. An urgent evacuative pericardiocentesis was performed, with positioning of a pericardial drainage. The drained material had a purulent aspect. Its microbiological exam was positive for *Fusobacterium nucleatum*. TB test, HIV-test, C. Pneumoniae, Toxo anti-bodies and blood cultures excluded other etiology. A therapy with piperacillin-tazobactam and daptomycin was started and the drainage was removed after 72 h. After 3 weeks, patient's clinical conditions were improved and pericardial effusion was almost absent on follow-up echocardiography.

Acute pericarditis is a clinical syndrome characterized by inflammation of pericardial leaflets and often associated with pericardial effusion; in developed country, it is caused mainly by viral agents (85-95%) while bacterial pericarditis is relatively uncommon in clinical practice, accounting only for < 1% cases. Among bacterial etiology, a new emergent species, even in healthy patients, is *Fusobacterium nucleatum*, an anaerobic bacterium commonly found as normal mucosal associated flora of the oral cavity. A prompt diagnosis, an adequate management of the possible complications (like cardiac tamponade) and a targeted antibiotic therapy are mandatory since they may affect patients' prognosis.

P2.21

Comparison of redo surgery to percutaneous valve-in-valve replacement in patients with failed aortic bioprosthetic valves

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Background: Repeat cardiac surgery to change failed aortic bioprosthetic valves is associated with significant morbidity and mortality. Since 2007, percutaneous valve-in-valve (VinV) replacement has emerged in high-risk patients.

Purpose: We sought to compare in-hospital and mid-term mortality and outcomes of patients who underwent redo aortic valve replacement (AVR) versus transcatheter VinV implantation in failed surgical aortic bioprosthetic valves.

Methods: We retrospectively reviewed consecutive patients, who underwent redo AVR or VinV replacement excluding active infective endocarditis, history of Ross surgery and multi-valvular disease from January 2006 to February 2016 in Bichat hospital for failed aortic bioprosthetic valves (aortic regurgitation grade > 2 and/or effective orifice area < 1 cm² according continuity equation).

Results: 234 consecutive patients with failed surgical aortic bioprosthetic valves were referred to our institution. We finally enrolled 128 patients: 45 underwent transcatheter VinV implantation and 83 underwent redo AVR. VinV group were significantly older (62 ± 19 versus 77 ± 13, $P < 0.0001$), had more previous CABG (36% versus 11%, $P = 0.002$), kidney impairment according to Cockcroft creatinine clearance (56 ± 28 mL/min versus 85 ± 47 mL/min, $P = 0.0005$), and atrial fibrillation (36% versus 17%, $P = 0.03$). Consequently, VinV group had higher risk scores than redo AVR group: mean logistic EuroSCORE 26 ± 15 % versus 18 ± 16%, $P = 0.0004$, mean EuroSCORE 2 13 ± 10% versus 10 ± 13%, $P = 0.002$ and mean STS Score 7 ± 6% versus 4 ± 6%, $P = 0.0001$ respectively. Overall mortality according to Kaplan-Meier survival test was 22% ($n = 10$) in VinV group and 17% ($n = 14$) in redo AVR group, $P = 0.38$. In-hospital and mid-term complications were not significantly different between the groups.

Conclusion: VinV may appear a promising alternative to redo surgery for patients with failed aortic bioprostheses. Future prospective studies are necessary to determine the place of TAVI VinV for failed aortic bioprostheses.

P2.22

Effect of polyphenol resveratrol on the isolated renal artery of normal and diabetic rats

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Aim: Resveratrol is a phytoalexin present in many different types of plants. It is suggested that chronic treatment of type 2 diabetes mellitus with resveratrol attenuates the inflammatory injury of the vascular wall. The aims of this study were to investigate the effect of mechanisms by which resveratrol relaxes the renal artery (RA) of normal and diabetic rats and testing the involvement of K-channels in this mechanism.

Methods: Samples of the renal arteries were isolated from normal and diabetic Wistar rats in which diabetes was induced by alloxan. Samples were mounted into organ bath for recording isometric tension. Resveratrol (1–100 μM) was added to the bath cumulatively. In order to test the involvement of K-channels in the mechanism of action of resveratrol, a selective blockers were used: for voltage-gated channels (Kv), margatoxin and phrixotoxin; for calcium-dependent big channels (BK_{Ca}) iberiotoxin, and for ATP-sensitive channels (K_{ATP}) glibenclamide. Experiments followed a multiple curve design. The presence and distribution of subtypes of K-channel was determined by immunohistochemical staining specific anti bodies.

Results: Resveratrol relaxed RA. Iberiotoxin, margatoxin and phrixotoxin antagonized the effect of resveratrol on RA of non-diabetic rats. However, only margatoxin antagonized the

effect of resveratrol on RA of diabetic rats. Immunohistochemical staining showed the presence of K_{ATP}, BK_{Ca} and Kv in RA of normal rats and only Kv1.3 subtype in RA of diabetic rats.

Conclusions: In conclusion, we have shown that resveratrol induced endothelium-independent relaxation of RA of normal rats, and that margatoxin-sensitive K-channels (kv1.3) are involved in this relaxation.

P2.23

SOD1, SOD2 and GPX1 genes expression in different form of atherosclerosis

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Introduction: Reactive oxygen species (ROS) are involved in the different living processes and low concentrations of ROS may be beneficial in intracellular signaling and antimicrobial defense but high amount of ROS plays a certain role in the human disease triggering, including atherosclerosis. Antioxidant system (AOS) including enzyme that serves as a safeguard against the accumulation of ROS but mechanisms of AOS dysregulation of in atherosclerosis of different localization still unclear and the results of studies are contradictory. So, the aim of this study was to examine the AOS enzymes gene activity (SOD1, SOD2, GPX1) in patients with cardiovascular diseases.

Materials and methods: Gene expression analysis was carried out on venous blood samples from 48 patients with confirmed multifocal atherosclerosis (IPA), 46 patients with acute coronary syndrome (ACS), 47 patients with risk factors for cardiovascular disease (RFCD) and 16 healthy donors. The relative gene expression level (RQ) evaluated by the 2deltaCt method and Student t-test ($P = 0.05$).

Results: Our results showed a lowering gene expression for all three studied genes ($RQ < 1$, $P < 0.05$) in both groups with different localization of atherosclerosis lesions (IPA and ACS) that can be possibly explained by exhaustion of antioxidant potential during atherosclerotic complications development and possibly due to chronic process and the area of atherosclerotic lesions in IPA patients. In patient with RFCD we also detected decreasing in activity of two AOS genes – SOD1 and GPX1 ($RQ < 1$) against the backdrop of increasing the activity of a SOD2 gene ($RQ 3.28$) but those differences were not statistically significant which may due varying of AOS activity due to some environmental factors not associated with atherosclerotic process.

Conclusion: Thus, gene expression study of AOS enzymes may have a prognostic value of AOS exhaustion in patients with atherosclerosis.

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P2.24

Silencing of the activated protein-1 transcription factor JunD exacerbates ischemia/reperfusion-induced cerebral injury

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Background: The activated protein-1 transcription factor JunD mediates inflammation, apoptosis and oxidative stress, which are crucial components to ischemia/reperfusion brain damage. In this study we investigate the role of JunD in ischemia/reperfusion-induced brain injury using a mouse model of ischemic stroke, primary human brain microvascular endothelial cells (HBMVECs) as well as peripheral blood monocytes (PBMs) from ischemic stroke patients.

Methods: Twelve-week-old male C57BL/6 mice underwent JunD silencing using small interfering RNA (siRNA) before transient (45 min) middle cerebral artery occlusion (MCAO) followed by 24 h of reperfusion; stroke size was assessed by TTC staining and neuromotor function by neurological score and Rotarod tests and compared to scramble siRNA-injected control animals (siScr, $n = 8$). Lastly, we examined vascular JunD responsiveness to ischemia/reperfusion in C57BL/6 wild-type (WT) mice, to hypoxia/reoxygenation in HBMVECs, and to ischemic stroke in PBMs isolated from patients.

Results: JunD silencing provided a $40 \pm 8\%$ reduction of JunD protein expression in murine aortae. After transient MCAO, JunD-silenced mice showed increased stroke volumes (siScr, $42 \pm 7 \text{ mm}^3$, siJunD, $69 \pm 9 \text{ mm}^3$) and decreased neuromotor function, compared with control animals. After transient MCAO, JunD expression was decreased in middle cerebral arteries of WT mice. Similarly, JunD was reduced in HBMVECs exposed to hypoxia/reoxygenation and in PBMs of ischemic stroke patients after 24 h of symptom onset, as compared to age- and sex-matched control subjects.

Conclusions: Brain ischemia/reperfusion reduces vascular JunD expression in WT mice as well as in primary human cerebrovascular endothelial cells and monocytes from patients with ischemic stroke. JunD silencing in mice augments stroke volumes and neuromotor deficits suggesting its involvement in the pathogenesis of ischemia/reperfusion-induced brain injury.

P2.25

Advanced oxidation protein products-modified albumin induces differentiation of RAW264.7 macrophage cell line into dendritic-like cells: role cell surface thiols

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Background: Advanced Oxidation Protein Products (AOPP) include oxidative modifications of proteins that mainly occur through myeloperoxidase-derived hypochlorite (HOCl). Local accumulation of AOPP induces pro-inflammatory and pro-fibrotic processes in patients with chronic kidney disease (CKD). In addition, circulating AOPP may be regarded as mediators of systemic oxidative stress, promoting the progression of atherosclerotic damage of arterial walls. Dendritic cells (DCs) are key cells in this process, due to their role in antigen presentation, inflammation resolution and T cell activation. Our work aims to evaluate whether AOPP-proteins induce activation and differentiation of macrophages into dendritic cells in vitro and the role of cell surface thiol groups and of free radicals in this process.

Methods: AOPP-proteins were prepared by in vitro incubation of human albumin (HSA) with HOCl. Mouse macrophage-like RAW264.7 were treated with AOPP-HSA with or without NAC. Following 48 h of HSA-AOPP treatment, morphology was evaluated with microscopic observation, while markers of dendritic lineage and activation (CD40, CD86, MHC classII) and allogeneic T-cell proliferation by flow cytometry. Cell surface thiols were determined as AlexaFluor-maleimide binding, ROS production as DCF fluorescence, hypodiploid DNA and cell cycle progression as Propidium Iodide staining by flow cytometry.

Results: HSA-AOPP induced the differentiation of RAW264.7 cells into a dendritic-like phenotype, as evidenced by morphological changes, by increase in CD40, CD86 and MHC classII surface expression as well as by induction of T cell proliferation. HSA-AOPP treatment decreased cell surface thiols and increased ROS production and the S-phase of the cell cycle. NAC prevented the AOPP-dependent surface thiols decrease, reduced ROS production, limited the cell cycle progression and the differentiation of RAW264.7 into DC-like cells.

Conclusions: Oxidized plasma proteins modulate immune responses of macrophages through a process involving thiol redox equilibrium. This mechanism may play a role in the rapid progression of atherosclerosis observed in CKD patients.

P2.26

Translational cardiovascular regenerative research: a call for a paradigm shift

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Potential cell products and potential new strategies have been successfully investigated in preclinical research. However, the translation into a successful registration of a therapy remains difficult. This is partly explainable by differences between

preclinical models and human beings. However, suboptimal quality of preclinical research and limited collaboration and sharing of knowledge also contributes to translational failure.

Stage-specific focus in preclinical research: In order to decrease translational failure, we should create a preclinical framework with a stronger translational focus. Therefore, pre-clinical research should be subdivided in three stages. Stage I aims to discover and develop a lead cell product and is mainly based on in vitro studies and zebrafish models. Stage II studies are exploratory studies in which one aims to establish a product and investigate safety, predominantly by using rodent models. Stage III are confirmatory studies which focus on large animal models and aim to investigate feasibility and efficacy. Within each stage one should focus on its specific strengths, and optimization of animal models and protocols should be part of pre-clinical research.

Rigor multicentre preclinical studies: Confirmatory studies should be considered as the preamble of clinical trials. These preclinical studies should be performed in a rigor multicentre large animal model setting. We need multicentre consortia, focusing on quality, rigor, standardization and reproducibility. All confirmatory studies should be registered in a prospective registration. This would be a first step towards the solution of publication bias in preclinical research, leading to a better translation into the clinical arena and will decrease the unnecessary repetition of animal research. The prospective registry should contain the major features of the study and should be managed by an independent surveillant in order to maintain a safe harbour. Protocols should be disclosed after set period of time.

P2.27

Aortic remodeling after hybrid treatment: a quantitative geometrical analysis

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Introduction: Hybrid arch (HA) repair is a less-invasive treatment that combines open and endovascular procedures to treat aortic arch (AA) diseases. Aim of the study is to evaluate whether thoracic stent grafts modify the physiological shape of AA after HA treatment.

Methods: A retrospective study was conducted on 26 HA cases (22 aortic aneurysms, 4 type-B aortic dissections) collected between 2012 and 2016 in Vascular and Endovascular Unit, IRCCS S. Martino, Genova. For each patient, the pre- and the first post-operative CT were analyzed: pre- and post-operative lumen and stents were reconstructed and registered in order to be superimposed. When the pre-operative 3D geometries involved saccular aneurysms, a virtual surface that excludes the aneurysm sac was created. Then, the two centerlines were created, from the aortic valvular plane to the plane passing the celiac tripod. The curvature parameter was introduced to characterize the centerline and evaluated as a function of the curvilinear abscissa s , which measures an approximate distance along the vessel. The punctual curvature was then evaluated in two characteristics points along the centerline: zone proximal (P1) and zone distal to the stent (D1). In case of 2 or more stents partially superimposed, evaluation was also performed in the proximal (P2) and distal portion (D2) of the overlapping zone. Global tortuosity of the AA segment and difference of centerline length were also quantified.

Results: Percentage of stent coverage was $59.60 \pm 17.14\%$, and 1.81 ± 0.69 stent grafts were used per patient. After intervention, centerline curvature was significantly greater in

correspondence of both P1 ($P < 0.05$) and D1 ($P = 0.01$). An average increase of the curvature was also observed in P2 ($P = 0.055$) and D2 ($P = 0.001$). Centerline length significantly increased (385.6 to 390.1 mm, on average). In contrast, tortuosity did not change significantly.

Conclusions: Our experience suggests that the physiological shape of the AA was altered after HA treatment.

P2.28

Mid-term follow-up geometrical analysis of thoraco-abdominal aortic aneurysms treated with multilayer flow modulator

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Objectives: Aim of the study is the analysis of the clinical results and aneurysmal sac evolution after placement of Cardiatis Multilayer Flow Modulator (MFM), in patients with thoraco-abdominal aneurysms (TAA).

Methods: All patients with asymptomatic TAA treated between October 2012 and November 2014 with MFM at the Vascular and Endovascular Unit, IRCCS San Martino-IST University Hospital, Genoa were retrospectively analyzed. 30 day evaluated outcomes were mortality and complications. Follow-up evaluated outcomes were mortality, aneurysm collateral branches patency and reintervention. A geometrical analysis of 2 years follow-up contrast-enhanced computed-tomography (CT) scans was carried out in order to evaluate the total aneurysm volume, the percentage of aneurysm growth and the evolution of maximum aneurysm diameter.

Results: 7 patients (3 male and 4 female, mean age: 71.8 years, range: 63-85 years) were considered in the study. Mean pre-operative aneurysm diameter was 6.8 cm (range 6-8.3 cm). No 30-day mortality or complications were observed. The mean clinical follow-up was 29.4 months. During the follow-up three deaths (42.8%) were observed, not related to aneurysm sac rupture or MFM complications. Reintervention rate was 42.8% (3 patients), occurred in all cases after two-year follow-up; in two cases the reintervention was necessary due to an excessive increase of the aneurysmal sac. During the follow-up, a mean growth rate of 6 mm/year (4 patients) for the diameter of the aneurysm external wall and a total aneurysm mean volume increase from $2.45 \times 10^5 \text{ mm}^3$ to $3.50 \times 10^5 \text{ mm}^3$ (4 patients) was evaluated.

Conclusions: Our results have shown no mortality related to aneurysm rupture during the follow-up and high rate of reinterventions after placement of MFM, partially linked to the growth of the aneurysm sac. Further geometrical analyses, based on the proposed approach, regarding a larger group of patients with long-term follow-up are required to draw indications about the MFM use.

P2.29

Patient-specific finite element analysis of transcatheter aortic valve implantation: a clinical case

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Introduction: Despite transcatheter aortic valve implantation (TAVI) has recently become the new standard medical treatment for patients affected by severe aortic valve (AV) stenosis who are not eligible for open-heart surgery, there are still some complications related with TAVI. For clinicians, such complications are difficult to predict due to patient variability, especially in terms of AV geometry, distribution and dimension of calcific plaques. For this reason, clinical operators look with enormous interest at tools able to give predictive evaluation of the prosthesis post-operative performance. This work concerns the implementation and validation of a systematic approach to perform patient-specific finite element analyses (FEA) with the aim of virtually reproduce the TAVI procedure and predict post-intervention outcomes. Focus is given on the patient-specific modeling of AV: in particular how the assumptions regarding the AV material model and properties, and its discretization strategy influence the outcomes of the structural FEA.

Methods: An "high-fidelity" model of a Medtronic CoreValve size 29 prosthesis is reconstructed from micro-CT images. CT images of a 76yo patient are used as starting point to create a patient-specific model of the AV, consisting of aortic root wall, leaflets and calcific plaques. Intraoperative angiographic measurements are used to correctly replicate with FE the real implantation procedure. The impact of modeling choices of the aortic district on simulation outcomes is investigated through a comparison between the simulation outcomes and the post-operative CT data.

The numerical simulations of TAVI are then performed using Abaqus 6.13 software.

Discussion: Although limited to only one patient, this study represents a further step towards the use of realistic computer-based simulations for virtual planning of TAVI procedures.

Our results indicate that specific modeling choices, such as the patient's calcifications, the AV material models and the discretization size have non-negligible effects on the simulation outcomes and, as a consequence, on the clinical indications.

P2.30

Longitudinal echocardiographic and clinical follow-up of patients undergoing mitral valve surgery without concomitant tricuspid valve repair

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Background: In patients with mild to moderate functional tricuspid regurgitation (TR) and absence of right ventricular dysfunction or tricuspid annulus (TA) dilatation, there is currently no indication for concomitant tricuspid valve (TV) repair during elective mitral valve (MV) surgery. However, long-term results

are conflicting. Here, we sought to determine the rate of progression in TR after MV surgery and the role of MV etiology.

Methods: Elective patients for MV surgery without concomitant TV repair were retrospectively analyzed with longitudinal echocardiographic and clinical follow-up, focusing on TR progression and MV etiology. Linear regression analysis was performed for change in TR at follow-up, using pre-determined variables and confounders.

Results: In total 213 patients without TV repair were analyzed. Development of more than moderate TR after 3.9 ± 2.5 years was rarely seen (2 patients, 0.9%). Overall, median TR grade pre- and late postoperative were equal ($P = 0.059$). Subanalysis showed no significant difference in MV etiology subgroups. Pre-operative TR grade and male gender were inversely correlated to change in TR. Mortality was not influenced by the TR severity at follow-up.

Conclusion: Our data showed that in a study population of patients with mild to moderate TR undergoing MV surgery without concomitant TV repair, significant late TR was rarely seen. Based on our study, it is safe to waive concomitant TV repair in this specific patient cohort.

P2.31

Atypical form of Kawasaki disease in an elderly womanD. Moustapha, H. Karim, M. Pascal & A.D. Von Düring
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Kawasaki disease is an acute febrile multisystem vasculitis of unknown etiology affecting most often children younger than 5 years of age. It is a unique clinical symptom complex characterized by persistent high fever, bilateral conjunctival hyperemia, and mucosal changes of the oropharynx, erythematous rash, erythema and indurative edema of the hands and feet, and cervical lymphadenopathy. Although Kawasaki disease is self limited, approximately 20-25% of untreated patients develop coronary artery changes, with a range of severity from asymptomatic coronary artery dilatation or aneurysm to giant coronary artery aneurysms with thrombosis, myocardial infarction and sudden death.

We reported an atypical case of an elderly woman of 86 years old, with a history of atrial fibrillation, hypertension and dyslipidemia, admitted for acute heart failure (proBNP 5200). An echocardiogram found inferior hypokinesia with a globally low normal left ventricular global function with diastolic dysfunction. She had mildly elevated troponin levels (68 ng/L troponin T-hs). An angiogram, surprisingly showed a giant aneurysm of the right proximal coronary artery and the left proximal artery descending [Fig A and B].

For patients with Kawasaki disease and coronary artery changes, the prognosis depends on the size of the aneurysms. If the aneurysm is very large, it's recommended to put the patient under anticoagulant treatment in order to prevent thromboembolic events.

P2.32

Implementation of a cardio-oncology healthcare pathway: a retrospective analysis of the first hundred patients

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Background: Anthracyclines are currently the cornerstone in the treatment of numerous haematological and solid cancers. However, one of the most feared side effects of these agents is cardiotoxicity. We have set up a specialized cardio-oncology healthcare pathway to improve the cardiac outcome in oncology patients by (1) identifying patients at high-risk of developing chemotherapy-related cardiac dysfunction (CTRCD) (2) screen and monitor high-risk patients to enable early detection of (sub-clinical) cardiac dysfunction and thereby (3) facilitate early treatment initiation.

Objective: To analyse the incidence of CTRCD, validate the Cardiovascular Risk Score (CRS) model and assess cardiac

outcome in patients that were referred to our cardio-oncology outpatient clinic between April 2015 and January 2016.

Methods: The Cardiovascular Risk Score (CRS) as proposed by the Mayo Clinics, was determined to estimate baseline CTRCD risk. All patients underwent extensive (strain) echocardiographic evaluation once every 3 months. Primary outcomes were defined as the development of CTRCD (LVEF < 45%) and a decline in global longitudinal strain (GLS) to < 18%.

Results: In total our cohort consisted of 100 patients, 55 males and 45 females. All patients were analysed within 3 months after having finalized their chemotherapeutic regimen and followed up to one year. LV impairment (LVEF < 45%) was seen in 11% of patients, LVEF < 50% in 27% and an abnormal GLS in 18%. All patients that developed CTRCD had a CRS ≥ 4 , with the highest proportion of CTRCD in patients with a CRS > 6. A reduced GLS was seen in > 50% of all patients with a CRS score of ≥ 4 , which may precede cardiac dysfunction on follow-up. Importantly, in patients with a CRS < 4, no CTRCD or abnormal GLS was observed.

Conclusion: The CRS model discriminated between high- and low-risk patients. Our new cardio-oncology healthcare pathway facilitated the early identification of all patients (11%) that developed CTRCD on follow-up.

Workshop 3: Diabetes with Italian Society of Diabetes

P3.01

Effect of pinacidil on the human saphenous veins obtained from patients with and without type 2 diabetes mellitus

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Aim: Although the mechanisms by which type 2 diabetes mellitus (T2DM) increases cardiovascular complications is not entirely explained, the change in the function of vascular smooth muscle is one of them. *In vitro* studies on animals have shown that the diabetic vascular dysfunction is associated with the reduced activity of the smooth muscle potassium (K^+) channels. Thus, the aim of our study was to investigate the involvement of the different K^+ channels in the relaxant effect of pinacidil on the human saphenous veins (HSV) obtained from the patients with and without T2DM.

Method: The rings of HSV obtained from the patients undergoing a coronary bypass surgery, were mounted in an organ bath system and an isometric tension was being recorded. The experiments followed a multiple curve design. The relaxation of HSV was produced by pinacidil, a K^+ channel opener. The HSV was precontracted with phenylephrine.

Result: Pinacidil produces comparable effects on the HSV from the patients with and without T2DM. The effect of pinacidil on the HSV from non-diabetic patients was antagonized by glibenclamide ($P < 0.001$), a selective blocker of ATP-dependent K^+ (K_{ATP}) channels and 4-aminopyridine (4-AP), a nonselective blocker of voltage-dependent K^+ (K_v) channels ($P < 0.05$), while margatoxin, a selective blocker of $K_v1.3$ channels did not inhibit the pinacidil effect. Tetraethylammonium, a nonselective blocker of Ca^{2+} -activated K^+ (KCa) channels and ibertotoxin, a selective blocker of large-conductance KCa channels did not antagonize pinacidil effects on the HSV from both groups of patients. However, the effect of pinacidil on HSV from diabetic patients was only antagonized by glibenclamide ($P < 0.05$).

Conclusion: Pinacidil produces dilatation of HSV from the patients with and without T2DM by activation of vascular K_{ATP} channels. However, in patients without T2DM, the 4-AP-sensitive K^+ channels are involved in pinacidil effects on HSV also.

P3.02

Platelets from diabetic patients show increased microparticle shedding and higher levels of Peroxiredoxin-2 and Heat shock cognate 71 kDa

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Background: Circulating microparticles (cMPs) are related to the pathogenesis of atherothrombosis. Hyperglycemia and/or

insulin resistance leads to increased oxidative stress. The pathophysiological changes elicited by diabetes may affect platelet function and reactivity, increasing the risk of atherothrombosis.

Purpose: To investigate the differential microparticle shedding and proteomic profile of platelets from diabetic patients treated as per guidelines and non-diabetic controls to identify novel molecular mechanisms behind the increased risk of atherothrombosis associated to diabetes.

Methods: Platelet-derived cMPs from 43 diabetic patients and 38 controls was analyzed by flow cytometry using the following platelet biomarkers: CD61, CD142, PAC1 and CD62P. The proteome of platelets from 10 diabetic patients and 10 controls was analyzed by 2-DE followed by MALDI-TOF/TOF identification. Changes in differential proteins were validated by western blot and serum protein levels were determined by ELISA.

Results: Diabetic patients were both type 1 ($n = 13$) and type 2 ($n = 30$) with similar clinical characteristics except for the percentage of metformin use (higher in type 2 diabetics). Platelet-derived cMPs were higher in diabetic patients for all biomarkers tested ($P < 0.005$, all). Proteomic analyses revealed significant differences between diabetics and controls in 15 proteins related to platelet aggregation, migration, and homeostasis. Diabetic patients showed higher levels of two key stress-related proteins, peroxiredoxin-2 (PRDX2) and Heat shock cognate 71 kDa (HSPA8). The intraplatelet increase in HSPA8 was associated to a decreased systemic protein levels in diabetic patients.

Conclusions: Platelets from diabetic patients are hyperreactive as shown by their increased MP release. In addition, we report for the first time that diabetic patients show increased levels of PRDX2 and HSPA8 in platelets, probably as a compensatory mechanism in response to an increased oxidative stress. Increased microparticle shedding and increased levels of PRDX2 and HSPA8 are mechanisms involved in platelet dysfunction associated to diabetes and its complications.

P3.03

Abcsic acid controls the release of insulin from beta-cells, Glucagon-Like Peptide-1 from intestinal L-cells and glucagon from alpha-cells

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Abcsic Acid (ABA) is involved in the regulation of glucose homeostasis in mammals, by stimulating insulin release (1) and glucose uptake (2). ABA is released by glucose- or GLP-1-stimulated β -cells (2). ABA plasma levels increase in healthy, but not in diabetic, subjects undergoing an OGTT (2,3) and oral ABA improves glycemic control in healthy humans (4). ABA stimulates GLP-1 release from the enteroendocrine L cell line

hNCI-H716, and oral ABA increased plasma GLP-1 and insulin levels in rats (5).

We investigated whether ABA regulates GLP-1 and/or glucagon secretion by the murine pancreatic α TC1-6 cell line. LANCL2, the mammalian ABA receptor, is expressed by these cells. Addition of exogenous ABA determined an increase of intracellular cAMP content, as occurred in β -cells and L-cells, and of the pre-proglucagon mRNA levels (1.5-fold relative to untreated controls). GLP-1 secretion was not significantly modified in the presence of 1 μ M ABA (for 2 or 24 h), neither at low (LG), nor at high glucose (HG) concentrations. Conversely, ABA consistently increased the release of glucagon (by 1.4 fold), both in LG and HG media.

Our most recent results indicate that perfusion of rat pancreas with a HG medium containing 10 μ M ABA resulted in a slow release of insulin. In addition, preliminary results indicated that perfusion of rat proximal small intestine with a medium containing 200 μ M ABA stimulated the release of GLP-1, when administered from the vascular side.

These results suggest a role for ABA in the dynamic interplay between insulin, glucagon and GLP-1 release, and may contribute to gain a deeper understanding of the mechanism of action of ABA in glycemia control.

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P3.04

Vascular damage in metabolically healthy and unhealthy obese subjects

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Obesity is an independent risk factor for cardiovascular disease (CVD) and type 2 diabetes mellitus (T2D). However, a subset of obese referred to as metabolically healthy obese (MHO), are characterized by a better metabolic profile. An objective method to identify these subjects is by stratifying the obese into quartiles of insulin sensitivity evaluated by Matsuda index. The aim of the present study was to evaluate the prevalence of sub-clinical target organ damage (TOD) in MHO subjects comparing them with MUHO. All patients underwent to OGTT and calculation of Matsuda index. From an initial cohort of 796 obese, (BMI ≥ 30 kg/m²), after stratification for quartiles of Matsuda, we considered patients in the 1st quartile ($n = 199$) as MUHO, and those in the 4th quartile ($n = 199$), as MHO. We included 182 males and 216 females, men age 47.7 ± 14.5 yrs, diabetic and drug-treated hypertensive patients were excluded from the study. Estimated glomerular filtration rate (e-GFR) was evaluated by CKD-Epi formula. To assess vascular function, arterial stiffness was obtained by measurement of carotid-femoral pulse wave velocity (PWV) by applanation tonometry and carotid intima media thickness (c-IMT) by a high-resolution ultrasound B-mode system. Compared with MUHO patients, MHO subjects presented a better metabolic and hemodynamic profile. In particular, they showed reduced levels of waist ($P < 0.0001$), triglyceride ($P = 0.001$), fasting glucose ($P = 0.001$), insulin ($P = 0.010$), and hs-C reactive protein ($P = 0.017$). Moreover, during OGTT MHO subjects showed lower values of area under curve (AUC) of glucose ($P < 0.0001$) and insulin ($P < 0.001$) than MUHO. Similarly, mean values of PWV (6.9 ± 1.8 vs 7.9 ± 2.1 ; $P = 0.017$) and c-IMT (0.6 ± 0.3 vs 0.8 ± 0.2 ; $P < 0.0001$) were

significantly lower and, on the contrary, values of e-GFR significantly higher ($P < 0.0001$) in MHO subjects. These findings may have important prognostic significance, thus a correct phenotyping of obese subjects is an essential tool to better characterize their cardiometabolic risk profile.

P3.05

Is type 2 diabetes a peripheral arterial disease risk equivalent?

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Aim: Both type 2 diabetes (T2DM) and peripheral arterial disease (PAD) have been considered coronary artery disease (CAD) risk equivalents. Here, we aimed at investigating whether T2DM also is a PAD risk equivalent.

Methods: Over a mean follow-up period of 6 years, we prospectively investigated a large series of 2137 patients, of whom 467 had sonographically proven PAD and 1670 did not have PAD.

Results: T2DM was significantly more prevalent among patients with than among subjects without PAD (44.3% vs. 27.4%; $P < 0.001$). The incidence of cardiovascular events was lowest in patients with neither PAD nor T2DM (21.6%); it was significantly higher in diabetic patients who did not have PAD (30.5%; $P < 0.001$), in nondiabetic PAD patients (38.7%; $P < 0.001$), and in patients with both PAD and T2DM (57.9%; $P < 0.001$), in whom the incidence of cardiovascular events also was significantly ($P < 0.001$) higher than in diabetic patients who did not have PAD or in nondiabetic PAD patients ($P < 0.001$). Importantly, cardiovascular event risk was significantly lower in diabetic patients who did not have PAD than in nondiabetic PAD patients ($P < 0.001$).

Conclusion: We conclude that PAD confers a higher cardiovascular event risk than T2DM; T2DM therefore is not a PAD risk equivalent. Cardiovascular risk is extremely high in patients with the combination of PAD and diabetes.

P3.06

Serum uromodulin is associated with impaired glucose metabolism

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Aim: Uromodulin is the most abundant urine protein under physiological conditions. It has recently been described as a serum marker of kidney disease. Whether uromodulin also is associated with impaired glucose metabolism is unknown.

Methods: We therefore measured serum uromodulin in a series of 529 patients who were undergoing coronary angiography for the evaluation of established or suspected stable coronary artery disease (CAD); in patients without established diabetes oral glucose tolerance tests were performed. Prospectively, diabetes incidence was recorded over 4 years.

Results: Serum uromodulin was significantly and inversely correlated with fasting plasma glucose ($r = -0.158$; $P < 0.001$), with plasma glucose 2 h after an oral 75 g glucose challenge ($r = -0.144$; $P = 0.002$), and with HbA1c ($r = -0.103$; $P = 0.018$). From our patients 146 (27.6%) had type 2 diabetes. Uromodulin was significantly lower in patients with T2DM than among non-diabetic patients (147.7 ± 69.9 vs. 171.4 ± 78.9 ng/mL, $P = 0.001$). Analysis of covariance confirmed that T2DM was an independent determinant of serum uromodulin ($F = 5.6$, $P = 0.019$) after multivariate adjustment including both the glomerular filtration rate and urinary albumin excretion. Prospectively, 21 patients of the initially non-diabetic subjects developed diabetes. Their uromodulin was intermediate (164 ± 67 ng/mL) between those who did not develop diabetes and those who already at baseline had diabetes (P for trend over these three categories < 0.001).

Conclusion: We conclude that serum uromodulin is significantly associated with impaired glucose metabolism; patients with T2DM have significantly higher levels of uromodulin than non-diabetic subjects.

P3.07

Single and joint effects of obesity and of the metabolic syndrome on the development of diabetes in patients with coronary atherosclerosis

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Aim: Obesity is a major risk factor for the metabolic syndrome (MetS), but some obese individuals do not have the MetS while others have the MetS but are non-obese. We prospectively investigated the single and joint effects of obesity and of the MetS on diabetes incidence in patients with angiographically proven coronary artery disease (CAD).

Methods: Diabetes incidence was recorded over 6.1 ± 3.7 years in a large cohort of 1063 patients with angiographically proven CAD. Obesity was defined as a BMI ≥ 30 kg/m²; presence of the MetS was defined according to the harmonized consensus definition.

Results: From our patients, 698 were non-obese and did not have the MetS, 62 were obese but did not have the MetS, 184 were non-obese but had the MetS, and 119 were obese and had MetS. During follow-up, the overall incidence of diabetes was 12.1%, corresponding to 1.9% per year. Diabetes incidence was 6.7% in non-obese patients without the MetS. It was significantly higher in obese patients without the MetS (16.0%; $P < 0.001$), in non-obese patients with the MetS (22.3%; $P = 0.009$), and in obese patients with the MetS (24.4%; $P < 0.001$). Diabetes incidence however did not differ

significantly between obese or non-obese MetS patients ($P = 0.303$) or between obese patients with and obese patients without the MetS ($P = 0.674$).

Conclusion: We conclude that the incidence of diabetes in patients with CAD is high, except among subjects who neither are obese nor have the MetS. Both obesity and the MetS increase diabetes risk; a metabolically healthy obese phenotype does not protect against the development of diabetes in this population.

P3.08

Dietary support at long term following bariatric surgery

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Background: The long term weight results after bariatric surgery are not always fully satisfactory, in some cases the weight regain or the obesity relapse being observed at one or more years after the operation.

Material and methods: Fifty patients were submitted to bariatric surgery, 35 undergoing Roux-en-Y Gastric Bypass (RYGBP) and 15 Sleeve Gastrectomy (SG), were accurately evaluated by an alimentary interview preoperatively and at 6 months and one year following the operation. The interview, carried out in all cases by the same person (MP), was designed for investigating both the energy content of the food eaten in the 24 h and the subject's alimentary habits and behaviors. The amount of calories intake was quantified by a food frequency questionnaire integrated by an accurate 24 h recall, eating habits and behavior were assessed with open questions exploring both the episodes of binge eating and night eating and the occurrence of nibbling and grazing behaviors and snaking between meals.

Results: At the sixth postoperative months, all patients experienced a satisfactory weight loss, the mean daily energy intake having become noticeable lower than the baseline one; however in a not small part of patients eating habits and behaviors remained unchanged in comparison to those recorded prior to the operation, without any evident difference between the RYGS and the SG individuals. At one year after the operation, in the subjects having reported abnormal eating behaviors the weight loss was noteworthy lower and the overall food consumption significantly greater in comparison with subjects showing a normal eating pattern.

Discussion: A long term satisfactory weight loss after RYGBP and SG requires a normalization of eating habits and behaviors. Therefore, an adequate educational/nutritional support may be of a great value for obtaining good results in the majority of the severe obese individuals after RYGBP and SG.

P3.09

Daytime ambulatory blood pressure variability predicts fluorophores advanced glycation end products (AGEs)-to-NADH ratio in type 2 diabetes and controls

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Background and aims: Higher serum advanced glycation end products to nicotinamide adenine dinucleotide hydride (AGEs-to-NADH) ratio was newly described in subjects with diabetes

and cardiovascular disease compared to controls. However, no data has been reported regarding the relation between the AGEs-to-NADH ratio and 24-h ambulatory blood pressure monitoring parameters. We aimed to assess the association between AGEs, NADH, the AGEs-to-NADH and ambulatory blood pressure control and variability in type 2 diabetes and control subjects.

Materials and methods: Serum AGEs and NADH were measured in type 2 diabetes subjects with cardiovascular disease ($n = 26$), without cardiovascular disease ($n = 37$) and controls ($n = 25$) using fluorescence spectroscopy. The excitation wavelength was set at 370 nm, and the emission was measured at 435 nm for AGEs and 460 nm for NADH. Daytime, nighttime and 24-h mean blood pressure and blood pressure variability were assessed in all subjects using 24-h ambulatory blood pressure monitoring.

Results: We found significantly higher AGEs (19.4 ± 3.1 vs. 18.0 ± 3.4 and 15.9 ± 1.5 ; $P = 0.002$) and lower NADH (14.9 ± 2.7 vs. 13.2 ± 2.6 and 19.6 ± 2.1 ; $P = 0.30$) in diabetic subjects with cardiovascular disease compared to those without cardiovascular disease and controls. The AGEs-to-NADH ratio was significantly higher in diabetic subjects with cardiovascular disease compared to those without cardiovascular disease and controls (1.38 ± 0.26 vs. 1.32 ± 0.21 and 0.82 ± 0.11 ; $P < 0.001$). The AGEs-to-NADH ratio was significantly and positively associated with mean daytime, night-time and 24-h systolic blood pressure ($r = 0.27$; $P = 0.01$), also with daytime systolic blood pressure variability ($r = 0.27$; $P = 0.01$) in all study population. In linear regression analysis, the AGEs-to-NADH ratio was significantly predicted by daytime systolic blood pressure variability ($P = 0.01$), even after adjustment for age, sex, smoking status, diabetes and hypertension duration ($R^2 = 0.19$; $P = 0.014$).

Conclusion: Our findings suggest that daytime ambulatory blood pressure variability might be implicated in increasing the newly reported fluorophores AGEs-to-NADH ratio in type 2 diabetes and control subjects.

P3.10

Diabetic milieu induces myostatin in renal proximal tubular cell line

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Background: Myostatin (MSTN), a member of the TGF- β family, regulates muscle homeostasis, protein synthesis, insulin sensitivity, glucose uptake and controls skeletal muscle development. MSTN was found to be upregulated in diabetes. The renal proximal tubular epithelial cells (PTEC) play an important role in the diabetic nephropathy (DN) pathogenesis. The aims were evaluate the involvement of diabetic milieu in MSTN expression in PTECs and the effects of MSTN on proliferation, cytokine expression and fibrosis.

Methods: HK-2 (human PTEC) cell line was exposed to low (LG 5 mmol) high (HG 30 mmol) glucose, and glycated albumin (GA 500 ng/mL) for 48 h. MSTN mRNA was evaluated by real time PCR and protein by western blot and immunocytochemistry. The effects of MSTN (500 ng/mL) on cell proliferation (CFSE incorporation), ECM production, cytokines expression, oxidative stress (rtPCR and immunocytochemistry, CellROX staining) were also studied. To verify the direct involvement of MSTN in fibrosis and inflammation, MSTN was down-regulated after 24 h of siRNA transfection.

Results: MSTN mRNA and protein were upregulated by HG, GA (by ~ 6 and ~ 2 folds respectively $P < 0.05-0.01$ vs LG and albumin). MSTN slowed proliferation: 76% MSTN-treated cells represented G2 + G3 generations of total cell population, against 54.2% of control cells ($P < 0.05-0.01$). MSTN was proinflammatory and profibrotic stimulus: MCP1 and RANTES mRNAs expression increased (respectively 4.4 ± 0.24 , 3.9 ± 0.05 , $P < 0.01$) and fibronectin mRNA and protein increased 11 and 3 folds respectively ($P < 0.01$). MSTN increased intracellular ROS (1.5 folds after 5 h) and Nox 4 mRNA and protein (5.6 ± 1.9 fold, $P < 0.05$). When MSTN knockdown HK-2 cells were exposed to HG or GA, fibronectin and MCP1 were significantly decreased ($\sim 60-80\%$, $P < 0.05-0.01$).

Conclusions: Diabetic milieu induces MSTN in PTECs. MSTN could be involved in diabetic alterations inducing: slowing in cell proliferation, inflammation and fibrosis and oxidative stress. MSTN could be a new trigger in DN.

P3.11

Hyperglycemia and AGEs negatively affect the angiotensin/Tie-2 system in endothelial cells

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Endothelial dysfunction is a major characteristic of diabetic microvascular complications. Microvascular changes start in the prediabetic state, become more complex with overt diabetes and remain even when glycemic control is reached. The latest condition is probably sustained by Advanced Glycation End-Products (AGEs), a heterogeneous group of compounds derived from the non-enzymatic reaction of reducing sugars with proteins, lipids or nucleic acids. The angiotensin growth factor-1 (ANG-1) contributes to vessel integrity by activating the tyrosine kinase receptor (Tie-2), conversely, ANG-2 acts as a dominant negative ligand of Tie-2 and promotes inflammation. The aim of the current study was to investigate whether exposure of endothelial cells to hyperglycemia and AGEs may alter the Angiotensin/Tie-2 system.

Human microvascular endothelial cell-1 (HMEC-1) were cultured for 5 days with Glycated serum (GS, which consists in a pool of AGEs), 16.7 mmol/L glucose (HG) or their combination (HG+GS). Then we evaluated: cell viability; mRNA levels of Vascular Endothelial Growth Factor A (VEGF-A) and Angiotensin-2 (ANG-2); TIE-2 protein expression; secretion of VEGF-A, ANG-1, ANG-2 and soluble Tie-2.

Viability of HMEC-1 cells wasn't affected by exposure to HG or GS. Culture in all of the diabetic conditions increased secretion of VEGF-A, and decreased secretion of ANG-2 and sTie-2. Exposure to GS increased mRNA expression of ANG-2; and decreased Tie-2 protein expression. Culture with HG up-regulated mRNA levels of VEGF-A, and decreased ANG-1 secretion. Combination of GS with HG had summative effects.

We show that both HG and AGEs negatively alter the Angiotensin/Tie-2 system favoring a molecular environment that may impair endothelial integrity. These results also highlight the importance of scavenging AGEs to prevent microvascular complications of diabetes.

P3.12

Abscisic acid improves glucose tolerance in rodents and in humans by increasing muscle glucose uptake

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Background: Abscisic acid (ABA) is a plant hormone also present in animals where contributes to the regulation of glycemia. Indeed: (i) nanomolar ABA stimulates GLUT4-mediated glucose uptake by myoblasts and adipocytes *in vitro*; (ii) plasma ABA increases in healthy subjects, but not in diabetic patients, after an oral glucose load (OGTT); (iii) oral ABA at 1 µg/Kg body weight (BW) significantly lowers glycemia and insulinemia in rats undergoing an OGTT.

Aims: To evaluate the effect of a nutraceutical containing ABA on glycemia and insulinemia in healthy volunteers consuming a standard meal.

To investigate the effect of chronic ABA treatment on blood glucose, lipids and BW in mice fed a high-glucose (HG) or a high-fat (HF) diet.

To measure the effect of a single oral ABA dose on muscle glucose uptake in rats by µPET analysis.

Results: 1. In healthy volunteers, intake of a nutraceutical containing 1 µg/Kg BW ABA before a standard meal significantly reduced both glycemia and insulinemia.

2. In both HG- and HF- fed mice ABA treatment significantly reduced HbA1c, lipidemia, glycemic profile during an OGTT and BW gain compared to untreated controls.

3. 1 µg/Kg BW ABA significantly increased FDG blood clearance and muscle uptake during an OGTT.

Conclusions: Oral ABA at 1 µg/Kg BW improves glucose tolerance in healthy humans fed a standard meal.

Chronic oral ABA administration reduces glycemia, lipidemia and BW gain in mice fed a HG or a HF diet.

µPET imaging reveals a significant increase of FDG blood clearance and muscle FDG uptake in rats: stimulation of tissue glucose uptake is likely the reason for the reduced insulinemia observed in ABA-treated rats and humans.

ABA administration could be a promising new intervention to improve glucose tolerance sparing insulin by stimulating tissue glucose uptake, which is usually compromised in pre-diabetic or diabetic subjects.

Workshop 4: Mitochondria Biology and Medicine

P4.01

IDH2 deficiency protects age-dependent weight gain

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Background: Reactive oxygen species (ROS) are a byproduct of normal metabolism and play important roles in cell signaling and homeostasis. Mitochondria, the main organelles involved in intracellular ROS production, play central roles in modulating redox-dependent cellular processes such as metabolism and apoptosis. We recently reported an important role for mitochondrial NADP⁺-dependent isocitrate dehydrogenase (IDH2) in cellular redox regulation.

Materials and methods: To explore the association between IDH2 expression and obesity, we measured energy metabolism, mitochondrial redox status, and modulation of signaling pathways in IDH2 knockout (*idh2*^{-/-}) and wild-type (*idh2*^{+/+}) mice.

Results: We show that mice with targeted disruption of *IDH2* exhibit resistance to obesity, with lower body weight and reduced visceral fat, and increased insulin sensitivity accompanied by enhanced energy expenditure relative to controls. This function of IDH2 is linked to its capacity to suppress lipogenesis in visceral adipose tissue, partly via transcriptional repression of SREBP1, and to increase thermogenesis in adipocytes by transcriptional activation of UCP1 via activation of the p38 signaling axis.

Conclusions: Our results highlight the importance of redox balance in the regulation of metabolism and demonstrate that IDH2 plays a major role in modulating both insulin sensitivity and fuel metabolism, thereby establishing this protein as a potential therapeutic target in the treatment of type 2 diabetes and obesity.

P4.02

Characterization of postnatal decline in hematopoiesis in human neonatal liver using RNA sequencing: a link to UCP2

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Liver plays a central role in metabolic homeostasis. In humans, the liver is also the primary source of red blood cells, starting from the 9th week of the prenatal development. During the 24th week, bone marrow replaces the liver as the main site of hematopoiesis, while the hematopoietic activity in the liver declines after birth. Using autopsy samples from a unique cohort of human (mostly premature) newborns, we previously found a strong correlation between liver hematopoiesis and tissue expression of the gene for mitochondrial uncoupling protein 2 (UCP2), suggesting a role of UCP2 in hematopoiesis (Brauner

et al., *Pediatric Research* 2001). The goal of this study was to verify the link between UCP2 gene and hematopoiesis using the available autopsy samples and RNA sequencing (RNAseq). Total RNA was isolated from the liver autopsy samples (stored in RNAlater at -80 °C for 15–20 years) from the previously used cohort of newborns (see above; *n* = 34). RNA integrity was checked using the Agilent 2100 bioanalyzer. Eventually, 16 samples were selected for RNAseq from the newborns that differed in gestational age at birth (22 – 39 weeks), birth weight (450 – 2060 g) and time of survival after birth (see below). 3 240 genes were identified showing a significant difference (*P* ≤ 0.05) in expression between the two subgroups differing in the survival time (45 min–1 day; *n* = 9 vs. 3.2–13.3 days; *n* = 7). Expression of a set of genes involved in heme biosynthesis declined during the postnatal life and correlated with the UCP2 gene expression. Thus, we have verified our previous results and identified novel genes involved in early postnatal development of human liver. We also proved the applicability of the RNAseq approach for the analysis of the whole transcriptome using the unique collection of human tissue samples.

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P4.03

Serum acylcarnitines and amino acids in patients with type 2 diabetes

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Serum acylcarnitines (AC) and amino acids (AA) levels (AC-AA-profile) mark both lipid metabolism and insulin sensitivity (Newgard et al., *Cell Metab.* 2009). The aim of this study was to characterize possible modulations of the AC-AA-profile by selected pharmacological or nutritional interventions that are relevant for the treatment of patients with type 2 diabetes (T2D).

Serum samples from two clinical studies involving patients with T2D were used. Serum AC-AA-profile was analyzed using a FIA-ESI-MS/MS. Multivariate statistical analysis (PLS-DA) was used to reveal changes of the AC-AA-profile in response to T2D or the selected interventions (see below).

The first study compared healthy adults (*n* = 30) and T2D patients (*n* = 30). PLS-DA revealed a separation between the two groups. Metabolites with a high discriminatory power were branched chain AA leucine-isoleucine and valin, showing higher serum levels in T2D patients (*P* < 0.05), and glutamine, C4OH and acetylcarnitine.

In the second study (see Veleba et al., *Nutr. Metab.* 2015), four groups (*n* = 13-17) of adult patients with T2D treated already with metformin were assigned to a 24-week-intervention using: (i) corn oil (5 g/day; Placebo), (ii) pioglitazone (15 mg/day; Pio), (iii) EPA+DHA concentrate (5 g/day, containing ~2.8 g EPA+DHA; Omega-3), or (iv) pioglitazone and EPA+DHA

concentrate (Pio&Omega-3). PLS-DA uncovered the effect of Omega-3 intervention, independent of Pio, which corresponded with preferential effects of Omega-3 on lipid metabolism but not on insulin sensitivity of T2D patients. The most discriminating metabolites were AC derived from either EPA or DHA, and also medium-chain AC.

Our results confirm that serum AC-AA-profile could serve as a biomarker of T2D and the state of lipid metabolism in T2D patients. They suggest that targeted analysis of the AC-AA-profile could be also used to monitor the effects of therapeutic interventions in patients with T2D.

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P4.04 Mitochondrial dysfunction and p53 signaling in mouse stem cell-derived cardiomyocytes exposed to doxorubicin

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Background: Doxorubicin (DOX) is a widely used anticancer drug although its clinical dosages are limited due to the development of delayed cardiotoxicity. Thus, it is crucial to investigate the mechanisms underlying DOX cardiotoxicity so that it can be better prevented. DOX cardiotoxicity has been studied in *in vivo* and *in vitro* models, including the rat cardiomyoblast H9c2 cell line. However, beating stem cell-derived cardiomyocytes may represent a better *in vitro* model to further uncover the mechanisms of DOX-induced cardiotoxicity.

Methods: In this work, we used cultured embryonic stem cell-derived mouse cardiomyocytes treated with 0.5 or 1 μ M DOX, and observed morphological, functional and biochemical changes associated with mitochondrial bioenergetics, DNA-damage response and apoptosis.

Results: DOX mostly depressed proteins and transcripts associated with mitochondrial bioenergetics and induced p53-dependent caspase activation. Moreover, DOX affected the expression of p53 target transcripts associated with mitochondria-dependent apoptosis and DNA-damage response, with the two concentrations showing interestingly different responses.

Conclusions: This cell model recapitulates mechanisms of DOX cardiotoxicity found in other biological models, including the commonly used rat H9c2 cells, but also presents some important differences that may be due to the lack of cellular proliferation and/or to the presence of a functional contractile machinery in Cor.At cardiomyocytes.

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P4.05 Implication of the mitochondrial 18-kDa Translocator Protein (TSPO) in the neurodegeneration induced by different states of aggregation of amyloid beta peptides in PC-12 cells

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Background: Amyloid beta peptides (A β) present one factor contributing to neuronal death in Alzheimer's disease, while the mitochondrial 18-kDa translocator protein (TSPO) is also involved in cell death and inflammation associated with neurodegenerative disorders.

Methods and results: Herein, untreated neuron-like PC12 cells showed clear 18 kDa TSPO expression with a maximal binding capacity of 4095 ± 276 fmol /mg for its ligand [³H]PK 11195. PC12 cells were also quite sensitive to A β (25–35), which was assayed by LDH cytotoxicity detection kit. A β -induced cell death of PC12 cells was associated with mitochondrial dysfunctions that affect metabolism (as studied by XTT assay), cardiolipin oxidation and the mitochondrial membrane potential ($\Delta\Psi$ m), as assayed by FACS analysis, processes that are essential for cell death functions of the 18 kDa TSPO. However, A β did not change the binding characteristics of the 18 kDa TSPO in PC12 cells. Interestingly, application of the TSPO ligand PK11195 by itself at 50 μ M induced an increased metabolic activity, while PK 11195 applied together with A β resulted in enhanced cell death processes of PC12 cells. U118MG cells of glial origin, which show TSPO expression and binding characteristics comparable to those of the PC12 cells of the present study, appeared to be strongly resistant to cell death induction by A β .

Conclusions: The present data corroborate previous suggestions that, other processes, in addition to those affected by A β , may contribute to Alzheimer's disease neuropathology. In particular processes modulated by the mitochondrial TSPO may be part of this relevant neuropathology.

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P4.06 Endoplasmic reticulum stress response during differentiation of P19 embryonic carcinoma cells

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Background: Since endoplasmic reticulum (ER) stress occurs during embryonic development and protein homeostasis is determinantal for avoiding cellular senescence, both events may play a role in the maintenance of the stem cell pool and generation of differentiated cells. In this work we evaluated the unfolded protein response (UPR) and the ubiquitin-proteasome degradation system during retinoic acid (RA)-induced early neural differentiation of P19 embryonic cells.

Material and methods: P19 stem cells (P19SCs) were differentiated (P19dCs) with 1 μ M of RA during 96 h. Markers of UPR (ATF6, P-eIF2 α , Ire1 α and CHOP) as well as protein ubiquitination were detected by immunoblotting and 20S proteasome activity was determined by using the substrate LLVY-7-Amino-4-methylcoumarin.

Results: We found that P19SCs presented higher protein expression of Ire1 α and CHOP while their differentiated counterparts showed higher levels of ATF6 and P-eIF2 α . We also found an accumulation of ubiquitinated proteins and higher 20S proteasome activity in P19dCs.

Conclusions: This indicates that ER stress accompanies stem cells differentiation and that the activation of ATF6 and P-eIF2 α branches of UPR probably facilitates adaptation to the stress induced by the accumulation of unfolded/misfolded proteins targeted for proteasomal degradation. The depletion of CHOP in P19dCs probably contributes to protect from ER stress-induced cell death. Since eIF2 α is required to activate CHOP, the results in P19SCs may suggest alternative roles for UPR. In fact, Ire1 signaling branch of UPR may play a role in stem cells maintenance.

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P4.07

Carvedilol regulates antioxidant proteins in a type I diabetes rat model

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Introduction: Patients with diabetes have a higher risk of developing both micro- and macro-vascular disease. Hyperglycaemia is the main factor in the pathogenesis of diabetic cardiomyopathy, often resulting from increased oxidative stress. Carvedilol, a β -adrenergic blocker, has intrinsic antioxidant properties and was previously described to be effective in the protection of cardiac mitochondria against oxidative stress. The objective of this study was to evaluate the effect of Carvedilol on hyperglycaemia-induced oxidative damage and mitochondrial protein alterations in cardiac and skeletal muscle in streptozotocin-treated rats.

Materials and methods: Body mass, blood glucose, the level of protein carbonylation, caspase 9 and 3-like activities, mitochondrial proteins, the status of antioxidant defense system and stress-related proteins were evaluated in streptozotocin vs streptozotocin+Carvedilol (1 mg/kg/day)-treated rats.

Results: Carvedilol decreased blood glucose in streptozotocin-treated animals. The protein amount of catalase in the heart, and SOD2, SOD1 and catalase in skeletal muscle were increased by Carvedilol treatment in streptozotocin-treated animals. At this particular time-point, streptozotocin-induced hyperglycaemia did not cause caspase activation or increase of protein carbonylation status.

Conclusions: The data showed that Carvedilol increased the level of antioxidant enzymes, what may contribute to preserve cell redox balance during hyperglycaemia. We also showed for

the first time that Carvedilol effects on streptozotocin -treated rats are tissue-dependent. Based on data showing modulation of the antioxidant network in the heart, Carvedilol may be beneficial in diabetic patients without advanced disease complications, delaying their progression.

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P4.08

Cytotoxicity of hyperglycemia on H9c2 cardiomyoblasts cultured in high-glucose media under different differentiation conditions

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Background: The H9c2 cell line is derived from the ventricular part of a rat embryo and presents a myoblastic proliferative phenotype while maintained in normal 10% fetal bovine serum (FBS)-containing culture media. H9c2 cardiomyoblasts have been extensively used as a model for cardiac cells in studies addressing not only mechanisms of cardiac differentiation but also cardiac toxicity and cardiovascular diseases. Reduction of FBS in the culture media alone or in the presence of retinoic acid (RA) can drive cell differentiation towards a skeletal muscle or cardiac cell phenotype, respectively. Chronic hyperglycemia (HG) can trigger maladaptive responses and oxidative stress in adult or progenitor cardiac cells, which can lead to mitochondrial dysfunction and cell death, with possible implications in the development of diabetic cardiomyopathy.

Objective: We intended to study the mechanisms of HG-induced metabolic dysfunction in H9c2 cardiomyoblasts investigating also the role of cell differentiation.

Materials and methods: H9c2 cardiomyoblasts were cultured in high-glucose media to which 22 or 33 mM glucose was added, in a total glucose concentration of 47 and 58 mM, respectively, mimicking HG conditions. Mannitol was used as osmotic control. Cells were differentiated in a skeletal muscle or cardiac phenotype according to standard protocols and cell mass, apoptotic signaling, mitochondrial protein expression and calcium uptake, and oxidative stress markers were evaluated.

Results: There was not an extensive toxicity of HG on H9c2 cardiomyoblasts, although interestingly a HG-mediated increased caspase-3 activation was observed when H9c2 cardiomyoblasts were differentiated towards a cardiac fate.

Conclusions: The lack of toxicity of HG on H9c2 cardiomyoblasts may stem from the culture conditions, which contained already a high-glucose concentration and which may have created metabolic adaptations per se, blurring the toxicity of HG. **Acknowledgments:** Funded by FEDER funds through the Operational Programme Competitiveness Factors-COMPETE and national funds by FCT under research grant PTDC/DTP-FTO/2433/2014.

P4.09

Validation of the human post-mortem brain samples usability to prove diagnosis of mitochondrial disorders

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Background: To confirm diagnosis of mitochondrial disorders often muscle, liver biopsies as well as patient's fibroblasts are commonly used. Taking in to account that manifestation of mitochondrial defects very often affect nervous tissue such material cannot be used to investigate mitochondrial abnormalities in live individuals. Inaccessibility of brain samples from living sick individuals causes that for diagnosis purpose post-mortem brain samples should be used.

Objective: To address this problem, the material (brains) was obtained from mice. After sacrificing, the animals were stored under the same conditions as in the procedures dealing with the remains of patients. Dissection material was collected in a similar time regime, to what is done when obtaining autopsy material from the deceased patients.

Results: We have found that post mortem interval (PMI) causes no significant alterations in the profiles of OXPHOS subunits levels. These data suggest, that the PMI regime in The Children's Memorial Health Institute could enable usage of postmortem human brain samples to prove or exclude mitochondrial disorder related to the OXPHOS dysfunction manifested in the brain tissue. Moreover, we did not find a significant effect of PMI on the protein and lipid oxidation damage status. Additionally, no effect of PMI has been observed on the level of antioxidant enzymes indicating that the level of proteins of interest is not changed with the increasing PMI as well as that the way of cryopreservation has little or no differential effect.

Conclusions: Our data show, that the post mortem interval does not influence significantly the quality of brain tissues as a material to study the OXPHOS protein pattern composition as well as manifestation of oxidative stress-related proteins, lipids and DNA damage.

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P4.10

Effects of mitochondrial uncoupling protein 2 inhibition in in vitro neuronal responses to different glucose conditions

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Though glycemic variability has been considered as an adverse factor for the development of several diabetes-related pathologies, its impact in the brain is still not fully elucidated. Considering the rationale that mitochondrial reactive oxygen species (ROS) production and alterations in the oxidative status are the adverse triggers for the development of diabetic complications, we hypothesize that mitochondrial uncoupling protein 2 (UCP2)-mediated effects may promote a cellular response to protect neuronal cells from metabolic insults. To achieve our goals, primary cortical neurons were submitted to glucose variations (GV) or to constant high (HG) or low glucose (LG) levels for 10 h, and the pharmacological inhibition of UCP2 was performed using genipin. Results obtained show that neuronal cells viability is only affected in the condition of GV, which also evoked a significant increase in intracellular and mitochondrial ROS production, alongside with a significant decrease in manganese superoxide dismutase activity. However, GV also promoted an adaptive response to cope with such metabolic insult, namely increased levels of the ratio GSH/GSSG, and an augment in the protein expression levels of nuclear factor E2-related factor 2 (NRF2) and UCP2, important regulators of oxidative stress protection. Conversely, genipin abrogated all the occurred compensations leading to a concomitant increase in the levels of caspase 3-like activity, and the loss of neuronal synaptic integrity while promoting an increase in UCP5 protein expression levels. Further, other effects of UCP2 inhibition were detected in the control and LG groups. Namely, genipin potentiated mitochondrial ROS production while increasing UCP4 protein expression levels in those groups. Overall, these data suggest that UCP2 is in the core of neuronal cells protection and/or adaptation against GV-mediated effects while suggesting that the different isoforms of neuronal UCPs may compensate each other's inhibition and/or inactivity.

P4.11

A protocol for human urine-derived stem cells isolation, expansion, and mitochondrial function profile analysis

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Background: Human urine contains a small population of cells with stemness properties, urine-derived stem cells (USCs). USCs can be non-invasively collected and used for personalized therapeutics. Only a small number of studies about USCs is available. Thus, a deeper characterization of these cells is essential, including in what regards to their mitochondrial function profile. Our aim is to establish a protocol to isolate and expand USCs, enabling for a mitochondrial metabolic characterization.

Material and methods: Urine from a female volunteer (32 years-old) was used to isolate and expand USCs using the

commonly used mixture of Keratinocyte Serum Free Medium and Embryonic Fibroblast Medium (KSFM+EFM) and a mixture of KSFM and DMEM, 10% FBS, 1% MEM-NEAA (KSFM+DMEM). Isolated cells were characterized by flow cytometry for mesenchymal and hematopoietic stem cells markers. For mitochondrial function profiling, different cell densities were used to determine the oxygen consumption rate (OCR) using the Seahorse XFe96 Extracellular Flux Analyzer. The optimized conditions used to measure OCR involved 20,000 cells/well, with oligomycin 2 μ M, FCCP 0.5 μ M and rotenone/antimycin A 1 μ M used to titrate respiration.

Results: USCs isolated and expanded from human urine were positive for CD73, CD44 and CD24 (>99%) for both media, 19%/38% positive for CD90, and 85%/65% positive for CD105, respectively for KSFM+EFM or KSFM+DMEM. We observed that 77.5% of the OCR was used by USC to sustain ADP phosphorylation. Also, we measured that USC basal respiration corresponds to 57.2% of their maximal capacity.

Conclusions: In our study, EFM could be replaced by the less expensive KSFM+DMEM for USCs isolation and expansion from human urine, with similar results regarding stem cells markers. USC that were isolated showed a respiratory reserve capacity which may be used for boosting mitochondrial metabolism after cell differentiation.

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P4.12

The effect of R5 in the preservation of cellular bioenergetics in a liver transplantation animal model

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Introduction: An important problem concerning liver transplantation and hepatic surgery is ischemia/reperfusion (I/R) injury. Mitochondria (M) become susceptible to damage during I/R.

Aim: to determine if the compound R5, would prevent impairment of liver M function following I/R injury.

Methods: Livers (L) of male Wistar rats were subjected to 3, 6 or 16 h of hepatic ischemia, preserved in Celsior solution (at 4°C) alone or with the addition of R5. During the 60 min of reperfusion (at 37°C), L were perfused with Krebs-Henseleit buffer at 37°C in a nonrecirculating fashion. Changes in membrane potential, M respiration as well as susceptibility to M permeability transition (MPT) induction were evaluated.

Results: In rats subjected to I/R, compared with the control group, a severe impairment of M bioenergetics was observed.

State 3 respiration was decreased and state 4 enhanced, associated with lower membrane potential developed following succinate energization. The injury presented does not seem directly related with the time of ischemia, but rather with the damage caused by the reperfusion at 37°C. If the reperfusion is conducted at 30°C, the I/R injury is significantly ameliorated. An increased susceptibility to MPT induction by calcium/phosphate was also observed. The effects of the reperfusion injury were ameliorated in the presence of the compound R5, under study.

Conclusion: R5 seems to prevent the most significant changes that occur in M during I/R, and the associated cell dysfunction, through their central role in cellular bioenergetics.

P4.13

Mitochondrial bioenergetics and biogenesis are involved in the regenerative response of the human liver in two-stage hepatectomies

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Introduction: Liver regeneration is an energy-consuming process. Mitochondrial status depends on adequate renovation of the mitochondrial pool, with biogenesis ensuring the renewal of damaged mitochondria. Two-stage hepatectomy with portal vein ligation (TSHPVL) is the only curative therapy for patients with malignant liver neoplasms and a predicted small future liver remnant (FLR). Inter-stages regenerative response is remarkable with this technique but the adaptations in energy metabolism and mitochondrial function taking place in the FLR are unknown.

Objective: Study liver mitochondrial bioenergetics and mitochondrial biogenesis in TSHPVL.

Methods: Five consecutive patients (three male; mean age 59) underwent TSHPVL. Measurement of mitochondrial membrane potential and respiration in intra-operative liver biopsies obtained in stage 1 (T1) and stage 2 (T2). Analysis of gene expression with RT-PCR and protein content with Western Blot of several factors involved in liver regeneration, energy metabolism and mitochondrial biogenesis.

Results: FLR volume increased from 330 cm³ (IQR 250–500) to 450 cm³ (IQR 350–610) ($P = 0.043$) between stages. Mitochondrial bioenergetics, namely lag phase and Respiratory Control Ratio improved from T1 to T2 ($P < 0.05$). We observed an increased expression of STAT3, Augmenter of Liver Regeneration, PGC-1 α , Cytochrome oxidase subunit 1 and NAMPT in T2 compared with T1 ($P < 0.05$). Content of PGC-1 α , Cytochrome oxidase subunit 1 and Sirtuin 1 was also increased in T2 ($P < 0.05$).

Conclusion: The remarkable regenerative response of the Human liver in TSHPVL is linked to an enhanced mitochondrial function, likely due to improved biogenesis. Energetic conditioning of the liver parenchyma could improve liver regeneration, expanding the limits of liver surgery.

Workshop 5: Internal Medicine with Fadoi

P5.01

Novel germline p.Ala72Glu AVP mutation in a family with autosomal dominant familial neurohypophyseal diabetes insipidus

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Autosomal dominant familial neurohypophyseal diabetes insipidus (adFNDI) is a rare cause of central diabetes insipidus (CDI). Patients with adFNDI harbour a mutation in the arginine vasopressin (AVP) gene. It is located on chromosome 20p13 and contains 3 exons coding for the arginine vasopressin (AVP) preprohormone which consists of signal peptide, AVP, neurophysin II (NPII), and a C-terminal glycopeptide, copeptin. Mutations of the AVP gene result in a lack of AVP. We report on a 66 year old female patient with the diagnosis of CDI in 1979 seeking for endocrine check-up. Laboratory investigations revealed no abnormalities on treatment with Desmopressin (1-deamino-8-D-arginine vasopressin). The father, brother and daughter of the index patient reported to have CDI as well but no genetic testing has been carried out. We performed DNA-extraction, PCR amplification and DNA sequencing of the AVP gene according to published methods. DNA sequencing revealed a heterozygous single nucleotide C > A change – c.215C > A; p.Ala72Glu – resulting in replacement of alanine to glutamic acid at codon 72, exon 2. In silico analysis (Alamut Visual Version 2.7.2) suggests likely pathogenicity based on AlignGVGD, SIFT, PolyPhen-2 and MutationTaster scores. The identified p.Ala72Glu AVP mutation impacts the NPII moiety of the AVP precursor and was not previously detected in patients with adFNDI. It affects a non-polar alanine, replaces it with the polar negatively charged glutamic acid and is located aside the Cys61-Cys73 disulfide bond. NPII is the carrier protein of AVP and is essential for its axonal transport from the hypothalamus to the neurohypophysis. The mutation might lead to conformational changes that impair the structure of NPII and therefore its function as a carrier protein. Genetic counselling and genetic cascade screening should be considered in familial cases of CDI to perform presymptomatic diagnosis in childhood and prevent growth retardation due to water deprivation.

P5.02

The value of uromodulin in serum as new biomarker to predict decline in renal function and cardiovascular events

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Aim: Uromodulin is the most abundant protein excreted in urine and, to a smaller amount, it is also released into blood. Only few and conflicting data exist addressing the association between uromodulin in urine and kidney disease, whereas uromodulin in serum has recently been demonstrated to be associated with kidney function. Whether serum uromodulin is also useful to predict a future decline in kidney function and cardiovascular events is not known.

Methods: We measured uromodulin in 529 angiographed patients and prospectively recorded kidney function and cardiovascular events over up to 8 years.

Results: Patients' uromodulin levels were significantly and inversely correlated with serum creatinine ($r = -0.288$, $P < 0.001$) and with ACR ($r = -0.120$, $P = 0.012$). We observed significantly lower serum uromodulin concentrations in patients affected with CKD compared to subjects with normal kidney function (71.9 ± 29.0 vs. 169.1 ± 76.1 ng/mL, $P < 0.001$), and also with respect to microalbuminuria (148.7 ± 72.2 vs. 167.9 ± 77.6 , $P = 0.008$) and hypertension (160.9 ± 74.0 vs. 181.8 ± 87.8 ng/mL, $P = 0.037$). Prospectively, serum uromodulin concentration was inversely associated with the incidence of CKD, even after adjustment for age, sex, BMI, CRP, blood pressure, ACR, the baseline stage of CKD, the angiographically determined CAD status, and the T2DM status (OR = 0.354 [95% CI 0.131–0.957], $P = 0.041$). The performance of a logistic regression model for predicting the incidence of CKD was significantly higher if uromodulin was included ($P = 0.049$). In addition, the creatinine to uromodulin ratio in serum significantly predicted cardiovascular events both univariately (HR 1.26 [95%CI 1.12–1.41], $P < 0.001$) and after multivariate adjustment including smoking, T2DM status, and CAD status (HR 1.29 [95%CI 1.12–1.49], $P < 0.001$), and the inclusion of this ratio also significantly increased the performance of a survival model for cardiovascular events ($P = 0.040$).

Conclusion: This study is the first demonstrating that uromodulin in serum is a novel and valuable predictive biomarker for the incidence of CKD and the cardiovascular event risk.

Workshop 6: Neurology

P6.01

Autonomic modulation via slow deep breathing and attention

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Introduction: The autonomic nervous system (ANS) is important in pain recruitment, processing and perception. Slow deep breathing (SDB) increases the parasympathetic tone, measured by cardiac vagal tone (CVT) and protects against oesophageal hyperalgesia (Botha et al, Gut 2014). However, the contribution of cognitive factors to the ANS effects of SDB are unknown.

Aim: To study the effect of SDB and attention on the ANS.

Methods: 21 healthy volunteers (age 23.9 ± 2.57 , 9 female), were randomised in a crossover design with 1 week washout period to: 1. 30 min SDB exercise (1-minute cycles of SDB at 0.1 Hz intercepted by 4 minutes of spontaneously paced breathing). 2. Attention task (One Back Task - Cogstate Ltd-USA). Autonomic variables were measured continuously (using Neuroscope™, Medifit Instruments, UK) at baseline, during interventions and 5 minutes after. Primary outcomes: CVT during interventions. Secondary outcomes: Cardiac sympathetic index (CSI), heart rate (HR), systolic blood pressure (SBP). CVT is presented as Median (IQR), other variables as Mean (SD).

Results: SDB increased CVT from baseline, 9 (7.25–15.5) vs. 8.5 (5.25–13.25), $P < 0.01$ and returned immediately to baseline at the end of the intervention. SDB also increased CSI, Δ CSI = 0.58, CI 0.56–1.1, $P < 0.03$ but had no effect on SBP or HR. Attention task did not affect CVT, however, after the intervention, CVT significantly increased compared to baseline 11 (8.25–12) vs. 9.5 (5.25–11.75), $P < 0.03$. Attention increased both HR and SBP, ($\Delta = 2.9$, CI 0.35–5.3, $P < 0.02$), ($\Delta = 10.7$, CI 5.2–16.2, $P < 0.001$) respectively, but had no effect on CSI.

Conclusion: SDB and not attention activated sympathetic and parasympathetic systems. CVT increase post-attention task may be due to relaxation and relief. Hence, attention contribution to ANS modulation during SDB task is minimal although the potential for post task increase in CVT being affected by cognitive factors should be considered in future studies.

P6.02

Nitric oxide has direct effect on electrical characteristics of neurons

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Discovery of the ability of mammalian cells to synthesize the free radical nitric oxide (NO) has stimulated significant efforts of researchers to study the role of NO in all areas of biology and medicine. It is found that NO is involved in behavioral programs, more and more data is accumulating that in the nervous system NO is involved in development, maturation and aging of the brain, in the processes of learning and memory. The aim of

this work was to study the effects of NO level on the membrane potential of the premotor interneurons of snail. We used an application of sodium nitroprusside (SNP), a donor of NO (at a concentration of 10^{-4} mol/l) and L-NAME, inhibitor of NO-synthase (at a concentration of 10^{-4} mol/l), into the solution that washing the preparation of intact snails, on the membrane potential (Vm) of premotor interneurons.

In the experiments it is found that application of NO donor SNP in a solution that washing the preparation of intact snails, caused the hyperpolarization on 3-5 mV for 10 minutes. In that time, inhibitor of NO-synthase L-NAME caused the depolarization on 4 mV. Thus, we have demonstrated that NO can cause hyperpolarization of membrane in certain neurons. It is assumed that the response of the neuron to NO depends on the location of it in the neural network.

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P6.03

Effects of serotonin depletion by p-chlorophenylalanine on reconsolidation of contextual memory

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It was found that long-term memory becomes variable after the reactivation, namely, after the remembering. The specific contextual learning and memory was also found in invertebrates. We conducted a study of the role of serotonin (5-HT) in context conditioning and its reconsolidation using inhibitor of 5-HT synthesis p-chlorophenylalanine (p-CPA). We elaborated the context learning in terrestrial snails, when the animals could distinguish the test signals used in different contexts (on ball and a flat surface). The next day, after testing confirming context learning, snails were placed for 20 minutes on the ball that served as a reminder, and then blocked the protein biosynthesis by injection of anisomycin. On the next day the preservation of context learning was tested. Disruption of memory demonstrated the process of reconsolidation.

Memory storage testing showed that anisomycin without reminders didn't disrupt the contextual memory, however it caused disrupting of memory if anisomycin injected simultaneously with reminder. Thus, we observe here the disruption of reconsolidation. But p-CPA injected 3 days before anisomycin (depletion of 5-HT) led to decrease of the behavioral reactions in response to tactile stimulation in 2 times on the second day after a reminder. The obtained results point to the necessity of 5-HT for the process of reconsolidation of memory.

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P6.04

Restriction of motor activity in rats: dynamics of nitric oxide production in the heart after recovery

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Hypokinesia (restricted of physical activity) is one of the most pressing medical and social problems caused by lifestyle, occupational activity, prolonged bed rest, and a range of diseases, etc. Today an important area of physiological research is to study the role of nitric oxide (NO) in the cardiovascular, nervous and other body systems. It has been found that NO impairs the progress of myocardial infarction, but is also an opposite point of view, according to which an excess of NO is a compensatory factor. The objective of the study was to investigate the role of NO in the processes under recovery after hypokinesia in heart tissues of rats growing under restricted physical activity.

The experimental group was divided into 3 sub-groups: 1) animals, kept under hypokinetic conditions for 30 days, 2 and 3) animals, kept for 1 and 2 week under recovering conditions after 30-day hypokinesia. NO content in the rat organs was determined by technique which uses spin trapping method. We have discovered that the hypokinetic regime leads to an increase in NO production in the tissues by two times. We have found that during the subsequent recovery from hypokinesia there is further increase in NO production in the heart tissues. This result indicates that the recovery from hypokinesia is also a significant immobilization stress for animals.

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P6.05

Opposite responses of interneurons of naive and learned animals to application of serotonin

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Serotonergic system plays an important role in the modulation of stress-induced excitability (arousal) and defensive behavior. It was shown that long-term facilitation of connections between sensory and motor neurons of gills withdrawal reflex was mediated by serotonin (5-HT) and this form of synaptic plasticity was found to be a critical cellular mechanism of behavioral sensitization. A lot of experiments were conducted using application of 5-HT for elaboration of cellular analogues of learning. These findings have induced us to investigate the role of 5-HT in mechanisms of learning by the analysis of changes of excitability of premotor interneurons to serotonin application in naive snails and animals after learning.

The decrease of interneuron's membrane potential was found to be caused by 5-HT application in isolated preparation from both naive and trained snails. At the same time, the application of 5-HT to preparation from trained snails caused the increase of threshold potential in opposite to naive snails where no changes

were revealed in the threshold potential. This effect means that the appearance of extracellular 5-HT, which could be emitted, for example, from modulator serotonin-containing neurons leads to a decrease in interneurons excitability which was increased after training procedures.

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P6.06

Connectionist model of the pattern generator of one muscle's specific activity profile

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The main goal of the project is to find common rules for building for creation of neural systems (composed from simple subsystems) with scalable cognitive qualities on the basis of simple formal neurons with spike activity. On the basis of the original software the variants of the neural structures configurations have been tested. These networks provide plasticity on the level of conditioning the signals coming from the neural blocks of different functions, which allows the implementation of the principle of Hebbian plasticity.

It was found a principal architecture of dynamic stochastic artificial neural network, which provides facilitation of the execution of the motor program which managed "outside". It was shown forming of the pattern generator of muscle activity with the activity profile of the single muscle of any specified waveform. The interconnections between the elements, which lead to the sequential activation of elements during the formation of the motor activity patterns present in proposed model. These interconnections allow for explaining the modulation polysynaptic responses observed in the electrophysiological experiments after applying of frequency stimulation. It is associated with the one of the main issues, namely with the recorded dynamic of changes of the motor responses of the spinal cord in the functional tests procedure, as well as after recovering of the complex changes of the structure of the nervous system, such as, for example, after a spinal cord injury. The described scheme also explains the necessity of optimal sensory input in restoring pre-existing motor programs, including motor programs after injuries, as well as the necessity of the additional activation or loading for improving of the motor response.

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P6.07

Influence of inhibitor of NO-synthase L-NAME on nitric oxide production in the rat hippocampus in acute phase of ischemic and hemorrhagic insult

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It is known that the prolonged deficit of oxygen leads to hypoxia of the brain, which is under certain conditions accompanied by the development of tissue ischemia. According to this the study of the pathogenesis, the methods of correction and the mechanisms of stroke is important both from the theoretical and practical points of view. Nitric oxide (NO) is an important signaling molecule that is widely used in the nervous system. Our investigation's main purpose is to study the processes of NO-synthase involvement in the control of NO levels in the hippocampus of rats after modeling both ischemic and hemorrhagic stroke.

By spectroscopy of electron paramagnetic resonance it was shown that in 5 h after the onset of symptoms ischemic and hemorrhagic stroke the level of NO in the hippocampus was reduced by 2-3 times and this reduction was maintained for 24 and 72 h. It has indicated that nonselective NO-synthase blocker L-NAME reduced the low level of NO production in 3 times by its administration in 72 h after post-ischemic and hemorrhagic stroke. But it was discovered that L-NAME returns the level of NO production to baseline (control) by its administration in 5 h after ischemia.

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P6.08

Management of acute ischaemic stroke at IRCCS San Martino in Genoa: from epidemiology to therapy

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Background: Thrombolysis improves functional outcome after acute ischemic stroke (AIS), but in Italy only the 12.5% of AIS patients undergo this treatment. Here, we studied the management of consecutive patients with suspect of AIS in order to identify factors associated with possible underutilization of thrombolysis.

Materials and methods: Among individuals ($n = 816$) arrived at the Emergency Room (ER) with a suspect of AIS from 1 January 2016 to 31 December 2016, we reviewed 458 records (264 females and 194 males) in which the diagnosis of AIS was confirmed.

Results: 49% of patients arrived at the ER within 4.5 h of symptoms onset and 49% of these were treated with systemic thrombolysis. In the remaining 51%, main reasons for NOT undergoing iv thrombolysis were: minor stroke or stroke in

rapid improvement, ongoing anticoagulant therapy, high risk of bleeding due to recent hemorrhage/ trauma or surgery, severe comorbidities or advanced age and epilepsy at onset. 17% patients had a wake-up stroke and for this reason were excluded from thrombolytic treatment. 67% patients who arrived at the ER in therapeutic window, underwent intracranial ANGIO-TC which revealed an occlusion of a proximal cerebral artery in 55% of cases. Among these patients who were eligible for endovascular revascularization, 57% underwent mechanical thrombectomy. TICI 2b/3 was obtained in 60% of patients who performed rescue thrombectomy. According to the SIST MOST definition, SICH rate was 1.8%. The mortality rate was 12%. Mean duration of hospitalization was 10 days. At discharge, 49% of patients returned home, 17% were transferred to Rehabilitation Centre and 12% in RSA.

Conclusions: A late patient presentation is a barrier for using thrombolysis in AIS. Intervenous thrombolysis was utilised in a higher rate of patients than expected. These data demonstrate how at the IRCCS San Martino the new ISO-SPREAD guidelines have been implemented.

P6.09

Sustained disease remission in aggressive multiple sclerosis after autologous haematopoietic stem cell transplantation

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Background: Despite the advent of new highly-active therapies for multiple sclerosis (MS), long-term disease remission remains elusive and only a small percentage of patients achieves the so-called no evidence of disease activity (NEDA) status. Against this scenario, autologous haematopoietic stem cell transplantation (AH SCT) has recently demonstrated the potential to maintain disease remission in aggressive MS patients.

Materials and methods: We analyzed data from 35 consecutive patients with aggressive MS (77% relapsing-remitting MS, 23% active secondary progressive MS) treated with AH SCT at Neurologic Departments of Genoa and Turin between 1998 and 2015. All patients underwent the same transplant protocol made of cyclophosphamide followed by carmustine-cytarabine-etoposide-melphalan (BEAM) plus anti-thymocyte globulin. NEDA status (a composite of absence of relapses, no sustained disability progression, and no new T2 or T1 gadolinium-enhancing lesions on MRI), disability scores and reports of adverse events were collected.

Results: NEDA status was achieved by 33 of 35 patients (94%) at 1 year, 21/25 (84%) at 3 years and 11/14 (79%) at 5 years. Improvement was noted in neurologic disability from a median pre-transplant disability score of 6.5 to 6 at 5 years. Adverse events were consistent with expected toxic effects associated with AH SCT and no treatment related mortality was reported.

Conclusions: Our data demonstrate that AH SCT is extremely effective for inducing long-term disease remission in aggressive RRMS patients and it is associated with improvements in neurologic functions.

P6.10

Delirium in the acute phase of stroke: comparison between methods of detection

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Background: Delirium is an acute neuropsychiatric syndrome, very common in hospitalized people with medical and neurological conditions. The identification of delirium after stroke is not an easy task and validated psychometric instruments are needed to correctly identify it. We decided to verify if (i) formal training in DSM-V criteria is needed to correctly identify post-stroke delirium, (ii) if the use of a brief psychometric instrument such as the 4AT improves its identification, (iii) the applicability of these scale in the stroke setting.

Methods: In the first phase of study we retrospectively studied 102 acute stroke patients in Stroke Units of San Martino Hospital (Genova, Italy) in order to evaluate delirium with clinical criteria, firstly by a neurologist without a formal training in DSM-V criteria and after training. Then, we enrolled 100 new acute stroke patients who underwent screening for delirium using 4AT scale and DSM-V criteria.

Results: In the first phase, DSM-V criteria training significantly increased the ability to capture delirium (5% vs. 15%; $P = 0.006$).

In the second phase, the 4AT was used for delirium screening revealing a 52% of cases of delirium, the same observed by the consensus diagnosis - in accordance to DSM-V criteria- of two senior neurologist (that was 50%). There was a significant difference in delirium incidence between 4AT and DSM-V criteria ($P = 0.009$).

In the second phase, the use of 4AT scale allowed to capture post-stroke delirium as well as the consensus diagnosis by two neurologists.

Conclusion: The identification of post-stroke delirium is not an easy task and requires both formal training in DSM-V criteria as well as the application of brief scales such as the 4AT.

P6.11

Differential use of the HCAR2 pathways triggered by monomethyl fumarate in different cells

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We recently demonstrated that monomethyl fumarate (MMF), the bioactive metabolite of the immunomodulatory drug dimethyl fumarate (DMF) approved for treatment of multiple sclerosis, modulates the activation of microglia towards a neuro-protective-like phenotype through a novel pathway triggered by MMF binding to the hydroxycarboxylic acid receptor-2 (HCAR2). In particular, we observed that HCAR2 activation by MMF leads to inhibition of the NF- κ B pathway and, thereby, the expression of inflammatory cytokines, via the AMPK/Sirt1 axis.

Increasing evidence associates signaling through HCAR2 in macrophages and dendritic cells (DC) with an anti-inflammatory phenotype; MMF could therefore exert its effect also in these cells by activating the AMPK/Sirt1 axis.

We show that MMF inhibits only partially the activation of bone marrow-derived macrophages, by reducing the expression of the M1-phenotype marker Nos2 without modulating the expression of other typical M1-/M2-markers, suggesting that in these cells MMF does not act through the AMPK-Sirt1 axis. Similarly, while MMF induced an anti-inflammatory phenotype in activated splenic DC, reducing the expression of Tnf, Il12 and Il23, it had no such effect on activated bone marrow-derived DC. Our ongoing experiments focus on understanding if MMF signals through the novel HCAR2-mediated pathway in splenic DC.

Since HCAR2 is also the receptor for butyrate, an anti-inflammatory commensal metabolite, we have speculated that the intestinal side effects associated with DMF treatment might be associated with competition of MMF with butyrate for HCAR2 binding, with MMF signaling in these cells through the prostaglandin D2/inflammatory pathway, whereas butyrate, which blocks NF- κ B activation in colonic cells, would signal through the AMPK/Sirt1-mediated pathway. To determine if butyrate could signal through this pathway, we have analysed its effect on activated microglia. Our preliminary results suggest that butyrate can trigger the AMPK-Sirt1 pathway upon binding to HCAR2.

Altogether these data suggest that HCAR2 signaling through different pathways could be cell-biased.

P6.12

Impact of teriflunomide treatment on innate and adaptive immune cell subsets in multiple sclerosis patients

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Teriflunomide, a drug approved for treatment of relapsing-remitting multiple sclerosis, inhibits dihydroorotate dehydrogenase, an enzyme involved in de novo biosynthesis of pyrimidines in highly proliferating cells, such as activated lymphocytes, without disrupting protective immunity. It does not affect resting cells, which use a different pathway to supply pyrimidine requirements. TEMSO and TOWER clinical trials showed that teriflunomide treatment was associated with a mean decrease in leukocyte counts of approximately 15 % compared to baseline, occurring within the first months of treatment and stabilizing thereafter. The effect of teriflunomide on immune cell subpopulations is not clearly understood. We aim at studying the effect of this drug on innate and adaptive immune cells. Recruited patients received teriflunomide (14 mg) once daily. Peripheral lymphocytes were isolated at baseline and at 3 and 12 months of treatment and adaptive and innate immune cell subsets were analyzed by flow cytometry as follows: T effector cells (classic CCR6-CD161-CXCR3 + and non-classic CCR6-CD161 + CXCR3 + Th1 cells, CCR6 + CD161 + CCR4 + Th17 cells, and CCR6 + CXCR3hiCCR4lo-CD161 + Th1-17 cells) and T regulatory cells (CD4 + CD25 + CD127- and CD8 + CD28-); B cells (CD19 + cells, including CD38-CD24 + memory, CD38lowCD24 + mature and CD38-highCD24 + regulatory B cells) and natural killer (NK) cells (CD56dim and CD56bright). Our results show that teriflunomide impacts B-cell subsets, significantly reducing absolute counts of total CD19 + B cells, and mature and regulatory B-cell subsets after 12 months. T cells were affected to a lesser extent,

with a trend in reduced absolute counts for both T effector CD4 + cells (Th1, Th17 and Th1·17) and T regulatory CD8 + and CD4 + cells. Teriflunomide had no detectable impact on NK-cell numbers. These data suggest that teriflunomide impacts on B cell population emphasizing their implication in MS pathogenesis and/or progression. Larger cohorts are necessary to confirm these findings and the impact of teriflunomide on the functionality of these cells.

P6.13

Prevention by creatine of statin-induced myopathy: a case report

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Statins are recommended in the prevention of cardiovascular diseases, yet they are generally under-used, one of the main reasons being the risk of statin-induced myopathy. The latter ranges in frequency between 1.5% and 13% of treated subjects, according to various reports. It ranges in severity from isolated elevation of serum creatine kinase (CK) to life-threatening rhabdomyolysis. This adverse event is one of the main reasons why physicians tend to under-prescribe statins. Preclinical evidence

suggests that creatine synthesis may be involved in the generation of statin-induced myopathy. A clinical study with cross-over design suggested recently that high-dose creatine prevents statin-induced myopathy. That finding prompted us to use creatine in the following clinical case. A 66 y.o. lady affected by chronic myeloid leukemia in nilotinib-induced clinical remission had amaurosis fugax in her right eye. The ipsilateral carotid artery was found to be 55% stenotic with an ulcerated plaque; the contralateral one was 45% stenotic. Serum cholesterol was 316 mg/dl (LDL-cholesterol 213 mg/dl). Nilotinib was replaced by interferon, and she received acetyl-salicylic acid, clopidogrel, and atorvastatin 40 mg/day. The statin, however, caused muscle pain and elevation of serum CK, and was replaced by ezetimibe. Two years later, serum cholesterol was still 211 mg/dl with LDL-cholesterol 126 mg/dl and normal CK. Simvastatin 5 mg/day was then started, but again the patient had muscle pain and her serum CK rose to 267 U/l (reference range 26-170). Simvastatin was stopped, and the next serum cholesterol measurement was 252 mg/dl. Creatine was then prescribed, 5 g b.i.d. for 5 days, then 5 g daily. After the first 5 days, Simvastatin 5 mg/day was reintroduced. One month after, serum cholesterol was 171 mg/dl, CK was 72 U/l and the patient had no muscle pain. Pending further follow up, this case confirms that creatine administration can prevent statin-induced myopathy.

Workshop 7: Lipidology and Atherosclerosis

P7.01

Apelin-13 enhances atherosclerotic plaques stability in ApoE-deficient mice

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Introduction: Atherosclerosis remains one of the main cause of death worldwide and substantial efforts have been made to identify novel approaches to improve the management of this disorder. Apelin is an endogenous peptidergic family with essential role on the cardiovascular hemostasis and pathologies. Recent studies pointed out a fundamental contribution of Apelin system on atherosclerosis development; however, such reports revealed contradictory data, and to date, it is difficult to accurately define the beneficial or deleterious role of Apelin in atherosclerosis.

Objective: To better understand the role of Apelin system on atherosclerosis, we aimed to investigate the actions of Apelin-13 treatment on atherosclerotic plaques composition, focusing on features of plaque vulnerability.

Methods: Apolipoprotein E gene-deleted mice ($n = 40$) were fed with western-type diet for 11 weeks. Atherosclerotic plaque formation was induced in the carotid artery by a shear stress modifier device, which exposed the vessel to distinct patterns of shear stress, resulting in plaque formation with different composition. The mice were treated with Apelin-13 (2 mg/kg/day) or vehicle for the last 3 weeks of experimental period.

Results: Apelin-13 treatment did not change atherosclerotic plaque size in the aorta, neither altered the lipid content of low shear stress and oscillatory shear stress-induced plaques in the carotid. However, Apelin-13 remarkably ameliorated plaque stability by increasing intraplaque collagen content, which was associated with a reduction of MMP-9 expression. Furthermore, Apelin decreased cell infiltration (neutrophil and macrophage) and intraplaque reactive oxygen species content. Interestingly, Apelin-13 treatment reduced total cholesterol, LDL levels and free fatty acids serum levels, while HDL, triglycerides serum levels were not significantly changed.

Conclusion: Apelin-13 treatment for 3 weeks did not alter the lesion size, but significantly enhances the stable phenotype of atherosclerotic plaques and improved serum lipid profile. These results indicate that activation of Apelin system enhances plaque stability.

P7.02

Low grade inflammation, oxidative stress and ultrasound assessment of abdominal fat tissues in non-obese subjects

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Aim: To compare “common clinical” markers of adiposity and ultrasonographic indices of adiposity (subcutaneous and visceral fat, liver steatosis) and to assess their relationships with metabolic abnormalities, low grade inflammation and oxidative stress in a healthy population of obese and non-obese.

Methods: We enrolled 60 subjects (age 43 ± 14 years, 26M / 34F). They were divided into 2 groups according to BMI (< 30 or ≥ 30 Kg/m²). The ultrasound measurement of abdominal fat tissues included subcutaneous fat (cutis-linea alba thickness), visceral fat (linea alba-posterior wall of abdominal aorta) and the presence of liver steatosis (assessment of Fatty Liver Indicator). Insulin resistance was defined for HOMA > 2.5 and dyslipidemia on the basis of ESC guidelines. Low-grade inflammation was determined by hs-CRP serum levels. Intracellular production of ROS and lipid peroxidation of blood cells was obtained by flow cytofluorimetric analysis.

Results: Non-obese differed from obese in term of all the abdominal adiposity indices and of metabolic abnormalities. Non-obese were compared on the basis of BMI values, waist circumference, FLI, visceral fat and subcutaneous fat. BMI and waist circumference were related to the different abdominal fat indices, in particular with subcutaneous fat ($R = 0.66$, $P < 0.001$ for BMI, $R = 0.63$, $P < 0.001$ for waist circumference), whereas FLI was only correlated to BMI ($R = 0.36$, $P = 0.025$). Visceral fat was associated with low-grade inflammation ($R = 0.61$, $P < 0.001$) and insulin resistance ($R = 0.55$, $P = 0.003$), even after adjustment for BMI and waist circumference. Subcutaneous fat correlated with blood cells oxidative levels ($R = 0.47$ to 0.59 , $P < 0.001$), even after adjustment for BMI and waist circumference.

Conclusions: Ultrasound evaluation of abdominal fat deposits may select non-obese patients, but metabolically obese. The subcutaneous fat is associated with increased oxidation of all blood cells.

P7.03

Hypertriglyceridemic waist phenotype and vascular damage in hypertensive patients

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Hypertriglyceridemic-waist (HTGW) phenotype, characterized by increased waist circumference (> 90 cm in males and > 85 cm in women) and hypertriglyceridemia (> 177 mg/dl), has been proposed to identify patients with excess intra-abdominal adiposity and associated metabolic abnormalities. HTGW

phenotype is associated with risk for type-2 diabetes (T2D) and cardiovascular disease (CVD) development. It's known that, early indicators of vascular damage so as endothelial dysfunction, increased arterial stiffness (AS) and carotid intima-media thickness (c-IMT), represent independent predictors of cardiovascular (CV) events. The aim of the present study was to evaluate the association between HTGW phenotype and vascular damage in newly diagnosed hypertensive patients. For this aim we enrolled 889 never-treated hypertensive subjects, 537 males and 352 females, (mean age 50.1 ± 10.1 years). According to HTGW phenotype, all patients were divided into two groups: 200 presenting phenotype and 689 without it. Endothelial function was estimated by a semi-plethysmographic method, using a peripheral arterial tonometer (PAT) with measurement of reactive hyperemia index (RHI). AS was evaluated with the measurement of carotid-femoral pulse wave velocity (PWV) by applanation tonometry (SphygmoCor) and c-IMT by a high-resolution ultrasound B-mode system. Patients with HTGW phenotype presented a worse metabolic and hemodynamic profile in comparison with the other group. Although there was no difference in clinical blood pressure (BP), HTGW phenotype presented increased values of central systolic ($P = 0.027$) and diastolic BP ($P = 0.034$). In addition, RHI was significantly reduced (1.8 ± 0.4 vs 2.1 ± 0.6 , $P < 0.0001$) and PWV increased (7.9 ± 2.1 vs 6.9 ± 1.7 m/s, $P < 0.0001$), so as c-IMT (0.8 ± 0.2 vs 0.7 ± 0.2 , $P = 0.001$). Considering a cut-off value of PWV > 10 m/s, the percentage of patients with increased AS was significantly higher in the HTGW group (25% vs 10.2%, $P < 0.0001$). In conclusion, in newly diagnosed hypertensive patients, HTGW phenotype was associated with a detrimental metabolic and inflammatory profile and vascular damage, resulting an increased risk for clinical events.

P7.04

Alterations of IKCa-mediated endothelial electrical signaling in a murine model of atherosclerosis

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Atherosclerosis-associated alterations of endothelial cell (EC) electrical properties, which are a key determinant of EC function, remain poorly understood. We aimed to explore the alterations in EC electrical signaling in apolipoprotein E-deficient mice (ApoE-ko). Membrane potential (MP) and currents were recorded using the perforated patch-clamp technique from in situ endothelium from excised mice aorta, C57Bl/6 mice served as a control. In aortic strips from C57Bl/6 mice, the mean resting EC MP was -36.7 ± 1.2 mV. In aortas from ApoE-ko mice, the MP was significantly less negative and averaged -31.4 ± 0.9 mV. In control group, the inhibitor of Ca²⁺-dependent K⁺ channel of intermediate conductance (IKCa) TRAM-34 (2 µM) produced cell depolarization by 7.6 ± 0.8 mV, while in aortic strips isolated from ApoE-ko group TRAM-34 failed to alter the EC membrane potential. In control group, voltage ramps from -90 to 80 mV elicited whole-cell currents of a significantly larger amplitude than that observed in ApoE-ko group.

The amplitude of hyperpolarization to 2 µM Ach was not significantly different between the two groups. However, lower concentrations of Ach (0.2 and 0.5 µM) elicited an increased hyperpolarizing responses in ApoE-ko mice (control: 15.7 ± 3.1 mV and 17.0 ± 3.7 mV, respectively; ApoE-ko: 23.4 ± 2.2 mV and 25.8 ± 2.4 mV, respectively). The hyperpolarizing responses to SKA-31, a direct IKCa/SKCa opener, were also significantly increased in ApoE-ko mice (3 µM, control: 12.1 ± 1.3 mV, ApoE-ko: 18.0 ± 2.1 mV). In conclusion, our study points to a dual modulation of endothelial IKCa by atherosclerosis. While the basal IKCa activity is compromised, contributing to a decreased resting membrane potential, IKCa stimulation reveals an enhanced hyperpolarizing response, pointing to upregulated IKCa activity, which diminishes the detrimental effect of atherosclerosis on vascular function.

P7.05

Remnant cholesterol predicts the development of type 2 diabetes mellitus in patients with established coronary artery disease

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Aim: Remnant cholesterol recently has attracted interest as a marker of cardiovascular event risk and is associated with the metabolic syndrome as well as with type 2 diabetes (T2DM). However, it is unknown whether remnant cholesterol also predicts the development of diabetes in patients who do not have diabetes yet.

Methods: We prospectively recorded incident diabetes over 6.1 ± 3.7 years in 855 consecutive non-diabetic Caucasian patients with angiographically proven coronary artery disease (CAD). Diabetes was diagnosed according to ADA criteria.

Results: At baseline, 41.3% of our non-diabetic CAD patients had impaired fasting glucose (IFG); remnant cholesterol was significantly higher in IFG than in NFG patients (23 ± 21 vs. 19 ± 22 mg/dl; $P < 0.001$). During follow-up, diabetes was newly diagnosed in 110 patients, i.e. in 12.9% of the study population. Remnant cholesterol strongly predicted diabetes both univariately (OR 1.88 [1.56-2.27]; $P < 0.001$) and after multivariate adjustment including both fasting glucose and HbA1c values (OR 1.40 [1.40-2.11]; $P < 0.001$).

Conclusion: We conclude that the incidence of diabetes is high in patients with established CAD and that remnant cholesterol strongly and independently predicts the development of diabetes in this population.

P7.06

Effect of cholesterol diet and corvitin at cardiohemodynamics and expression of H2S-synthesizing enzymes genes in aorta of spontaneously hypertensive rats

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Arterial hypertension is accompanied with increased stiffness of vessels which might occur due to disturbances in lipid metabolism and impaired vasorelaxant agents' synthesis, like hydrogen sulfide (H2S). 'Corvitin' (BCPP, Ukraine), the modified form of flavonoid quercetin, was shown to correct the blood flow disturbances. The aim of the study was to evaluate the effect of cholesterol rich diet and 'Corvitin' at expression of H2S synthesizing enzymes genes cystathionine gamma lyase (CSE) and mercaptopyruvate sulfur transferase (MPST) in aorta of Wistar and spontaneously hypertensive rats (SHR).

4 month old SHR and Wistar rats were kept on 3%-cholesterol rich diet (CRD) for 8 weeks. Another SHR group received 'Corvitin' in dose of 15 mg/kg/day for 8 weeks as well. Total mRNA was extracted from aorta and real time PCR analysis performed. The 26 hemodynamic parameters were monitored using catheter (Millar Instruments, USA).

Arterial elastance was 4.92 times lower in SHR comparing to Wistar rats. CRD increased arterial elastance in 2.3 times in Wistar, however, opposite effect was observed in CRD SHR: arterial elastance was decreased twice. 'Corvitin' prevented increase of stiffness of vessels in SHR. Total cholesterol and low density lipoproteins were significant increased (2- and 4,5-times) in CRD SHR.

Expression of CSE and MPST genes were 8- and 2- times decreased in aorta of SHR comparing to Wistar rats. 'Corvitin' showed normalizing effect on CSE and MPST expression levels in SHR aorta. Cholesterol containing feeding was accompanied with decreased mRNA levels of CSE in 4,5 times and increased MPST levels in 2,5 times in Wistar rats' aorta, however, no notable changes were observed in SHR aorta under cholesterol diet.

Thus, dysregulation of H2S seems to be important in pathogenesis of genetically determined hypertension. Consumption of 'Corvitin' might correct the blood flow disturbances influencing H2S system normalizing CSE and MPST genes expression.

P7.07

Follicular Treg cell controls Tfh, Breg and marginal B2 cells during atherosclerosis development in mice

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Atherosclerosis is a major trigger of myocardial infarction and constitutes the leading cause of cardiovascular mortality. Atherosclerosis is a chronic inflammatory disease involving the infiltration of immune cells, such as monocytes/ macrophages, neutrophils, T and B cells, into the inner layer of vessel walls. The function of B cells in the process of atherogenesis has been investigated but several aspects remain to be clarified. In the present study, we show that follicular regulatory T cell (Tfr) regulates atherosclerosis lesions in ApoE^{-/-} mice models under high cholesterol diet by modulating regulatory B cell (Breg), marginal zone B cell (MZ B cell) and follicular B cell

(FO B cell) population. Spleen and lymph node (LN) follicular T cells (Tfh) are actually decreased by growing Tfr population in advance atherosclerosis. This modulation of Tfh is correlated with the development of atherosclerotic plaque size in thoraco-abdominal aortas and aortic root plaques suggesting that Tfh are atheroprotective. High cholesterol diet induces a strong increase of Breg and MZ B cells in secondary lymphoid organs while FO B cells are decreased. In this context, the treatment of mice with Bcl-6 inhibitors leads to intensification of atherosclerotic plaque size, to an increase the frequency of MZ B cell and to a decrease of Breg in spleen and LN. Consistently, adoptive transfer of isolated Tfr from 11 weeks ApoE^{-/-} mice induces atherosclerotic plaque size, MZ B cells and Breg cells augmentation in ApoE^{-/-} mice under low cholesterol diet. Our results demonstrate that Tfh and Tfr modulate anti- and pro-atherosclerotic immune process in ApoE^{-/-} mice model. This study further suggests that Tfr cells positively regulate Breg cell population and *ex vivo* expansion of Tfh could be use for cellular therapy against atherosclerosis.

P7.08

Anti-PCSK9 antibodies in heterozygous familial hypercholesterolemia treatment: efficacy in LDL-C reduction and clinical safety

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Background: Familial Hypercholesterolemia (FH) is a form of hypercholesterolemia due to an autosomal dominant mutation in LDL-R, APOB or PCSK9 gene. A higher cardiovascular risk characterizes FH patients. Standard lipid-lowering therapies are usually ineffective to reduce LDL-C blood levels in these patients. Anti-PCSK9 monoclonal antibodies can lead to consistent reductions in LDL-C plasma levels in HeFH and hypercholesterolemic patients unresponsive to or intolerant to conventional lipid-lowering treatments.

Aim of the Study: To assess Alirocumab and Evolocumab lipid-lowering efficacy, safety and adverse events in two groups of patients who underwent to an anti-PCSK9 treatment.

Methods: Anti-PCSK9 drugs were prescribed in addition to previous lipid-lowering therapy. We assessed baseline Total and LDL-Cholesterol in each patient before the first administration of these drugs. Efficacy has been expressed as Total and LDL-Cholesterol reduction. The afore mentioned levels have been evaluated after 12 weeks in Alirocumab treated patients and after 16 weeks in Evolocumab treated ones.

Results: In Alirocumab-treated patients the mean LDL-C reduction was of 103.1 mg/dL, -49.9% vs baseline ($P = 0.001$), and the mean TC reduction was of 103.5 mg/dL, -36.6% vs baseline ($P = 0.002$). In Evolocumab-treated patients the mean LDL-C reduction was of 135.14 mg/dL, -65.56% vs baseline ($P = 0.018$), and the mean TC reduction was of 143.71 mg/dL, -50.4% vs baseline ($P = 0.018$). In both groups, there were no adverse events and both drugs were tolerated and safe.

Conclusion: Therapy with anti-PCSK9 mAbs resulted in a remarkable and effective LDL-C reduction in our patients leading to achievement of therapeutics targets in a large number of patients. LDL-C therapeutic targets achievement is the characteristic clinical marker of cardiovascular risk reduction. Currently, definitive clinical cardiovascular risk reduction data is unknown and it could be confirmed only by ongoing prospective clinical studies conclusions.

P7.09

Lomitapide in homozygous familial hypercholesterolemia: our clinical experience

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Background: Homozygous Familial Hypercholesterolemia (HoFH) consists in an alteration in lipid metabolism correlated with elevated risk of cardiovascular disease in early ages. Conventional therapies are not able to determine significant cholesterol reduction, for this reason chronic LDL-apheresis is usually necessary. Lomitapide (L) represents a new treatment perspective.

Aim of the Study: To evaluate the clinical efficacy of L in terms of Total and LDL cholesterol reduction, and the occurrence of treatment-related side effects.

Methods: Effects of L were assessed in two brothers with HoFH (FH-Pavia mutation), undergoing apheresis from the age of 4 associated with pharmacological therapy. Patient A started L at the age of 19; previously mean LDL-c levels were 486 mg/dL. Patient B started L therapy at the age of 18, with mean LDL-c levels of 435 mg/dL. We considered lipid parameters, hepatic cytolysis and cholestasis markers, gastrointestinal side effects, variation of interapheretic interval and impact on QoL.

Results: In A, introduction of lomitapide brought to significantly lower mean lipid values in pre-apheresis samples: LDL-c 270 mg/dL (–45%), CT 308 mg/dL (–45%), TG 62 mg/dL (–62%). At 30 mg mean LDL-c values were 187 mg/dL (–62%). The most remarkable side effect observed was an elevation in transaminases levels, usually not higher than 3xULN: the rise was temporary and related to L dose escalation or to a decrease in low-fat diet adherence. A better lipid profile allowed extending the interval between apheresis procedures (from 9 to 28 days), improving the QoL.

In B mean pre-apheresis lipid levels were: CT 386 mg/dL (–24%), LDL-c 330 mg/dL (–24%), TG 125 mg/dL (–30%). At 20 mg mean LDL-c concentrations were 231 mg/dL (–47%). The interval between apheresis was implemented from 8 to 14 days. No transaminases elevation occurred.

Conclusion: Lomitapide produced a strong impact on lipid profile allowing the extension of the interval between apheresis procedures and, consequently, ameliorating QoL. Adverse reactions were mild and transient and never required discontinuation of therapy.

P7.10

Histopathological analysis of skeletal muscles after injection of adipose-derived mesenchymal stem cells transduced with LV-VEGF165 and LV-SDF1A

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Prevalence of peripheral arterial disease has been increasing over decades, that stimulates interest to therapeutic angiogenesis. Combination cell and gene-based therapeutic angiogenesis may enhance therapeutic effect. The aim of our project was histopathological analysis of skeletal muscles after native

adipose-derived mesenchymal stem cells (ADMSC), VEGF165-ADMSCs, or VEGF165-SDF1A-ADMSCs injection in rat hindlimb ischemia model.

ADMSC were isolated from the Wistar rats. Co-transduction of lentiviruses encoding VEGF165 or VEGF165 + SDF1A was performed together with the EGFP transduction. Hindlimb ischemia model was performed on Wistar rats by hindlimb vessels ligation. On the 7th day after the second stage of the operation gastrocnemius muscle was injected with 2×10^6 of VEGF165-ADMSC, VEGF165-SDF1A-ADMSC or native ADMSC. The calf muscles of the operated and intact limbs were taken on the 3, 7, 14, 21, 28th days after injection.

Histopathological analysis identified that number of centronucleated fibers decreases in «VEGF» and «VEGF+SDF1a» groups while in the native ADMSCs group remains high by day 28. Mallory staining revealed interstitial fibrosis in the native ADMSCs group by day 28. Increased vessel number was demonstrated by anti-aSMA staining by day 3 and 7 in «VEGF» group. In the «VEGF+SDF1a» group number of vessels is decreased till the 14th day, but significantly increased on the 21st day. Evaluation of Ki-67 expression shows decreasing proliferative activity in «VEGF» and «VEGF+SDF1a» groups. In the native ADMSCs group we observed increased proliferation rate by day 28. Anti-fast/slow myosin heavy chains staining showed increasing number of slow fibers in all groups.

Thus, transduction of ADMSCs with LV-VEGF165 and LV-VEGF165 + LV-SDF1A increases their regenerative potency. This work was funded by RSF grant (14-15-00916).

P7.11

Multiplex analysis of skeletal muscles after intramuscular injection of adipose-derived mesenchymal stem cells transduced with LV-VEGF165 and LV-SDF1A in rat hindlimb ischemia model

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Implementation of gene-cell-based therapy of peripheral arterial disease into clinical practice requires pre-clinical investigations for better understanding of curative effect mechanisms. The aim of our investigation was to study expression of growth factors after injection of native adipose-derived mesenchymal stem cells (ADMSC), VEGF165-ADMSCs or VEGF165-SDF1A-ADMSCs into gastrocnemius muscle in rat hindlimb ischemia model.

ADMSCs were isolated from adipose tissue of rats. Cells of the 3rd passage were transduced with recombinant lentivirus LV-VEGF165 or with combination of LV-VEGF165 and LV-SDF1A. Hindlimb ischemia model was performed on rats by hindlimb vessels ligation. On the 7th day after the second stage of the operation gastrocnemius muscle was injected with 2×10^6 of VEGF165-ADMSCs, VEGF165-SDF1A-ADMSCs or native ADMSCs. The calf muscles of the operated and intact limbs were taken on the 3, 7, 14, 21, 28th days after injection. Multiplex analysis of cytokines/chemokines CAV-1, CTGF, IL-6, MCP-1, TNF- α , GROKCCINC, TIMP-1, tPAI-1, VEGF production level in the muscle homogenates was performed using MILLIPIX kits, results were presented as the ratio of cytokines/chemokines levels in the muscle of operated limb to intact limb.

Analysis showed significantly increased level of caveolin-1 (4.18 ± 1.79 by day 28), CTGF (4.23 ± 0.04 by day 21) expression, decreased level of GROKCCINC (0.48 ± 0.07 by day 21)

expression in group with native ADMSCs compared with skeletal muscles of animals in groups with genetically modified ADMSCs indicating retarded muscle regeneration and angiogenesis and activated fibrogenesis in absence of growth factors overexpression. In addition there was no significant difference of proinflammatory cytokines MCP-1, IL-6, TNF α , tPAI-1 levels revealed between operated and intact hindlimbs in all groups. This work was funded by RSF grant (14-15-00916).

P7.12

Use of evolocumab in a patient with homozygous familial hypercholesterolemia

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Introduction: Homozygous familial hypercholesterolemia (FH) is a rare (1:160,000-360,000) but serious disorder with a substantial reduction in LDL receptor function and severely elevated LDL-C. Response to conventional lipid-lowering agents is modest. Novel monoclonal antibodies to PCSK9 have shown ability to substantially reduce LDL-C in heterozygous FH. We evaluated the efficacy of evolocumab in a patient with homozygous familial hypercholesterolemia.

Materials and methods: A 49 years old homozygous FH patient with a missense mutation in the LDLR gene (Gly352Asp) and with severe cardiovascular disease (3 vessels coronary artery disease, aortic mechanical valve replacement for severe stenosis). His pre-treatment LDL-C level was 420 mg/dl. Usual lipid-lowering therapy comprised atorvastatin (80 mg) plus ezetimibe (10 mg) daily in association with LDL-apheresis performed every tenth day. The patient was monitored with three different regimens: I) 42 days with LDL-apheresis + atorvastatin/ezetimibe; II) 61 days with same therapy plus subcutaneous 420 mg evolocumab every 30 days; III) 65 days with 420 mg evolocumab every 15 days.

Results: Regimen I: mean LDL-C 165 mg/dl in pre-apheresis, 77 mg/dl in post-apheresis, 136 mg/dl intermediate.

Regimen II mean LDL-C 149 mg/dl (n.s.) in pre-apheresis, 64.5 mg/dl ($P < 0.01$) in post-apheresis, 118 mg/dl ($P < 0.01$) intermediate.

Regimen III mean LDL-C 164 mg/dl (n.s.) in pre-apheresis, 63 mg/dl ($P < 0.01$) in post-apheresis, 123 mg/dl ($P = 0.01$) intermediate.

Cumulative load in the three periods, mean LDL-C was 129 mg/dl in the first period, 113 mg/dl in the second period (13% reduction), 117 mg/dl in the third period (9% reduction).

Discussion: The selection of the patient for therapy with evolocumab was based on the assumption that his LDLR mutation was receptor-defective. The lipid profile was slightly but significantly improved by evolocumab 420 mg administered every 30 days (13% LDL-C reduction) or every 15 days (9% LDL-C reduction) but still far from LDL-C target (< 70 mg/dl).

P7.13

Evaluation of efficacy and safety of evolocumab 140 mg in genetically characterized He-Fh patients

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Introduction: Primary objective was to establish how lipid profile is modified in patients affected by Heterozygous Familial Hypercholesterolemia (He-FH) treated with high intensity hypocholesterolemic therapy (statin in association to ezetimibe) after four and 8 weeks of therapy with Evolocumab, the new antiPCSK9 monoclonal antibody; secondary objective was to verify the tolerability of the product.

Methods: We enrolled 7 genetically characterized He-FH patients treated with high intensity hypocholesterolemic therapy, who were not reaching low density lipoprotein cholesterol (LDL-C) recommended goal. 5 patients were in secondary prevention, 2 patients were in primary prevention. At baseline the mean of LDL-C levels was 140.14 ± 26.44 mg/dL.

Results: After 4 weeks of treatment with Evolocumab 140 mg every 14 days, LDL-C was decreased to 41.57 ± 20.54 mg/dL with a reduction of 98.57 mg/dL. After 8 weeks of treatment with Evolocumab 140 mg every 14 days, LDL-C was decreased to 39.20 ± 22.01 mg/dL with a reduction of 100.94 mg/dL. In all patients, the recommended LDL-C target was reached. No differences of triglycerides and high density lipoprotein cholesterol (HDL-C) levels were observed. Determination of Lipoprotein (a) levels is in progress. In 3 patients we stopped the administration of ezetimibe and/or reduced the statin dose because of LDL-C < 25 mg/dL. No adverse effects were reported by patients.

Conclusions: Evolocumab 140 mg every 2 weeks is a very effective and safe therapy to obtain the reduction of LDL-C levels in He-FH.

P7.14

Hypertriglyceridemia in the genomic era: risk of ischemic heart disease and pancreatitis

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In observational epidemiological studies mild to moderately high levels of plasma triglycerides are associated with increased risk of cardiovascular disease (CVD). That this is a causal association is supported by numerous genetic studies using Mendelian randomization. The most likely explanation for this is that high levels of triglycerides is a marker of remnant cholesterol, defined as the cholesterol content in the triglyceride-rich lipoproteins, that is intermediate density lipoproteins (IDL) and VLDL in the fasting state, and these two together with chylomicron remnants in the nonfasting state. In contrast, extremely high levels of triglycerides, above 10 mmol/L, are associated with chylomicronemia and an increased risk of recurrent acute pancreatitis. Moderately elevated plasma triglyceride levels are typically due to interactions between genetic susceptibility factors: an increased burden of rare heterozygous variants of large effect plus a high cumulative burden of common SNPs of small effect. The presence of a number of environmental factors, such as insulin resistance, nephrotic syndrome, alcohol intake, and pregnancy may exacerbate the biochemical phenotype. In contrast, extreme triglyceride levels can be monogenic, typically autosomal recessive. While mild to moderate

hypertriglyceridemia can be treated with statins or fibrates, there are currently no efficient therapies for the treatment of extreme triglyceride levels such as those found in chylomicronemia, except for extreme restriction in consumption of dietary fat occasionally supplemented with fish oil or apheresis. Therefore, the recent findings that antisense inhibition of *APOC3* profoundly reduces plasma levels of triglycerides over a wide spectrum of plasma triglyceride levels, including in familial chylomicronemia due to *LPL* mutations, holds great promise for the future treatment of high and very high triglycerides, and for the reduction of residual CVD risk and acute pancreatitis.

P7.15

Effect of statin treatment on arterial stiffness in individuals with newly-diagnosed Heterozygous Familial Hypercholesterolemia

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Background and aim: Pulse wave velocity (PWV) is considered a major indicator of arterial stiffness, which quickly responds to therapeutic interventions. We sought to assess the short-term effect of the high-intensity statins on PWV in individuals with newly diagnosed familial hypercholesterolemia (FH).

Methods: In this prospective single-center observational study, statin-naïve individuals with a probable or definite diagnosis of FH (including a LDL cholesterol ≥ 190 mg/dl and a Dutch Lipid Clinic Network score ≥ 6), without a history of diabetes or cardiovascular diseases, underwent a clinical, biochemical and instrumental evaluation including carotid-femoral PWV assessment at baseline and after a 3-month treatment with atorvastatin or rosuvastatin.

Results: Among more than 500 individuals screened for FH in a 1.5 year period, 22 fulfilled our strict inclusion and exclusion criteria and were enrolled in the study. Twenty completed the 3-month treatment period and evaluations, including 7 men and 13 women, with a mean age of 45 ± 14 years. At 3-month follow-up there was a significant 46% reduction in LDL cholesterol (from 255.8 ± 42.1 to 138.6 ± 41.2 , $P < 0.0001$ for both). Concomitantly, we observed a significant 14% reduction in PWV (from 8.30 ± 1.4 to 7.13 ± 0.97 m/sec, $P < 0.0001$), which persisted after adjustments for mean blood pressure and heart rate. There was no significant relationship between the change in PWV and in LDL cholesterol levels.

Conclusions: Our preliminary results suggest that in individuals with FH, a 3-month treatment period with high-intensity statins determines a significant reduction in arterial stiffness, possibly through its pleiotropic vascular effect beyond lowering of plasma cholesterol.

P7.16

Addition of omega-3 fatty acid and coenzyme Q10 to statin therapy in patients with combined dyslipidemia

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Background: Statins represent a group of drugs that are nowadays indicated in primary and secondary prevention of cardiovascular events. Their administration can be associated with side effects, but also not sufficient reduction of triacylglyceride (TAGG) levels. This study was aimed to assess the effect of triple combination of statins with omega-3 fatty acids and coenzyme Q10 (CooQ10) on parameters associated with atherogenesis and statin side effects.

Methods: In this pilot randomized double-blind trial, 105 subjects who met the criteria of combined dyslipidemia and elevated TAAG levels were randomly divided into 3 groups. In the control group, unaltered statin therapy was indicated. In the second group, omega-3 PUFA 2.52 g/day was added and the third group omega-3 PUFA 2.52 g + CoQ10 200 mg/day (Pharma Nord ApS). At the end of the 3-month period (± 1 week), all patients were evaluated.

Results: Significant reduction of hepatic enzymes activity, systolic blood pressure, inflammatory markers and TAG levels were detected in both groups in compare to the control. Activity of SOD and GPx increased significantly after additive therapy. CoQ10 addition significantly reduced most of above-mentioned parameters (systolic blood pressure, total cholesterol, LDL, hsCRP, IL-6, SOD) in comparison with the statin + omega-3 PUFA group. Intensity of statin adverse effects were significantly reduced in the group with addition of CoQ10.

Conclusion: The results of this pilot study suggest possible beneficial effects of triple combination on the lipid and non-lipid parameters related to atherogenesis and also side effects of statin treatment as well.

P7.17

Clinical and epidemiological investigations of an outbreak of whooping cough in Stara Zagora region, Bulgaria

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Introduction: Whooping cough is a vaccine-preventable disease which affects small children age. It is manifested with characteristic coughing paroxysms.

Aim: To show the peculiarities of clinical course and to clear up the origin of epidemic outbreak of whooping cough in Stara Zagora region.

Materials and Methods: In a period from 28.08. to 28.09.2016, 31 children with Whooping cough were passed through the Clinic of Infectious diseases of University hospital, Stara Zagora. They were aged between 2 months and 14 years. An even distribution by sex was demonstrated. The diagnosis was confirmed by clinical, laboratory, serological and molecular-genetic investigations. A careful epidemiologic research was done at the epidemic outbreak.

Results and Discussion: Usual clinical picture was demonstrated in all patients. We observed a mild clinical form in 9, moderate – in 10 and severe in 6. A typical “visiting card” of

the disease - leukocytosis plus lymphocytosis - was established in 17 patients. Seven patients had pulmonary complications. More of our patients were partially immunized or not immunized. All of them belonged to Roma society and lived in poor housing conditions.

Conclusion: Whooping cough is a disease which usually has a benign course and favourable outcome. Recently we have seen a

strange, but widespread course against immunizations. The lack of adequate specific prevention could lead not only to whooping cough but could result in many serious and life-threatening diseases.

Keywords: whooping cough, clinical peculiarities, vaccine

Workshop 8: Gerontology

P8.01

Post-operative delirium in a cohort of elective surgical oncogeriatric patients: is it still a neglected issue?

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Introduction and aim: Post-operative delirium (POD) is a prevalent geriatric issue, especially in the geriatric wards, along with neurological and orthopedics wards; however, it is widely neglected in routine clinical practice, leading to poorer clinical outcomes. The present study was aimed at assessing the incident rate of POD and the correlation analysis of associated predisposing risk factors in a cohort of older adults, eligible for elective surgical intervention for solid gastro enteric tumors.

Subjects and methods: 90 consecutive subjects were enrolled, after obtained written informed consent. The mean age was of 80.26 ± 0.65 years; female:68 and male:12. The rapid assessment test for delirium (4AT) was performed after 48 h to assess POD. Each patient underwent the Comprehensive Geriatric Assessment (CGA) and frailty assessment with Rockwood Frailty Index (FI). CGA was aimed to assess cognitive status, functional status, comorbidity, depression, malnutrition, risk of falls and pain. The following clinical assessments were also performed: ECOG-PS, Dindo-Clavien, ASA and TUG-test to assess physical performance.

Results: 4AT: 3.61 ± 0.25 mean score; MMSE: 27.12 ± 0.37 ; CIRS: 4.53 ± 0.19 ; CDT: 2.56 ± 0.16 ; Barthel: 97.56 ± 0.70 ; Tinetti: 24.09 ± 0.61 ; MNA: 28.52 ± 4.17 ; ECOG-PS: 0.37 ± 0.24 ; BI: 97.23 ± 0.85 ; GDS: 3.62 ± 0.32 ; CGA: 3.61 ± 0.25 ; TUG: 11.13 ± 0.63 ; Rockwood-FI: 0.24 ± 0.01 ; ASA: 2.30 ± 0.08 ; Dindo-Clavien: 0.88 ± 0.16 ; NRS: 0.65 ± 0.19 .

The incident rate of POD for elective surgical patients was 21.11%.

The results showed the following significant correlations with 4AT test:

Age: $P < 0.005$.

TUG: $P < 0.0003$.

CGA: $P < 0.0001$.

Barthel: $P < 0.0007$.

MMSE: $P < 0.0001$.

CDT: $P < 0.002$.

Tinetti: $P < 0.007$.

ASA classification did not predict POD.

Discussion: The study showed the high incidence of POD in elective surgical oncogeriatric patients. There is a significant correlation between delirium incidence and impaired CGA, age, cognitive status, functional status and the impaired performance physical assessment. Even if preliminary, the results originally confirmed that POD is a highly incident geriatric syndrome. The accurate analysis of in hospital precipitating factors and associated clinical outcomes (one-month mortality, overall mortality, functional status, cognitive dysfunction) in such a vulnerable population will help understanding the predictive role of delirium in the oncogeriatric surgical setting.

P8.02

Effectiveness of parenteral nutritional supplementation in elderly patients with hip fracture

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About 60% of older patients, hospitalized for hip fracture, are malnourished at hospital admission. Trauma and surgical stress induce a catabolic state that negatively affects muscle mass and strength, resulting in reduced rehabilitation capacity. Currently, several studies based on oral nutritional supplementation led to contrasting results in terms of effects on muscle mass or functional recovery, and they do not provide clear recommendations.

The aim of the study is to evaluate if a parenteral nutritional intervention produces significant changes on some biochemical markers of nutritional status, reducing as well the incidence of post-operative complications in hip fractured patients.

The prospective observational study includes 92 hip fractured patients over 75 years old, admitted from March to August 2016 at the Department of Orthopedics and Traumatology of San Martino Hospital of Genoa, Italy. The mean age is 86.6 ± 5.9 years and the 80% are female. At the time of admission (T0), all patients undergo abbreviated comprehensive geriatric assessment and blood sampling for the assay of the three nutritional status biomarkers (albumin, transferrin and lymphocytes). The intervention group consists of 40 patients: mean age is 87 ± 6.18 years with 75% of malnourished or at risk of malnutrition. The intervention consists of a three-compartmental parenteral nutrition (volume 1000 mL; 700 kcal), infused during the first five postoperative days, compared to control group (52 patients), only on standard diet (meanKcal1100). After seven postoperative days (T7), all patients undergo blood control of the nutritional markers and the postoperative complications were recorded before discharge.

By comparing the changes of the three nutritional markers (T7-T0), albumin presents a significant decrease ($P < 0.001$) in both groups. Lymphocyte count increases with an increase of 23% in the intervention group ($P < 0.001$) compared to an increase of 8.7% in the control group. Patients in the intervention group have fewer fracture-related complications than patients in the control group. In particular delirium shows statistical significance (incidence of 27.5% vs 50%; $P < 0.0339$ chiQuadroTest) suggesting that intravenous nutrition may play a role in reducing the risk of developing postoperative delirium.

P8.03

A challenge in oncogeriatrics: is Rockwood frailty index an accurate tool to predict clinical outcomes?

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Introduction: Comprehensive geriatric assessment (CGA) is the gold standard for elderly assessment in oncology to predict chemotherapy tolerance and the main clinical outcomes (survival,

functional status and quality of life). CGA is also able to stratify elderly patients according to their biological condition (frail, pre-frail, fit). However, the method is of specialist expertise and it is still poorly incorporated into routine clinical practice.

Aim: The study is aimed to compare different evaluation scales to assess the best predicting oncogeriatric tool.

Setting: Surgical oncological ward of the IRCCSS AUO San Martino Hospital, Genoa, Italy.

Subjects and methods: Before the start of chemotherapy (T0) each patient underwent CGA, Rockwood Frailty Index (40 item FI), ECOG Performance Status (to investigate physical performance) and Short Form Health Survey-36 (to evaluate quality of life). Patients were assessed after 1 month for mortality, after 3 and 6 months for chemotherapy toxicity and after 12 months for quality of life, functional status and overall mortality.

Results: 215 consecutive patients (124 females, 91 males), with solid tumors, mean age of 71 ± 0.39 years, were enrolled from May 2015 in an Italian hospital. Respectively, 6,9% of patients by ECOG PS, and 57,7% by CGA were frail. Interestingly, by IF, 73 patients were frail (34%), 114 pre-frail (53%) and 29 were fit (13%). Our study originally showed a significant positive correlation between Rockwood FI and the gold standard ($n = 215$; $R=+0.69$; $P < 0.0001$).

CGA unmasked several clinical problems in 169 out of 215 examined oncogeriatric patients, such as nutritional deficit (21,5%), pain (14%) and mood disorders (14%).

Conclusions: The study results indicate a significant correlation between CGA and Rockwood IF and address a different predictive accuracy of IF in stratifying the pre frail patients' category. The larger enrollment and follow-up of the study will allow to identifying the best predicting tool in oncogeriatrics, improving as well the clinical assessment and management of the pre frail oncogeriatric patients.

P8.04

H2S donor restores redox status of heart tissues, eNOS coupling and endothelium dependent vasorelaxation in old animals

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The aim of the study was to investigate the effect of NaHS as exogenous H₂S donor on the heart redox status, cNOS coupling, cardiodynamics and vasorelaxation in old rats. The study was conducted on adult (6-8 months old) and old (22-24 months old) male Wistar rats. To evaluate the systolic and diastolic function of the heart in experiments in vivo, we used pressure-volume (PV) conductance catheter system (Millar Instruments, USA). Markers of oxidative and nitrosative stress determined by biochemical methods. The cNOS coupling index calculated as relation $-cNOS / \bullet O_2^-$. It has been revealed that a combined oxidative and nitrosative stress develops in the heart of old rats, leading to cNOS uncoupling, which correlates with a decrease in diastolic function (dp/dt_{min} decreased by 33%, end-diastolic pressure increased in 3 times, the time constant of left ventricular relaxation (Tau g) increased by 44%). At the same time acetylcholine induced vascular strips relaxation was significantly inhibited. Hydrogen sulphide donor (NaHS) increased H₂S pools in heart, suppressed oxidative stress ($\bullet O_2^-$ generation decreased in 7,4 times, hydrogen peroxide - 3,3 times, reactive hydroxyl radical ($\bullet OH$) reduced in 4,3 times). NaHS inhibited nitrosative stress: cNOS activity increased in 2,8 times; NO₂-pools (constitutive synthesis marker) increased in 3,8 times, iNOS activity reduced in 4 times. The cNOS coupling index increased in 8 times, that indicate restoring cNOS coupling

and promoted to improvement of heart diastolic function and endothelium-dependent vasorelaxation in old rats. It was shown that dp / dt_{min} increased by 20% ($P < 0.05$), Tau decreased by 13% ($P < 0.05$). NaHS also increased endothelium-dependent vasorelaxation. Thus, hydrogen sulphide inhibits oxidative and nitrosative stress, restores cNOS coupling and increases constitutive de novo synthesis of nitric oxide, improves diastolic heart function and endothelium-dependent vasorelaxation in old rats. That stimulation could be as a result of cNOS recoupling.

P8.05

Association of TERT gene polymorphism and relative telomere length with ageing in healthy donors of Volga Tatars ethnicity

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Introduction: The role of telomeres in genomic stability is an established fact and a few genome wide association studies (GWAS) have been verified the association between telomere length and genes polymorphism with ageing. One of these genes is *TERT* that encodes the reverse transcriptase component of the telomerase, enzyme that protects DNA from damage through each replication cycle. Recently, short telomeres have been linked to the etiology of many multifactorial diseases but to date there was no clear evidence for the relationship between TL and *TERT* gene polymorphism in healthy donors. So, the aim of this study to determine the relative telomere length and *TERT* gene polymorphism in Volga Tatars (Russia).

Materials and methods: Relative telomere length (RTL) measurement and genotyping of *TERT* gene polymorphism (rs2736100) was assessed by real-time PCR in 380 healthy Tatar individuals (169 male and 211 female) aged between 4 to 77 years old divided into 5 groups: from 7 to 11 yrs. ($N = 60$), II - 11-20 yrs. ($N = 75$), III - 20-45 yrs. ($N = 78$), IV - 45-60 yrs. ($N = 89$) and V - above 60 years old ($N = 78$). The average RTL was calculated as described previously. Statistical analysis done with packet program RStudio.

Results: Genotypes frequencies for rs2736100 was under Hardy-Weinberg equilibrium in all studied groups ($P > 0.05$) with predominance of G allele genotypes carriers. The average meaning of RTL was 1.164 ± 0.11 and didn't vary significantly within and between studied groups ($P > 0.05$). Also no correlation was determined between biological age and RTL in studied groups with certain genotype carriers ($r^2 < 0.5$, $P > 0.05$).

Conclusion: Our results are preliminary however they can possibly indicate that rs2736100 in *TERT* gene determined by GWAS is not actually associated with ageing process and RTL in healthy individuals of different ethnicity but it needs to be further investigated.

P8.06

Resistin, oxidative stress and amyloid beta: a possible link in Alzheimer' disease

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Alzheimer's disease is a life threatening neurodegenerative disorder and traditional cardiovascular risk factors along with epigenetic modifications are associated to the increased risk for

dementia. Namely, subjects with diabetes mellitus exhibit an increased risk for developing Alzheimer's disease, but the relationship between the two clinical entities is still partially understood. The metabolic syndrome is also considered a risk factor for dementia and mild cognitive impairment. In addition, resistin, a serum adipokine, has been proposed as a potential biomarker of the metabolic syndrome and diabetes; high serum resistin levels have been observed in both clinical conditions.

So far, scant data address the potential link between resistin and dementia, with insufficient investigation from in vitro and in vivo models.

The present study was aimed at assessing the potential role of resistin in the pathogenesis of Alzheimer's disease, by analyzing an in vitro model of neuroblastoma cells.

SH-SY 5Y cells were treated with different resistin concentrations (100, 150 ng/mL) for 24 and 48 h, moreover N-Acetylcysteine was added to the cell, as anti-oxidant agent, 30 min. before resistin treatment, at the final concentration of 500 μ M.

The results showed an increase of oxidative stress (+40.7% and +53%) followed by up-regulation of BACE1 (+42.7% and +70.1%) and increasing Abeta 42 levels (+245.2% and +285%), after 24 h of treatment with pharmacological concentrations of resistin with a mechanism dependent on NF- κ B activation. The results were confirmed using a NF- κ B inhibitor.

The present data originally indicate resistin as playing a key relevant role in brain oxidative stress burst, promoting neurotoxic amyloidogenic processing. The results move a step forwards in the understanding of the potential link between Alzheimer's and some risk factor such as metabolic syndrome and diabetes.

P8.07

Catheter-related bloodstream infections in hospitalized elderly patients receiving total parenteral nutrition

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Background: Catheter-related bloodstream infections (CRBSIs) are a common complication in patients receiving total parenteral nutrition (TPN).

Objective: The aim of this study was to assess epidemiology and clinical associations between CRBSIs and TPN in a cohort of hospitalized elderly patients.

Methods: An observational and prospective study was carried out, including 45 elderly patients admitted from March 2016 to January 2017, at the Geriatric Clinic of San Martino Hospital. Patients underwent abbreviated Comprehensive Geriatric Assessment (CGA): Barthel-index, Cumulative Illness Rating Comorbidity Scale (CIRS-C), Mini Nutritional Assessment Short Form (MNA-SF) and polypharmacy. Inclusion criteria was: elderly patients above 65 years old; clinical diagnosis of malnutrition (MNA-SF); TPN(central or peripheral catheter; PEG and NGT); clinical diagnosis of bloodstream infections (including blood cultures). Exclusion criteria was: end-stage chronic diseases (NYHA IV; Stage 5 CKD and MELD > 40).

Results: Forty-five consecutive patients were included (15 men and 30 women); a mean age of 83.52 \pm 1.03 years old. Patients' clinical characteristics were the following: functional status (Barthel 28.89 \pm 1.16); nutritional status (MNA 2.5 \pm 2.27), comorbidity (CIRS C 4.66 \pm 0.22) and mean average hospital stay of 35.78 \pm 2.32 days. Among 45 patients in TPN 84% (38/45) developed CR-BSIs and respectively 47% were positive for Staphylococcus species (epidermidis), 16% Staphylococcus

Aureus, 16% Candida species and 6% Klebsiella Pneumoniae. Only 15% had negative blood cultures. Mortality was higher post-discharge (31%), while hospital mortality was 14%.

Conclusion: To our knowledge, few studies in literature focused on hospitalized elderly patients receiving TPN, and few data indicates the incidence of CRBSIs associated to TPN with inconclusive remarks. Current results showed the high incidence of CRBSIs in hospitalized elderly patients receiving TPN. The study confirmed that most CRBSIs cases were caused by Staphylococcus species. Our study is preliminary but further patients' enrollment and risk factors analysis will help identifying the role of TPN in predicting blood stream infections in elderly comorbid patients.

P8.08

A case of sepsis due to Leuconostoc Medenteroides ssp Cremoris in a 90-year-old patient

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Introduction: Leuconostoc is a genus of Gram-positive, anaerobic, vancomycin-resistant cocci, catalase-negatives. Leuconostoc is usually multi-drug resistant, especially to glycopeptides. Since 1985, a variety of cases of bacteremia have been reported, especially in immunocompromised, oncologic, critically ill patients, often with intravascular devices and use of total parenteral nutrition.

Case report: A 90-year-old woman was referred to Emergency room because of dyspnea. She had history of Parkinson's disease, previous ischemic stroke with aphasia and total dysphagia, fracture of the left femur with entrapment syndrome. When she entered the ward, she was alert, aphasic, uncooperative, feverish (39.1°C), bedridden, in poor general conditions. She had a peripheral venous access with total parenteral nutrition. The multidimensional assessment showed severe cognitive impairment (MMSE and 4AT test not applicable) with severe functional decline (ADL 0/6; IADL 0/8, Barthel Index 0/100).

In the potential occurrence of an aspiration pneumonia, an antibiotic therapy with Piperacillin/Tazobactam was started. After nine days of persisting fever, the blood culture showed to be positive for Staphylococcus Epidermidis, Candida Parapsilosis and Leuconostoc Mesenteroides ssp Cremoris.

According to the antibiogram, Leuconostoc turned out to be Glycopeptide-resistant, but Penicillin-sensitive. Therefore, the patient was treated with Vancomycin, Caspofungin and Ampicillin/Sulbactam 1,5gx3/day (targeting Leuconostoc).

Results: In the following days, blood cultures resulted negative for bacteria (including Leuconostoc). After 24th day of recovery, the patient died for Candidemia.

Conclusions: We originally reported a case of Leuconostoc sepsis in an oldest old patient. In literature, only few cases of Leuconostoc sepsis are described with the majority in immunocompromised infants. The report confirms that Leuconostoc species can be also cause of bacteraemia in elderly patients with high comorbidity, frailty due to critical ill conditions. Further research is needed to clarify whether Leuconostoc may be preferentially cause of sepsis in immunocompromised subjects with peripheral or central devices and under parenteral nutrition.

P8.09

Toxicity of glibenclamide on H9c2 cells includes alterations in energy metabolism

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Background: It is known that glibenclamide, an antidiabetic sulfonylurea drug, may be cardiotoxic by binding to and inducing the closure of the ATP-sensitive potassium channels (K_{ATP}) expressed by cardiomyocytes. Prompted by the recent observation that glibenclamide impairs mitochondrial bioenergetics of renal cells, we investigated whether it also deranges energy metabolism of cardiac cells.

Methods: H9c2 cardiomyoblasts were left untreated or exposed to glibenclamide for 24 h. Then, the following parameters were assessed: ATP and AMP levels (enzyme coupling method); activity of the main glycolytic enzymes, mitochondrial complexes I to IV, and Fo-F1 ATP synthase (enzymatic assays); oxygen consumption stimulated by exogenous pyruvate-malate or succinate (amperometric electrode); phosphorylation of AMP-

activated kinase (AMPK) (western blotting); mitochondrial structure (immunofluorescence for JC-1); and apoptosis (annexin V/propidium iodide flow cytometry). Apoptosis was also evaluated after culturing control or glibenclamide-treated cells in the absence of oxygen for 5 or 24 h. Gliclazide, a sulfonylurea with no affinity for the K_{ATP} expressed by H9c2 cells, was used as negative control.

Results: Incubation with 50-250 μ M glibenclamide dose-dependently decreased the ATP/AMP ratio with ensuing activation of AMPK. By contrast, gliclazide did not affect intracellular ATP and AMP. The ATP/AMP drop induced by glibenclamide was related to a dose-dependent reduction in oxygen consumption and ATP synthesis. Direct measurement of mitochondrial complexes activity indicated that glibenclamide treatment led to inhibition of complex I and/or II and disruption of mitochondrial organization. Phosphofructokinase, pyruvate kinase and lactic dehydrogenase activity was dose-dependently enhanced by glibenclamide, possibly in a compensatory manner. The same concentrations of glibenclamide perturbing energy metabolism also dose-dependently worsened apoptosis of H9c2 cells following anoxia.

Conclusions: Glibenclamide blocks mitochondrial energy metabolism of cardiac cells by a mechanism requiring the interaction with K_{ATP} . This kind of toxicity should be taken into account in future studies evaluating glibenclamide effects on the heart.

Workshop 9: Cancer Immunoregulation and Immunotherapy

P9.01

New kinases targeted compounds as possible antiangiogenic and antimetastatic agents

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Background: The intracellular activation of Akt, ERK1/2 and p38MAPK are crucial for neutrophil chemotaxis, tumor growth, angiogenesis and metastasis formation. Aiming at interfering with cancer growth and metastasis formation, we designed and synthesised a new library of pyrazolylureas and imidazopyrazolcarboxamides differently decorated in respect to previous analogue derivatives, which were able to interfere with neutrophil migration.

Materials and methods: New compounds were screened on primary VEGF-stimulated endothelial cells (HUVEC), by western blotting (to test their capacity of interfering with PI3K/Akt and MAPK pathways) and in wound healing assay (to evaluate their migration). The most active compound (named GeGe3) was subjected to further biological studies. HUVEC and different cancer cell lines (MCF7, DLD1, B16, LLC1) proliferation, as well as cytotoxicity activity were evaluated. In addition, GeGe3 was tested in vitro on an angiogenesis assay and in vivo on a tumor mouse model. Finally, it was screened on a panel of kinases with the PAMGene[®]12 and the candidate targets emerged were analyzed by western blotting.

Results: Most of synthesized compounds were able to interfere with the PI3K/Akt and the MAPK pathways. The pyrazolyl derivative GeGe3 was the most active in inhibiting the HUVEC cell migration, endothelial and cancer cell proliferation without any toxic effect. It also inhibited angiogenesis in vitro and tumor growth in vivo. In addition, in the presence of GeGe3, Aurora B, Aurora C, NEK 10, PLK3, PLK2, DMPK and CAMK1 activity was blocked. Western blotting assay revealed that the possible target of our compound is DMPK, a kinase never described in endothelial cells, but probably involved in tumor angiogenesis and metastasis formation. All biological results will be reported in poster session.

P9.02

Anti-PD1 therapy effects on T cell repertoire and functions in patients with NLCS cancer: a preliminary study to identify biomarkers of efficacy

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Background: Immune responses protect against tumors. Conventional chemotherapy may treat cancer but its efficacy is

compromised by tumor relapse. Chemotherapy “per se” have immunostimulatory effects and sustain an antitumor T cell response. Anti-PD1 antibodies are used in clinics to boost immune responses blocking of an inhibitor receptor on T cells. We evaluated the T cell repertoire and cytokines in eight NSCLC patients who underwent anti-PD1 therapy after chemotherapy. **Methods:** We used PBMC to study T cell repertoire by “Spectratyping” a PCR based technique, and production of γ -IFN, IL-2, IL-4, IL-12, IL-13 and IL-17 by Quantitative PCR. Presence of cytokine message was then confirmed measuring the protein in the sera. Each patient was studied at the end of chemotherapy and after each anti-PD1 shot.

Results: We found that chemotherapy shaped a specific T cell repertoire in these patients, expanding several T cell clonotypes that were maintained by anti-PD1 administration undergoing a long-lasting expansion. Of note, a prolonged effect in term of clinical outcome was paired by a consolidated production of IL-12 and γ -IFN.

Conclusions: These data show that chemotherapy reshapes a T cell repertoire involved in antitumor response and the functional profile of these cells marked a prolonged efficient anti-tumor T cell response. Although preliminary, these results help to understand how monitor the patients undergoing therapy with anti immune-checkpoints. This is of critical importance due to the need to identify biomarkers and monitoring tools to optimize the use of these drugs, considering the high costs of these therapies.

P9.03

Analysis of biological properties and anti-cancer activity in vitro of mesenchymal stem cells primed with cisplatin

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Tumor microenvironment consists of various cell types such as endothelial cells, fibroblasts, immune cells and mesenchymal stem cells (MSCs). Recent studies showed that MSCs have a tropism for tumor and can be used to deliver chemotherapeutic drugs in metastatic, pre-metastatic and tumors niches. One of the effective anticancer drugs is cisplatin.

In this research were investigated the cytokine profile of MSCs primed with cisplatin, and their antitumor activity in vitro. Investigation of the cytokine profile of primed MSCs will allow to understand the possible mechanisms of interaction with the tumor cells after migration to the tumor niche.

MSCs from adipose tissue were isolated using enzymatic digestion with collagenase. Isolated MSCs expressed markers of mesenchymal stem (CD44, CD73, CD90, CD29, CD166) and do not express hematopoietic cells markers (CD34, CD11b, CD19, CD45, HLA-DR).

Nontoxic concentration of cisplatin (5 mg/mL) for MSCs was determined using MTS-test. After priming, cells were

trypsinized, washed out of the drug, and transferred to a new culture flask. After 48 h of incubation, conditioned medium was collected and applied on neuroblastoma SH-SY5Y cells. Viability of SH-SY5Y after incubation with conditioned medium decreased by 20% compared to control.

Conditioned medium of primed and untreated MSCs was analyzed using Bio-Plex Pro Human Cytokine 21-plex Assay (Bio-Rad). Multiplex analysis showed significant increase of IL-2R α , IL-3, IL-16, HGF, MIF, SCF, SCGF- β and TRAIL in primed MSCs compared to control. These cytokines are involved in the proliferation and differentiation of various cells. TRAIL is capable of inducing apoptosis of tumor cells.

Thereby, we demonstrated antitumor activity of cisplatin-primed MSCs toward SH-SY5Y, suggesting that primed stem cells can be a promising approach for delivering of chemotherapeutic compounds. The work was funded by a grant RFBR №16-34-60201.

P9.04

Nicotinic acid phosphoribosyltransferase is overexpressed in solid tumors and regulates cancer cell metabolism and susceptibility to FK866

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In the last decade, substantial efforts to identify NAD⁺ biosynthesis inhibitors, specifically NAMPT inhibitors, have been made, as preclinical studies indicated that they could be effective cancer drugs. However, the clinical activity of NAMPT inhibitors proved limited, suggesting that alternative NAD⁺ production routes could be exploited by tumors, conferring drug resistance. Our data show that the expression of nicotinic acid phosphoribosyltransferase (NAPRT), a second NAD⁺ producing enzyme, is elevated in a subset of common types of cancer and that both NAPRT and NAMPT increase intracellular NAD⁺ levels. NAPRT silencing, or inhibition, are shown to reduce energy status, protein synthesis and cell size in ovarian and pancreatic cancer cells, and to sensitize them to the NAMPT inhibitor FK866 both in vitro and in vivo. Thus this knowledge could be used to overcome the resistance observed with NAMPT inhibitors in clinical trials.

P9.05

Colorectal cancer microenvironment expressing BTN3A1 stimulate effector $\gamma\delta$ T cells with anti-tumor activity upon zoledronate treatment

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$\gamma\delta$ T lymphocytes are involved in stress responses to injured, infected or transformed cells. The most representative $\gamma\delta$ T cell subset in the blood is the V γ 9V δ 2 which recognizes unprocessed non-peptide molecules, including phosphoantigens (PAG) derived from the mevalonate pathway in mammalian cells, and via the 1-deoxy-D-xylulose-5-phosphate pathway, in bacterial cells. Besides their effects of inhibiting osteoclastic bone

resorption, amino-bis-phosphonates (N-BPs) such as zoledronate (Zol) have been employed in anti-cancer clinical trials due to their ability to up-regulate isopentenyl-pyrophosphate (IPP) accumulation promoting anti-tumor V γ 9V δ 2 T cells. Recently, the butyrophilin 3A1 (BTN3A1) through the B30.2 domain has emerged as important structure contributing to V γ 9V δ 2 T cell stimulation. Aim of this study was to investigate the relevance of BTN3A1 in N-BPs-induced anti-tumor immune response in colorectal cancer (CRC); indeed, we have analyzed whether the immunosuppressive tumor microenvironment, composed of CRC cells and tumor associated fibroblasts (TAF), can become a stimulator of $\gamma\delta$ T cells when primed with Zol.

Herein, we show that: i) CRC, exposed to Zol, stimulate the expansion of V δ 2 T lymphocytes with effector memory phenotype and anti-tumor cytotoxic activity, besides sensitizing cancer cells to $\gamma\delta$ T cell-mediated cytotoxicity; ii) this effect is partially related to BTN3A1 expression and ability of synthesizing IPP; iii) BTN3A1 is detected in CRC at the tumor site, both on epithelial cells and on TAF, close to areas infiltrated by V δ 2 T lymphocytes; iv) Zol is effective in stimulating anti-tumor effector V δ 2 T cells from ex-vivo CRC cell suspensions; v) both CRC cells and TAF can be primed by Zol to trigger V δ 2 T cells.

These findings strongly suggest that Zol can work as anti-tumor immune-stimulator in vivo; the in situ analysis of BTN3A1 expression and IPP production can help to select responders to Zol treatment among CRC patients.

P9.06

ADAM10 new selective inhibitors sensitize Hodgkin lymphoma cells to NKG2D-mediated killing and reduce their growth and metabolism

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Members of the "A Disintegrin And Metalloproteases" (ADAMs) family, mainly ADAM10 or ADAM17, have been proposed as therapeutic targets in solid tumors and some ADAMs inhibitors have been shown to exert anti-tumor effects. We have described overexpression of ADAM10 in HL, together with increased release of NKG2D ligands (NKG2D-L) and reduced activation of effector anti-lymphoma T lymphocytes. Aim of this work was to verify whether ADAM10 inhibition in HL cells could restore the triggering of NKG2D-dependent anti-lymphoma T cell response and reduce Reed-Sternberg (RS) cell growth. We synthesized the two hydroxamate compounds LT4 and MN8 with selectivity for ADAM10 over metalloproteases, LT4 showing higher specificity for ADAM10 over ADAM17.

Herein we show that: 1) HL lymph nodes and cultured HL cells express high levels of the mature active membrane form of ADAM10, that is also release in exosomes; 2) ADAM10 is the major sheddase for the NKG2D-L in HL cells; 3) the new LT4 and MN8 compounds co-localize with ADAM10 in the endo-lysosomal compartment, and in the exosomes, in both RS cells and mesenchymal stromal cells of the lymph node; 4) these inhibitors strongly reduce the shedding of NKG2D-L by HL cell lines and enhance the binding of NKG2D receptor; 5) of note these new ADAM10 inhibitors increase the sensitivity of HL cell

lines to NKG2D-dependent cell killing exerted by natural killer and $\gamma\delta$ T cells; 6) they can also reduce in vitro the metabolism and growth of RS cells. Inhibition of cleavage of other ADAM10 substrates, such CD30, a target for antibody-based anti-lymphoma therapy, might also be useful in HL. Thus, we think that selective ADAM10 inhibitors may be proposed as part of anti-lymphoma therapeutic schemes and contribute to the enhancement of anti-tumor immune response.

P9.07

Effect of novel polyphenol compound from yeast-like fungi *Nadsoniella nigra* sp. X1 (Antarctica) Melanin on serum cytokine profile in patients with advanced urological cancer in the adjuvant setting

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Background: Cancerogenesis occurs against deterioration of immunosurveillance and growing immunodeficiency. This requires addition of adjuvant treatment correcting immune status in patients. It was postulated that polyphenol substances yield positive effects on cancer pathogenesis exerting anti-proliferative, DNA-damaging, anti-angiogenic, anti-metastatic and other functions.

Purpose: To assess prospectively the immunomodulating effect of novel polyphenol substance Melanin on cytokine profile in patients with advanced urological cancer in the adjuvant setting.

Methods: Fifteen random patients with advanced urological cancer (kidney, bladder, prostate) scheduled for cancer treatment (surgery, chemotherapy) were administered Melanin from yeast-like fungi *Nadsoniella nigra* sp. X1 (Antarctica), one capsule (10 mg) twice daily for 30 days after initiation of treatment. Controls were twenty random uro-oncological patients of identical clinical stages subjected for the same treatment protocol, receiving placebo, and ten healthy individuals. We drew plasma samples in all groups at the beginning of the study and after 1 month of treatment. We analyzed serum cytokines (IL-1 β , IL-12, IFN- γ , TNF- α , IL-4 and IL-10) by ELISA.

Results: Administration of Melanin in study group caused statistically significant changes in cytokine profile. The pro-inflammatory IL-1 β dropped by 24 %, IFN- γ rose by 50 %, and IL-12 stayed unchanged. All anti-inflammatory cytokines showed increase: TNF- α rose by 184 %, IL-4 rose by 44 %, and IL-10 rose by 85 %.

Conclusions: Application of novel polyphenol substance Melanin 10 mg as cancer treatment adjuvant in patients with advanced urological cancer exerts evident immunomodulating effect, affecting the level of cytokines in peripheral blood. Considering state of decreased immunosurveillance, studied polyphenol substance may be considered a beneficial immune-rehabilitative compound deserving further study of its biochemical and clinical effects.

P9.08

Oxaliplatin neuropathic pain: involvement of the P2X7 receptor and recruitment of pannexin1

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Development of neuropathic syndrome limits anticancer therapy with oxaliplatin. Increasing evidence indicates that complex mechanisms and maladaptive plasticity of the central nervous system, including central sensitization, are involved in the pathophysiology of chemotherapy-induced neuropathies. The insufficient information on the pathophysiology and molecular basis of the chemotherapy-induced peripheral neuropathy is an important limit to the development of new effective treatments.

In a rat model of oxaliplatin-induced neuropathy, we found that activation of presynaptic P2X7 receptors for ATP evoked an increased glutamate release from cerebrocortical nerve terminals. The release was abolished by the P2X7 antagonists Brilliant-Blue-G and A-438079, and reduced by carbenoxolone and the Pannexin1 selective inhibitors erioflaucine and ¹⁰Panx, suggesting the recruitment of the accessory protein Pannexin1. Aimed to evaluate the significance of P2X7-Pannexin1 system activation in pain induced by oxaliplatin, pharmacological modulators were intrathecally infused in oxaliplatin-treated animals. Brilliant-Blue-G, erioflaucine and ¹⁰Panx reverted oxaliplatin-induced pain. Finally, the influence of the P2X7-Pannexin1 system blockade on oxaliplatin anticancer activity was evaluated on the human colon cancer cell line HT-29. Prevention of HT-29 apoptosis and mortality was dependent on concentration of P2X7R antagonists. On the contrary, the inhibition of Pannexin1 did not alter oxaliplatin lethality in tumor cells.

In summary, glutamate release dependent on P2X7 receptor is increased in cerebrocortical nerve terminals from oxaliplatin-treated rats; the increase is mediated by functional recruitment of the accessory protein Pannexin1; P2X7 antagonists and Pannexin1 inhibitors revert oxaliplatin-induced neuropathic pain; Pannexin1 inhibitors did not alter the oxaliplatin-induced mortality of cancer cells HT-29. In conclusion, our results highlight the relevance of P2X7-Panx1 complex in the maladaptive response of central nervous system to oxaliplatin neurotoxicity. P2X7 receptor- Pannexin1 participates in alteration of neuronal functions leading to central sensitization and pain chronicization. The selective inhibition of Pannexin1 channel is suggested as new pharmacological target for oxaliplatin-induced neuropathic pain relief.

P9.09

Regulation of p53-dependent genes expression in multiple sclerosis: the effect of MDM2 inhibitor Nutlin-3a

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Transcription factor p53 is a well-known oncosuppressor protein with thoroughly explored role in cancer. However, recent

advances suggest that both p53 and its negative regulator MDM2 might be involved in autoimmune processes. Inhibition of MDM2 (i.e. by small molecule Nutlin-3a) and associated activation of p53 are considered as a promising therapeutic approach for treatment of autoimmune diseases, i.e. multiple sclerosis.

In the current study we compared Nutlin-3a-induced expression of p53-dependent genes (*p21*, *mdm2* and *PUMA*) in peripheral blood mononuclear cells (PBMCs) from patient diagnosed with multiple sclerosis (MS) and a healthy volunteer. The results indicate that gradual increase of Nutlin-3a concentration (5uM, 10uM, 20uM, 40uM) leads to higher expression levels of *p21*, *mdm2* and *PUMA* genes in MS samples compared to healthy control. These preliminary data suggest that the transcription functions of p53 protein might be enhanced in patients with MS. Data obtained by TaqMan real-time PCR technique using CFX96 Touch Detection System (Bio-Rad). The study was funded by RFBR research grant 16-34-60213 mol_dk.

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P9.10

Autoimmune component of essential hypertension and prostate cancer: tyrosyl-trna synthetase and their fragments are novel subpositional antigens

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There are growing evidences that etiology and pathogenesis of essential hypertension and prostate cancer include autoimmune component. However, autoantigens involved in these processes are still largely unknown. Separated fragments of tyrosyl-tRNA synthetase (TyrRS) miniTyrRS and C-terminal domain (CTD) provide non-canonical functions such as immune cell signaling and can potentially serve as autoantigen.

The aim of the study was to investigate antibodies to full-length TyrRS and its domains in sera of patients with essential hypertension and prostate cancer.

Patients with essential hypertension and prostate cancer, as well as healthy subjects were recruited to participate in the study. The recombinant proteins generated by *Escherichia coli* were purified by chromatography on Ni-NTA-agarose. Specific autoantibodies (aAbs) were measured by ELISA and confirmed in an immunoblotting assay. From 6% to 12% of subjects with elevated levels of aAbs against the full-length enzyme were detected in all the cohorts studied. 52 % of patients with essential hypertension were identified as immunoreactive against miniTyrRS and 50 % - against CTD ($P < 0.01$). In 41.8 % of individuals with prostate cancer the levels of anti-CTD aAbs were elevated, meanwhile, only 22.4% of these oncology patients were immunoreactive for miniTyrRS ($P < 0.01$).

We propose that autoantibodies to Tyrosyl-tRNA synthetase and their fragments provide one of the triggers for auto-sensibilization in essential hypertension and prostate cancer which may well extend to other autoimmune disease in humans.

P9.11

Cytokine balance as criterion for colorectal cancer immunomodulation

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Background: Mucosa-associated lymphoid tissue is the largest reserve of immune cells along the gastro-intestinal system to front microorganisms that are confined in gut lumen by a thin epithelial barrier. In colorectal cancer (CRC) mucosa equilibrium is subverted becoming antigen-permeable, switching-on a strong inflammatory response that can inhibit the anti-tumor T-cells response. COX-2, the key enzyme of PGE2 synthesis, and IL8 are highly expressed in CRC and linked to bad prognosis. Both factors can recruit immunosuppressive cells and recent studies showed a correlation between COX-2 and IL8 mRNA in CRC samples, though their relation has neither been investigated at protein level, nor used for defining CRC clusters.

Methods: COX-2 and IL8 modulation by IL1b, IL17 and IL22 were tested *in vitro* both on CRC cell lines and on primary CRC-associated fibroblasts (CAF) to identify different responses of cancer and stromal cells. COX-2, IL8 and IL1b were also measured in 100 human CRC specimens by quantitative western blot and ELISA. Their expression was related to each other and to clinicopathologic parameters to define clusters of tumors with a different immune-modulatory microenvironment.

Results: We found that IL1b strongly induces a synchronous expression of COX-2 and IL8 in CAF, while its activity in cancer cells is lower and cell line-specific. We also observed that IL22 is active only on cancer cells, triggering STAT-3 and down-regulating COX-2.

Protein analysis of CRC samples identified two subsets of tumors: one where COX-2 and IL8 are modulated in tandem and associated with IL1b levels, the other with dominant COX-2 or IL8 alone, showing a more aggressive phenotype (prevalence of stage IV/worse prognosis).

Conclusions: The evaluation of COX-2/IL8 balance in primary tumors represents a new criterion for CRC patients clustering after surgery, providing a rationale for the experimentation of immune-modulatory targeted therapies according to a defined cytokine balance.

P9.12

Combined immunotherapy with anti-PDL-1/PD-1 and anti-CD4 antibodies cures syngeneic disseminated neuroblastoma

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Purpose: Anti-PD-1 or anti-PD-L1 blocking monoclonal antibodies (mAbs) have shown potent anti-tumor effects in mouse tumors and adult cancer patients. Clinical studies have recently been started in pediatric cancers, including high-risk or relapsing neuroblastoma (NB).

Experimental Design: We studied the effects of anti-PD-1 or anti-PD-L1 mAbs in two syngeneic model of disseminated NB generated by the injection of either Neuro2a or NXS2 cells, which express PD-L1. In addition, we tested the combination of these agents with the immune-enhancing cytokine IL-21, with the Ecto-NTPDase inhibitor sodium polyoxotungstate (POM-1), with an anti-CD25 mAb targeting Treg cells or with an anti-CD4 mAb. We previously showed that CD4-transient depletion removes CD4⁺CD25⁺ Treg cells and other CD4⁺CD25⁻ regulatory subsets.

Results: Mono-therapy with anti-PD-1 or anti-PD-L1 mAbs had no effect on systemic NB progression *in vivo*, and their combination with IL-21, POM-1 or anti-CD25 mAb was also ineffective in both syngeneic models of NB. However, the combined use of

anti-PD-1 with an anti-CD4 mAb mediated a very potent, CD8-dependent synergistic effect, leading to significant elongation of tumor-free progression of mice, and, in Neuro2a model, complete tumor regression in 100% of mice and durable anti-NB immunity. Similar results were obtained by combining the anti-PD-L1 and anti-CD4 mAbs.

Conclusions: These findings indicate that both PD-1/PD-L1 and CD4⁺ T cell-related immune-regulatory mechanisms play an essential role and must be simultaneously blocked to mediate therapeutic effects, in these models. Therefore, our syngeneic models represent excellent platforms to study new combined therapies that can be exploited also for those adult cancer patients resistant to anti-PD-1 mAb treatment.

Workshop 10: Phagocyte Biology

P10.01

Homing regulation of distinct macrophage subsets in infection and cancer

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Tumor associated macrophages (TAMs) share a typical M2-skewed phenotype with lipopolysaccharide- (LPS-) tolerant macrophages. Both populations display impaired capacity to mount an effective M1 inflammatory response and this phenotype, often referred to as "tolerance", is driven by nuclear accumulation of p50 NF-κB inhibitory homodimers. It's widely accepted that TAMs recruitment into tumors correlates with poor prognosis, however, the detailed mechanisms underlying the recruitment of M2-like macrophages to inflamed tissues, including tumor microenvironment, are not fully understood. Given the importance of p50 NF-κB in driving macrophage's tolerance, we investigated whether nuclear accumulation of p50 may also mediate the recruitment of M2-like macrophages to inflamed tissues. This study demonstrates that p50 NF-κB accumulation is a necessary event guiding the chemotactic responsiveness of tolerant macrophages to complement anaphylatoxins C3a and C5a. By using an in vivo model of LPS-tolerance, we demonstrate a differential recruitment into the inflammatory sites of distinct F4/80⁺ macrophage subsets characterized by different expression levels of F4/80 and the C5aR (CD88), respectively defined as F4/80^{high}CD88^{high} and F4/80^{low}CD88^{low}. Of relevance, we observed systemic accumulation of the F4/80^{high} mono/macrophage population only in tolerant conditions, both in mouse and human. Differential expansion of the F4/80^{low}CD88^{low} and F4/80^{high}CD88^{high} populations was confirmed also in the preclinical MN/MCA1 fibrosarcoma model. Consistent with the LPS-tolerance model, ablation of p50 NF-κB resulted in significant impairment of F4/80^{high}CD88^{high} TAMs accumulation in primary tumors, which correlated with inhibition of tumor growth and vascularization. Moreover, transcriptome analysis proved that F4/80^{high} and F4/80^{low} TAMs express different genetic programs, supporting putative different roles in tumor progression. Thus, our study provides evidence that distinct macrophage subset arise during infection- and cancer-driven inflammation, in a p50 NF-κB-dependent manner and that complement-mediated pathways drives their infiltration of inflammatory sites, including solid tumors. Future studies will provide the functional characterization of these populations in infection and cancer.

P10.02

The fibrinolytic and innate immune systems cooperate to control and eliminate microbial skin infections

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NFAT is activated in innate immune cells, but little is known about NFAT's functions. Here, we show that early activation

of NFAT balances the two major phases of the innate response to *Candida albicans* skin infections: the protective containment (abscess) and the elimination (expulsion) phases.

During the early containment phase, TGF-β activates fibroblasts that deposit collagen around recruited polymorphonuclear cells for preventing microbial spreading. If the TGF-β pathway is blocked, the protective collagen deposits are not formed, microbial infections are not appropriately contained and diffuse inside the subcutaneous space where multiple granulocyte accumulation and larger lesions can be observed.

During the elimination phase, IFN-γ, by antagonizing TGF-β, dampens PAI-1 production and permits plasmin formation. Plasmin, in turn, allows abscess capsule digestion and microbial discharge from the skin. In the absence of IFN-γ, the continuous signaling of TGF-β not only impedes plasmin formation but also induces the differentiation of fibroblasts into myofibroblasts with the generation of a very thick capsule around the abscess that hampers skin ulceration and microbial elimination. Therefore, microbes persist alive inside the abscess.

NFATc2 controls innate IFN-γ production and microbial expulsion. This crosstalk between the innate immune and the fibrinolytic systems also occurs during infection with *Staphylococcus aureus* and is a protective response to minimize tissue damage and optimize pathogen elimination.

TGF-β is fundamental during the inflammatory process to contain the infection, reduce tissue damage, and favor microbial discharge. TGF-β is mainly considered a cytokine that negatively regulates the inflammatory process. Here we show that, by exerting its profibrotic functions (fibroblast activation, collagen deposition, inhibition of the fibrinolytic system), TGF-β is fundamental to increase the effectiveness of the inflammatory process.

P10.03

CD115+ macrophages promote tumour growth via the suppression of the adaptive immune system

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Tumour-associated macrophages (TAMs) constitute a major proportion of tumour-infiltrating leucocytes and TAM-infiltration has been identified as a poor prognostic factor in several human cancer types. Colony-stimulating factor-1 (CSF-1) and its receptor, CSF-1R (CD115), regulate the differentiation, survival and migration of macrophages. Here we tested the role of the CSF-1/CD115 axis in TAMs in a syngeneic mouse model of colorectal adenocarcinoma (MC38) *in vivo*.

Csf1^{-/-} MC38 tumour cells were generated using the CRISPR/Cas9 system. Parental or Csf1^{-/-} MC38 cells were implanted into wild-type (WT) and Rag2^{-/-} mice subcutaneously. TAMs were isolated from enzymatically-digested primary tumours using discontinuous Percoll-gradient and CD115-based positive magnetic selection. Immune cells isolated from primary tumours were stained with fluorochrome-conjugated antibodies and acquired on a BD LSRFortessa Flow Cytometer. Lymphocytes

were isolated from spleens and lymph nodes. Cytokines were detected in the culture supernatant by ELISA. PLX3397, a CSF-1R inhibitor, was orally administered once a day in 50 mg/kg dose from when tumours were palpable.

CSF-1, the major survival factor for macrophages, was secreted in high levels into the culture supernatant by several tumour cell lines capable of syngeneic transfer. In a mouse model of colorectal carcinoma, deletion of CSF-1 in the cancer cells strongly reduced tumour growth, CD115⁺ TAM-infiltration and significantly increased CD4⁺ T lymphocyte numbers in the primary tumours of WT mice. Surprisingly, the growth of the Csf1^{-/-} tumours was unaffected in the Rag2^{-/-} animals. CD115⁺ TAMs isolated from the primary tumours secreted high levels of TGF- β 1 into the culture supernatant and strongly expressed PD-L1. Administration of the CSF-1R inhibitor, PLX3397 led to a significant reduction of tumour growth in the MC38 model.

These data suggest that tumour-derived CSF-1 can recruit CD115⁺ macrophages which then support tumour growth by inhibiting T lymphocytes via the secretion of TGF- β 1 and expression of PD-L1.

P10.04

Unconventional PI3K-activated Erk signalling controls immune complex-induced apoptosis in human but not mouse neutrophils

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Neutrophils are peripheral blood leukocytes that represent the first line of defense against bacterial and fungal infections and are also key regulators of inflammatory response. Immune complexes are important mediators of many neutrophil effector functions; immune complexes are critical in driving a range of chronic autoinflammatory disorders that rely on neutrophilic inflammation. However, insoluble immune complexes are also known to induce neutrophil apoptosis, an essential process in the resolution of inflammation.

Here we present a novel, non-canonical signaling pathway, FcgR – PI3Kb/d – Cdc42 – Pak – Mek – Erk, that promotes immune-complex induced apoptosis in human neutrophils. This pathway represents an example of Ras- and Raf-independent Erk activation. Instead, an alternative small GTPase (Cdc42) and an alternative MAP3K (Pak) are involved. The non-canonical pathway is moreover a rare example of PI3K-driven activation of Cdc42 rather than Rac.

Mechanistically, we show this pathway drives apoptosis of human neutrophils by regulating the ratio of the Bcl2 family proteins Mcl1 and Bax1. We conclude that our novel, non-canonical signaling pathway may be important for the resolution of inflammation in chronic inflammatory diseases that rely on immune-complexes driven neutrophil activation. In addition, we demonstrate that other effector functions induced by immune complexes (ROS generation, selectin shedding, cytokine release) are also PI3Kb/d dependent but employ separate pathways downstream of PI3K.

We also analysed mouse neutrophils with a view to study the novel pathway genetically by using the available knock-out models. Although immune complexes drive PI3K-dependent apoptosis of mouse neutrophils, the two species use separate pathways. Control experiments performed with fMLF-stimulated neutrophils demonstrated that fMLF-stimulated Erk activation is also controlled by separate pathways in human and mouse neutrophils. Our work therefore emphasizes that results

obtained with mouse neutrophils need to be interpreted with caution, as they may or may not be conserved in human.

This work is in press. CELREP_CELL-REPORTS-D-16-01826.

P10.05

The Mcl-1 Δ Myelo mice are highly susceptible to microbial infections

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Background: Genetic deletion of specific leukocyte lineages contributes to understanding the role of various leukocyte subsets in physiological and pathological conditions. We have recently found that the myeloid-specific deletion of the anti-apoptotic Mcl-1 protein in LysM^{Cre/Cre}Mcl-1^{flox/flox} (referred to as Mcl-1 ^{Δ Myelo}) mice leads to dramatic reduction of circulating and tissue neutrophils without affecting other leukocyte lineages. These mice were also completely protected in two known neutrophil-dependent, autoantibody-induced *in vivo* inflammation models. The aim of the present experiments was to test the long-term viability of these mice, and their susceptibility in microbial infection models.

Materials and methods: The long-term viability and fertility of the mice was monitored for 12 months under SPF and conventional conditions. The bodyweight was measured for 3 months and was compared to the littermate controls. The susceptibility of the Mcl-1 ^{Δ Myelo} mice to infections was tested following intraperitoneal injection of 2×10^7 *Staphylococcus aureus* bacteria/mouse or intravenous injection of 10^5 *Candida albicans* cells/mouse.

Results: Mcl-1 ^{Δ Myelo} mice were viable in SPF and conventional conditions, though their long-term survival was somewhat reduced compared to wild type mice. There was only a slight reduction in the body weight in the Mcl-1 ^{Δ Myelo} mice compared to controls. Importantly, Mcl-1 ^{Δ Myelo} mice were fertile even in homozygous form, allowing efficient maintenance of the colony without routine genotyping. Following infection with *S. aureus*, all Mcl-1 ^{Δ Myelo} mice died on the first day. The neutrophil-deficient mice were also significantly more sensitive compared to the control group in the candidemia model. In both infection models, there was a significant increase in the CFU counts between the control and Mcl-1 ^{Δ Myelo} mice.

Conclusions: The Mcl-1 ^{Δ Myelo} mutation leads to a reduced innate immune response, making these mice more susceptible for bacterial and fungal infections. However, Mcl-1 ^{Δ Myelo} mice are viable and fertile, making them a very useful novel model of genetically determined neutrophil deficiency.

P10.06

Understanding the signaling mechanisms of NETosis by using selective target inhibitors

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Neutrophils are essential cells of the innate antimicrobial defense. A recently identified anti-microbial function is the

formation of Neutrophil Extracellular Traps (NETs) that are composed of decondensed chromatin and antimicrobial granular contents. NETs can capture and kill invading pathogens. However, the granule proteins on the NETs can also serve as auto-antigens leading to autoimmunity. NETs are released from neutrophils undergoing a unique kind of cell death the so called NETosis. The mechanisms involved in NETosis are poorly understood.

In the present study the signaling mechanisms involved in NETosis were investigated. NETosis was induced with PMA and immobilized immune complexes (iC) which both induce Reactive Oxygen Species (ROS) dependent NETosis. The aim was to identify signaling events downstream of ROS. A library of 141 inhibitors of known signaling pathways was screened for molecules that inhibit the formation of NETs but do not inhibit ROS production. Two inhibitors D14 and D83 were found to inhibit both PMA- and iC-induced NETs in a dose dependent manner without inhibiting ROS release. D14 and D83 are known to target PDGFR and Topoisomerase II, respectively. However, our experimental results suggest that the inhibitory effects of these inhibitors on NETosis are independent of an action on the known targets. D14 and D83 are not cytotoxic for neutrophils and do not inhibit basic neutrophil functions like activation, migration, and phagocytosis. The inhibitor D83 was also able to inhibit ionomycin-induced NETosis which is a known ROS-independent NETosis indicating that this molecule might serve as a general NETosis inhibitor. Elucidating the molecular targets of the inhibitors D14 and D83 could lead to the identification of novel signaling events involved in NET formation.

P10.07

Treatment with Oxaprozin reduces metalloproteinase-9 expression and activity in immune-complex activated human monocytes

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Background and aims: As compared to normal joints, matrix metalloproteinase-9 (MMP-9) is highly expressed during rheumatoid arthritis. The collagenase activity of MMP-9 strongly promotes joint inflammation and enhances the local recruitment of circulating inflammatory cells. Here, we evaluated the potential effect of oxaprozin, a non-steroid anti-inflammatory drug (NSAID), on MMP-9 expression and activity in immune-complex (IC)-activated human monocytes.

Materials and methods: Human monocytes were isolated from blood buffy coats of healthy donors by Ficoll-Percoll gradient. Monocytes were cultured for 12 h in the presence or absence of oxaprozin at different concentration (up to 100 μ M). In selective experiments, other NSAIDs (ibuprofen and naproxen) and inhibitors of intracellular signaling kinases (Akt, IKK) were tested as potential inhibitors in parallel to oxaprozin. Finally, 25 μ g/mL IC was added as a cell-activating stimulus for 12 h. The MMP-9 activity was measured by zymography, whereas MMP-9 expression was assessed by RT-PCR.

Results: After 12 h, the exposure of human monocytes to IC increased MMP-9 mRNA levels (2.1 ± 0.46 fold increase) as

compared to control medium. Similarly, also MMP-9 activity was increased in IC activated monocytes (11.9 ± 3.1 fold increase) as compared to control medium. Treatment with 50 mmol/l oxaprozin reduced the mRNA levels of MMP-9 by $40 \pm 10\%$ as compared to IC-stimulated monocytes. Furthermore, oxaprozin dose-dependently inhibited the release of active MMP-9. Among intracellular kinases inhibitors, only pre-treatment with IKK inhibitor was shown to abrogate the MMP-9 activity induced by IC. Pre-treatment with ibuprofen or naproxen had no effects of MMP-9 mRNA levels or release.

Conclusions: Overall, those data indicate a potential inhibitory effect of oxaprozin on MMP-9 mRNA expression and activity, likely due to an inhibitory effect on IKK signalling pathway. This raises promising opportunities to develop new strategies to reduce MMP-9-mediated effects in rheumatoid synovitis.

P10.08

Fc receptors on neutrophils: two sides of the same coin?

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Background: Neutrophils express Fc receptors, which recognize the antibodies IgA (Fc α RI) or IgG (Fc γ receptors). We previously demonstrated that neutrophil migration is only induced after IgA activation, which may play a role in IgA-autoantibody mediated autoimmune diseases.

Aim: The aim of this study is to investigate in depth the differences between IgA- versus IgG-induced neutrophil effector functions and to elucidate the specific signaling pathways of Fc α RI in neutrophils, responsible for IgA-induced neutrophil migration and activation.

Methods: The quantitative number of Fc receptors expressed was determined with a Qifikit. Furthermore, neutrophils were stimulated with complexed serum IgA or IgG to determine the differences in phagocytic index, induction of reactive oxygen species (ROS), NETosis, cytokine release, metabolite release, calcium flux and induction of signaling.

Results: Neutrophils express a higher number of Fc γ receptors (Fc γ RII and Fc γ RIII) compared to Fc α RI. No differences were observed in phagocytosis of IgA- versus IgG-coated beads, but triggering the Fc alpha receptor with IgA led to enhanced release of cytokines, chemokines and pro-inflammatory metabolites. Additionally, IgA stimulation resulted in a higher induction of calcium flux. Furthermore, crosslinking the Fc α RI led to a stronger and more sustained phosphorylation of different signaling molecules.

Conclusions: Activating neutrophils with IgA leads to increased pro-inflammatory effector functions and enhanced signaling, which could not be explained by a higher expression of Fc α RI on neutrophils. This suggests that the signaling routes after IgA and IgG triggering are different, which is against the current dogma which dictates that both Fc α RI and Fc γ receptors use similar signaling pathways via immunoreceptor tyrosine-based associated motifs (ITAMs). The delineation of the exact

signaling pathways of Fc α RI and Fc γ receptors in neutrophils is the subject of further studies, as specifically targeting IgA signaling pathways may represent a novel therapeutic strategy to prevent tissue damage in IgA-mediated autoimmune diseases.

P10.09

IL-21 promotes Granzyme B-dependent NK/plasmacytoid dendritic cell functional interaction in cutaneous lupus erythematosus

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Autoimmune skin lesions are characterized by a complex cytokine milieu and by the accumulation of plasmacytoid dendritic cells (pDCs). Granzyme B (GrB) transcript is abundant in activated pDCs, though its mechanisms of regulation and biological role are largely unknown. Here we report that IL-21 was the only Th1/Th17 cytokine able to induce the expression and secretion of GrB by pDCs and that this action was counteracted by the autocrine production of type I interferons (IFNs). In lupus erythematosus (LE) skin lesions, the percentage of GrB⁺ pDCs directly correlated with the IL-21/MxA ratio, indicating that the interplay between these two cytokines finely tune the levels of pDC-dependent GrB also *in vivo*. In LE, pDCs colocalized with professional cytotoxic cells at sites of epithelial damage, suggesting a role in keratinocyte killing. In accordance, we demonstrate that supernatants of IL-21-activated pDCs promoted autologous keratinocyte killing by NK cells and this action was dependent on GrB. These results propose a new GrB-dependent functional interaction between pDCs and NK cells and highlight a negative feedback regulation by type I IFNs *in vitro* and *in vivo* that may function to limit excessive tissue damage.

P10.10

ARAP3 regulates inflammation by neutrophil-autonomous and neutrophil non-autonomous mechanisms

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ARAP3 is a dual GTPase activating protein for RhoA and Arf6 that is regulated by phosphoinositide 3-kinase and Rap. We previously showed that ARAP3-deficient neutrophils are characterized by increased β 2 integrin ligand binding affinity and avidity. This resulted in enhanced adhesion-dependent pro-inflammatory effector functions (e.g. degranulation, ROS production) of ARAP3-deficient neutrophils plated onto integrin ligands or immobilised immune complexes and a chemotaxis defect [1, 2].

We now show that in-line with increased adhesion and spreading to surfaces coated with integrin ligands, ARAP3-deficient neutrophils also adhered more strongly than control neutrophils to activated endothelial cells. ARAP3-deficient neutrophils were further characterized by reduced transendothelial migration *in vitro*. Together, these observations suggested a hypo-inflammatory response due to a recruitment defect *in vivo*, although enhanced adhesion-dependent

neutrophil effector functions were suggestive of a hyper-inflammatory response.

We analysed neutrophil recruitment and neutrophilic inflammation in two short models of neutrophil-dependent inflammation, thioglycollate peritonitis and LPS-induced acute lung injury. In addition, as a model of prolonged neutrophil-dependent inflammation, we used a model of rheumatoid arthritis induced by anti-collagen antibody complexes.

As expected, all of these models identified an ARAP3-dependent neutrophil-autonomous recruitment defect to sites of inflammation. Using a combination of conditional *Arap3* deletion and adoptive transfer, we also observed a separate, ARAP3-dependent, neutrophil non-autonomous defect that led to increased inflammation in these models. This is likely due to increased cytokine production by cells other than neutrophils, which acts in combination with the enhanced pro-inflammatory effector functions observed with ARAP3-deficient neutrophils in adhesion-dependent situations. Together, this causes increased inflammation, as opposed to protection from inflammation in the ARAP3-deficient mice.

[1] Gambardella et al, 2011, *Blood* 118, 1087-98.

[2] Gambardella et al, 2013, *J. Immunol* 190, 381-91.

P10.11

Development of iPSC-derived monocytes/macrophages: a new reliable tool to study rare diseases and for drug screening

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Background: Human induced pluripotent stem cells (iPSCs) are generated from adult somatic cells genetically reprogrammed by enforcing expression of transcription factors important to maintain embryonic stem cell's properties. Because iPSCs are self-renewing and pluripotent, they can serve as an unlimited source of personalized human cells and tissues for cellular therapies, drug screens and disease modelling. In particular, we took advantage of this modern technology to develop a protocol to generate iPSC-derived monocytes and macrophages (iPSC-MM). **Methods:** Dermal fibroblasts from healthy skin were reprogrammed to iPSCs and then differentiated in myeloid precursors. We then generated iPSC-monocytes (M-CSF, for 7 days) that were subsequently differentiated in resting macrophages (M0), pro-inflammatory (M1: IFN γ +LPS) and anti-inflammatory macrophages (M2: IL-4). We then analyzed by flow-cytometry the expression of monocyte-macrophage lineage and polarization markers. Their functional relevance was evaluated by analyzing their allo-stimulatory and phagocytic properties and their cytokine profile. Finally, we compared the susceptibility of iPSC-MMs derived from either healthy donors or Δ 32 patients to be infected by HIV-1

Results: Similar to blood monocyte-derived macrophages (MDM), iPSC-MMs were able to correctly differentiate toward M0, M1 or M2 following incubation with the above-mentioned stimuli. Even at functional level, iPSC-MMs were similar to MDM as they were able to serve as professional phagocytes,

promoted T-cell proliferation in mixed lymphocyte reactions and showed a proper cytokine profiles reflecting their polarization status. Finally, iPSC-MMs were susceptible to HIV infection only when derived from healthy donors, but not from $\Delta 32$ patients.

Conclusions: This newly developed protocol is able to generate iPSC-MMs phenotypically and functionally similar to blood monocytes and therefore, could represent an alternative and reliable tool to study the monocytic-macrophagic compartment both in physiological and pathological conditions in the context of rare human diseases as well as for testing drug efficacy.

P10.12

Bacterial DNA impairs neutrophil phagocytosis, phagocytosis-induced neutrophil apoptosis and resolution of *E. coli*-induced acute lung injury

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Background: Neutrophil dysfunction, resulting in inefficient clearance of invading bacteria and delayed apoptosis, is a characteristic feature of severe pathologies, including sepsis and cystic fibrosis where bacterial DNA was detected in the blood and tissues even in the absence of bacteria. We investigated the impact of bacterial DNA on phagocytosis, phagocytosis-induced neutrophil apoptosis and clearance of *E. coli*, critical events in timely resolution of infections.

Methods: Human neutrophils were cultured with bacterial DNA (0.1-3.2 $\mu\text{g}/\text{mL}$) and surface expression of complement receptors, phagocytosis of *E. coli* and phagocytosis-induced apoptosis were studied in the presence of bacterial DNA. Acute lung inflammation was produced by intratracheal instillation of live *E. coli* (5×10^6 CFU) +/- bacterial DNA (1 $\mu\text{g}/\text{g}$ body weight, i.p.) in wild type and TLR9-deficient mice.

Results: Culture of human neutrophils with bacterial DNA resulted in decreased phagocytosis and killing of opsonized *E. coli*. Bacterial DNA up-regulated C3R (CD11b) expression, down-regulated C5aR (CD88) expression and evoked release of neutrophil elastase. C5aR cleavage was prevented by a specific neutrophil elastase inhibitor and PMSF. Consistently, bacterial DNA reduced phagocytosis-induced neutrophil apoptosis through attenuating NADPH oxidase-mediated activation of caspase8 and caspase-3. These actions of bacterial DNA were blocked by the telomere-derived TLR9 inhibitory oligonucleotide 5'-TTT AGG GTT AGG GTT AGG G-3'. In wild type mice, bacterial DNA impaired pulmonary clearance of *E. coli*, suppressed neutrophil apoptosis and delayed resolution of lung injury evoked by *E. coli*. Genetic deletion of TLR9 rendered mice unresponsive to bacterial DNA.

Conclusions: Our results identify a novel mechanism, neutrophil elastase-mediated inactivation of C5aR-mediated phagocytosis, by which bacterial DNA could contribute to neutrophil dysfunction and prolongation of tissue injury. These findings also suggest a therapeutic potential for TLR9 antagonists or neutrophil elastase inhibitors for enhancing clearance of bacterial infections in an environment where bacterial DNA is abundantly present.

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P10.13

Dynamics of ezrin location at the plasma membrane: relevance to neutrophil spreading

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Background: The spreading of neutrophils onto a surface involves a massive change in cell shape. As ezrin forms crucial crosslinks between the plasma membrane and cortical F-actin, in neutrophils and other myeloid cells, it is thought to maintain the structure of cell surface microridges which act as a reservoir of plasma membrane for spreading. It has been suggested that elevating the cytosolic $[\text{Ca}^{2+}]$, thereby activating the Ca^{2+} -activated cysteine protease calpain, would break the ezrin link and permit cell spreading. In this work, we have expressed fluorescent constructs of ezrin to investigate the dynamic relationship between elevated $[\text{Ca}^{2+}]$ in the microdomain of ezrin and its release from the cell periphery.

Materials and methods: RAW 264.7 cells were transfected with plasmids encoding ezrin-mEmerald, mCherry-ezrin, ezrin-mCherry, GFPi-ezrin and ezrin-CEPIA3 using the Cell Line Nucleofector™ Device (Lonza). Elevation of cytosolic $[\text{Ca}^{2+}]$ was achieved using a high Ca^{2+} cocktail with thapsigargin and ionomycin.

Results and conclusions: Ezrin constructs with available N-FERM domains (e.g. ezrin-mEmerald, ezrin-mCherry and GFPi-ezrin) located to the cell membrane in transfected myeloid cells, whereas ezrin constructs with a fluorophore linked to this domain (mCherry-ezrin) remained cytosolic. This suggests that ezrin localisation to the cell edge is primarily achieved through its N-FERM domain binding to the plasma membrane. Elevation of cytosolic $[\text{Ca}^{2+}]$ resulted in rapid loss of ezrin from the cell cortex. This may result in the reduction of membrane surface microridges, and contribute to the observed increase in cell size. By attaching a low affinity Ca^{2+} -sensing fluorophore to ezrin (ezrin-CEPIA3), the cytosolic $[\text{Ca}^{2+}]$ beneath microridges of myeloid cell membranes was measured for the first time, and exceeded 20 μM before release of ezrin and cell expansion. The dynamics of ezrin localisation and response to $[\text{Ca}^{2+}]$ are relevant to neutrophil spreading during extravasation, where cytosolic $[\text{Ca}^{2+}]$ is elevated.

P10.14

Chemokine receptors expression on circulating monocytes and neutrophils in high grade glioma patients

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High grade gliomas (HGGs) are the most frequent brain tumors and include the more aggressive form glioblastoma multiforme (GBM) with short survival time after diagnosis. One of the characteristics of HGGs is the loss of blood-brain barrier and the vascularization of the tumoral tissue. Leukocytes infiltrate the tumor and are key modulators of tumor growth and responsiveness to therapy. The objective of the study is to correlate circulating myeloid cells phenotypes to HGG aggressiveness. HGG patients had an increase rate of classical CD14 + monocytes

compared to the non-classical CD16⁺ subset with a dysregulated expression of inflammatory chemokine receptors. We also found three different neutrophil subpopulations with differential expression of activation state markers and chemokine receptors in HGG patients compared to healthy controls. The presence of differentially activated myeloid cells in the blood of HGG patients could be used as a prognostic indicator of HGG aggressiveness.

P10.15

Nano- and microparticles of different sizes and shapes induce neutrophil necroptosis and neutrophil extracellular traps

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The human body is exposed to a wide range of nano- and microparticles of industrial, environmental or internal origin such as titanium dioxide, asbestos, alum, silica or crystals of urate, calcium phosphate, calcium oxalate, cystine or cholesterol. Phagocytic clearance of such particles involves neutrophils and macrophages. We report that neutrophils that encounter such particles of diverse sizes and shapes frequently undergo necrotic cell death, a process associated with the formation of neutrophil extracellular traps (NETs). In human neutrophils receptor-interacting protein kinase (RIPK)-1 inhibition with necrostatin-1s or RIPK-3 inhibition with necrosulfonamide abrogated both cell death and NET formation induced by all of the aforementioned nano- and microparticles. Similar results were obtained with mouse neutrophils deficient in kinase active RIPK3 or mixed lineage kinase domain-like (MLKL) for all nano- and microparticles *in vitro*. Furthermore, lack of MLKL abrogated NET-driven tophus formation upon injection of either of the aforementioned particles into subcutaneous air pouches of mice *in vivo*. These findings imply that nano- or microparticle induced NET formation is the consequence of neutrophil necroptosis, a regulated form of cell necrosis defined by RIPK1-RIPK3-MLKL signaling. Interestingly, this finding was consistent across different particles sizes and shapes. RIPK1, RIPK3, and MLKL may represent potential therapeutic targets in nano- or microparticle-related diseases (crystallopathies).

P10.16

Tumor-associated M2-like macrophages express Molecule 1: a possible new marker for M2-polarization affecting monocytes motility

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Increasing evidences correlated the levels of tumor-associated macrophages (TAMs) with bad prognosis. TAMs are key orchestrators of the tumor microenvironment, directly affecting different biological activities, including neoplastic cell growth, neoangiogenesis, and extracellular matrix remodelling. Recently, we observed that TAMs display a gene profile similar to M2 polarized macrophages, with selective up-regulation of a set of genes. In this study we focused on one of the genes up-

regulated in M2 macrophages and TAMs, here called Molecule-1 (Mol-1). The selective expression of Mol-1 by M2-like TAMs prompted us to test whether the protein could act as novel marker of macrophage polarization.

We developed a monoclonal antibody (MoAb) directed against human Mol-1 and used in immunohistochemistry and immunofluorescence to detect the endogenous protein in tumor tissues (lung, breast and colon) as well as in normal tissue samples (liver, skin and decidua of first trimester). An heterogeneous pattern of Mol-1 expression was observed in tumor specimens where the protein is expressed by cells lining the glands, infiltrating tumor cells, stromal cells, fibroblasts and TAMs. Interestingly in double immunostaining most CD68, CD206 and CD163 positive macrophages were found to express Mol-1. Recombinant human Mol-1 was expressed and purified by immunoaffinity using the specific MoAb. The biological activity of purified Mol-1 was confirmed in migration and invasion assays with monocytes and cancer cells, using Boyden chambers or transwells respectively. By RNA and protein analysis we confirmed Mol-1 expression by M2 polarized macrophages, where is induced by M-CSF, IL-4, and IL-10 but not by proinflammatory stimuli (INF-gamma). Our data suggest that Mol-1 could represent a novel marker of macrophage polarization. In addition, modulating monocyte migration, Mol-1 might promote and/or sustain a permissive microenvironment for cancer cell invasion and metastasis.

P10.17

Role of hypoxia and the triggering receptor expressed on myeloid cells in human macrophage polarization

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Background: Macrophages (Mf) are a major component of the leukocyte infiltrate at sites of inflammation and tumor growth, where they differentiate from recruited monocytes (Mn). Mf can undergo diverse forms of activation in response to environmental factors, polarizing into specialized functional subsets. A hallmark of the pathologic environment is represented by hypoxia. Little is known about the impact of the hypoxic environment on Mf polarization.

Objective: The objective of this study was to elucidate the effects of hypoxic conditions reflecting those occurring *in vivo* in diseased tissues on the ability of Mn-derived Mf to polarize into classically activated (proinflammatory M1) and alternatively-activated (anti-inflammatory M2) subtypes.

Methods: Human peripheral blood Mn were cultured for 6 days with M-CSF under normoxic (20% O₂) or hypoxic (1% O₂) conditions and for additional 24 hr with LPS (for M1 polarization) or IL4 (for M2 polarization) and then phenotypically and functionally characterized.

Results: We demonstrated that hypoxia hindered Mf polarization toward the M1 phenotype by decreasing the expression of T cell costimulatory molecules and chemokine homing receptors and the production of proinflammatory, Th1-priming cytokines typical of classically activated Mf, while promoting the

acquisition of phenotypic and secretory features of alternative activation. Furthermore, we identified the triggering receptor expressed on myeloid cells (TREM)-1 as a marker of hypoxic Mf and showed that its engagement by an agonist Ab reversed the M2-polarizing effect of hypoxia reprogramming hypoxic Mf towards a M1 proinflammatory state. Finally, we provided the first evidence that Mf infiltrating the inflamed hypoxic joints of children affected by Juvenile Idiopathic Arthritis express TREM-1 and are predominantly polarized towards a M1 proinflammatory phenotype, pointing to a potential pathogenetic role of this molecule in the disease.

Conclusions: These results highlight the fine regulatory control exerted by the pathologic hypoxic environment on Mf polarization.

P10.18

Exploring neuro-immune networks in colorectal cancer

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The central nervous system reflexively regulates the inflammatory response, via a direct modulation of immune cells by peripheral nerves. However, in the context of cancer, where it is already known that inflammation plays a key role, the regulation of immune cells by the nervous system is still largely unexplored. Macrophages, professional phagocytes involved in the inflammatory response, hold a key position also in homeostatic and tissue responses, including neural-mediated circuits. To define whether a neural control of macrophage functions in tumors exists, we have investigated the macrophage-neural interaction in a preclinical model of colorectal cancer. In a model of AOM/DSS-induced colorectal cancer, we have visualized the spatial interaction of the complex intestinal neural networks with macrophages through a 3D spatial distribution analysis. F4/80⁺ macrophages were found in close proximity to S100⁺ neural cell bodies and fibers throughout the intestinal wall, with distinct distributions according to different layers, suggesting intra-tissue specialization of these phagocytes. Compared to control mice, we found a closer association of macrophages to nerves in tumor bearing mice ($P = 0.016$). Interestingly, macrophages of treated mice also showed an increased expression of the $\alpha 7$ subtype nicotinic acetylcholine receptor ($\alpha 7nAChR$), a neural receptor that plays a key role in the anti-inflammatory reflex in the gut. These results suggest a modification of neural-macrophage networks in colorectal cancer that could be important in the regulation of macrophage function in tumors.

P10.19

Regulation of the neutrophil NADPH oxidase and release of S100A8/A9

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S100A8 and S100A9, two EF-hand Ca²⁺-binding proteins, are abundantly expressed in the cytosol of neutrophils. At the intracellular level, S100A8/A9 are notably described to regulate NADPH oxidase (NOX2) activity, the major source of reactive

oxygen species (ROS) in neutrophils. ROS are designed to kill invading pathogens but can lead to tissue damages when they are excessively produced. S100A8/A9 are also secreted in the extracellular environment and are considered as damage-associated molecular pattern molecules, which amplify the pro-inflammatory response. High concentrations of S100A8/A9 are indeed found at local sites of inflammation or in the serum of patients with inflammatory diseases and can be used as biomarkers. Although the role of S100A8/A9 is prominent in the pathophysiology of many inflammatory diseases, the mechanisms by which they are released remain unknown.

In this work, we focused both on the role of S100A8/A9 in the regulation of NOX2 activity and on their secretion.

We demonstrated that NOX2 activation is regulated by the integration of diverse signaling pathways, and that S100A8/A9 may constitute the molecular switch between the Ca²⁺-dependent signaling cascade and NOX2 activation.

Second, our results on differentiated HL60 cells and purified human neutrophils show a time-dependent secretion of S100A8/A9 and the formation of neutrophil extracellular traps (NETs) when induced by PMA, but not by fMLF or LPS. S100A8/A9 secretion is only detected in conditions where the cells also produce NETs, supporting the hypothesis that S100A8/A9 could be released by NETosis in neutrophils.

S100A8/A9 are involved in the pathophysiology of many inflammatory diseases. Understanding the mechanisms by which they are secreted as well as their various functions will be a major advance for the development of respective selective therapy.

P10.20

Intratracheal instillation of alveolar type II cells safely improves recovery from acute lung injury in rats

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Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are acute inflammatory lung diseases characterized by excess production of inflammatory factors in lung tissue. Decades of research failed to find effective therapies that reduced mortality in established ALI/ARDS. Alveolar type II (ATII) cells help repair damaged lung tissue, rapidly proliferating and differentiating into alveolar type I cells after epithelial cell injury. In ALI, the lack of viable ATII cells favors progression to ARDS.

In the present investigation, we have performed intra-tracheal transplantation of isolated alveolar type II cells in a two hits rat model of Hydrochloric acid and lipopolysaccharide (HCL/LPS)-

induced ALI. The current study evaluated the anti-inflammatory effect and reparative effect produced by ATII transplantation in a sustained model of ALI.

Our experiments showed that tracheal instillation of isolated ATII cells in rats with ALI promoted lung recovery; paracrine effects of prostaglandin (PGE₂) and surfactant protein (SP) A from ATII modulate alveolar macrophages to the anti-inflammatory phenotype. Macrophages significantly decreased the expression of pro-inflammatory markers (IFN γ , IL-1 β , IL-4 and IL-6). Moreover, ATII transplantation increased the expression of SPA and SPC after transplantation and significantly reduced permeability and peribronchiolar and interstitial infiltration with inflammatory cells, hemorrhage and the interstitial edema.

Our findings strongly suggest that ATII transplantation is safe and may have therapeutic potential for ARDS. The mechanism for this effect may be due to the secretion of paracrine soluble factors such as PGE₂ and SPA by the ATII cells that may modulate alveolar macrophages to an anti-inflammatory phenotype.

P10.21

The role of Galectin-3 in acute and chronic lung injury

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Acute lung injury (ALI) is characterised by inflammatory cell infiltration and host tissue damage that can result from multiple causes such as trauma, sepsis, ventilator-induced lung injury as well as a myriad of other medical conditions. ALI can develop into chronic lung injury, such as idiopathic pulmonary fibrosis (IPF), a condition with limited treatment options that severely compromises the patient's respiratory health.

Galectin-3, a beta-galactoside binding lectin, has been shown to be highly expressed in macrophages and epithelial cells and is dramatically up-regulated during lung injury¹. Previous work has shown that inhibiting galectin-3 reduces collagen deposition in a bleomycin-induced pulmonary fibrosis model, however whether galectin-3 inhibition is protective during the acute phase of lung injury remains unknown. We demonstrate, using an LPS-induced model of ALI, that a global deletion of galectin-3 inhibits alveolar neutrophil recruitment and myeloperoxidase release, which correlated with a reduction in vascular permeability. However, we saw no change in the number of interstitial neutrophils in the lung suggesting that galectin-3 affects the emigration of neutrophils into the alveolar space which is a critical mediator of lung injury. A reduction in neutrophil, lymphocyte and monocyte/macrophage recruitment alongside a reduction in vascular permeability (as measured via total protein) was also observed with galectin-3 inhibition during the acute phase of bleomycin-induced lung injury. We demonstrate that galectin-3 activates neutrophils and reduces their rates of apoptosis in vitro, thus prolonging acute inflammation. Inhibiting the function of galectin-3 may therefore offer an attractive novel therapeutic target for the treatment of ALI to help prevent chronic disease.

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P10.22

CD47-SIRP α checkpoint blockade involves kindlin3-dependent enhancement of CD11b/CD18-integrin affinity and cytotoxic synapse formation

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Recently, we established that neutrophils kill antibody-opsonized tumour cells by a novel cytotoxic process that we have termed *trogoptosis*. This previously unknown killing mechanism involves trogocytosis (from Greek *trogo*, gnaw), where fragments of target cell membrane are actively taken up by the neutrophil and this contributes to the destruction of cancer target cells. Trogocytosis and subsequent killing is strictly dependent on antibody-opsonization of the tumour cells, neutrophil Fc γ -receptor signalling and CD11b/CD18 integrin-dependent cytotoxic synapse formation. Furthermore, it is promoted by CD47-SIRP α checkpoint inhibition. Here, we present evidence that CD47-SIRP α interactions act by controlling the initial stage of CD11b/CD18-dependent cytotoxic synapse formation. In particular, CD47-SIRP α interactions negatively regulated the CD11b/CD18 inside-out activation that occurred as a consequence of Fc-receptor signalling in neutrophils. Moreover, the inhibitory effect acted via the integrin-associated protein kindlin-3, as demonstrated, amongst other things, by using neutrophils from rare LAD-III patients that have mutations in *FERMT3* and lack kindlin-3 expression. Collectively, these findings demonstrate that CD47-SIRP α interactions control a kindlin-3-dependent pathway of CD11b/CD18-integrin activation, and that targeting the CD47-SIRP α checkpoint primarily improves integrin activation, and therefore also the resultant cytotoxic synapse formation, trogocytosis and killing during neutrophil ADCC towards cancer cells.

P10.23

Microglia and circulating phagocyte metabolic profile in rats with MPTP-induced Parkinson's disease and concomitant ulcerative colitis

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Background: Peripheral inflammation, including chronic inflammatory bowel diseases, could be implicated in the origin and development of neurodegenerative conditions such as Parkinson's disease (PD). Activation of microglial cells as well as peripheral blood phagocytes plays a crucial role in the pathobiology of PD. The aim of this study was to evaluate and compare the functional state of microglia and peripheral blood phagocytes in rats with PD and concomitant ulcerative colitis (UC).

Materials and methods: Wistar rats were injected subcutaneously with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

(MPTP) at the dose of 20 mg/kg to induce PD-like dopaminergic neurons loss. Experimental colitis was induced by intracolonic administration of 0.1 mL of 6% of iodoacetamide on the 7th day after MPTP injections. Metabolic profile of microglial cells, circulation phagocytes were characterized by reactive oxygen species (ROS) generation and phagocytosis activity that were evaluated by flow cytometry. CD69 early activation marker and CD14 expression by these cells were also determined. NO production by rat microglial cells was assayed in Griess reaction. **Results:** PD development (17th day after MPTP injection) was associated with the increase of phagocytosis intensity in microglia. The proportions of CD14^{high} and CD 69 + microglial cells in these animals were also dramatically increased. Statistically significant alterations in peripheral blood phagocyte functions were not observed. Microglial cells from rats with PD and concomitant UC were characterized by 4 times upregulated CD69 expression along with increased ROS and NO production. In peripheral blood of those animals monocytes upregulated (by 7.5 times) CD69, and the number of CD14 + granulocytes was 2-fold decreased. Decreased phagocytosis activity of circulating phagocytes was accompanied by their slightly enhanced ROS production.

Conclusions: Peripheral inflammatory response, which was initiated by UC, potentiated inflammatory process in CNS of rat with PD that was characterised by their microglial cells strong activation.

P10.24

Development of a fluorescence-based osteoclast fusion assay

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Background: Osteoclasts are phagocytic cells capable of degrading bone tissue. They develop from myeloid progenitors via biochemical differentiation followed by intercellular fusion. During the biochemical maturation several factors are expressed and activated, committing the cells towards the osteoclast fate. During fusion, mononuclear cells fuse into multinuclear cells that can perform bone resorption. Osteoclasts can be differentiated *in vitro* from bone marrow cells cultured in the presence of M-CSF and RANKL. Such cultures are usually stained histochemically for the osteoclast-specific TRAP enzyme. Osteoclasts are then defined as TRAP-positive cells containing at least three nuclei. Despite its wide use there are limitations of this approach. Therefore, our aim was to develop and characterize a novel, fluorescence-based assay for the identification of osteoclasts in *in vitro* cultures.

Materials and methods: We have used the mTmG and the mG mice for these studies. mTmG mice express a membrane-targeted tdTomato protein, whereas mG mice (generated from mTmG using a germline Cre transgene) express membrane-targeted EGFP. We cultured mTmG, mG or mixed mTmG+mG bone marrow cells towards osteoclasts. We then examined osteoclast morphology. We have also exposed osteoclast cultures to dasatinib, a known inhibitor of osteoclast maturation.

Results: Bone marrow cells of mTmG and mG mice remained red and green, respectively. However, culturing mixed mTmG+mG cells gave rise to large "yellow" cells carrying both

red and green fluorescence signals. This indicated the fusion of mTmG and mG cells. The mixed mTmG+mG osteoclast cultures showed normal kinetics of osteoclast development, were normally sensitive to the inhibitory effect of dasatinib, and could be re-stained with TRAP. Importantly, quantification of the mTmG+mG cultures was significantly easier than that of the TRAP-stained cultures.

Conclusions: We have set up a dual fluorescence-based fusion system that allows analysis of intercellular fusion *in vitro*, even under microscopy, overcoming the limitations of TRAP-staining.

P10.25

On the production of the C-C motif chemokine ligand 23 (CCL23) by human neutrophils

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CCL23, also known as myeloid progenitor inhibitory factor (MPIF)-1, macrophage inflammatory protein (MIP)-3 or CKβ8, is a member of the CC chemokine subfamily exerting its effects via CCR1 binding. By doing so, CCL23 selectively recruits resting T lymphocytes, monocytes and dendritic cells, inhibits proliferation of myeloid progenitor cells and promotes angiogenesis. Previously, we and other groups have reported that human neutrophils produce a number of chemokines upon appropriate activation, including CCL2, CCL3 and CCL4, which also bind CCR1. Herein, we report that human neutrophils display the capacity to additionally produce and release CCL23 when *in vitro* activated by R848, a ligand of TLR8, and, to a lesser extent, by other proinflammatory agonists, including LPS, Pam3CSK4 and TNFα. Notably, we show that, on a per cell basis, R848-activated neutrophils produce higher CCL23 levels than autologous R848-activated CD14⁺-monocytes, unlike other chemokines/cytokines which are typically much more expressed by R848-activated monocytes than neutrophils. On the other hand, we found that neutrophils do not produce CCL23 in response to IL-4, in contrast to CD14⁺-monocytes, thus indicating that CCL23 expression is regulated in a stimulus- and cell-specific fashion. Finally, we show that the production of CCL23 by R848-stimulated neutrophils is amplified by endogenous TNFα, but negatively modulated by exogenous IFNα. Together, data extend our knowledge on the chemokines potentially produced by neutrophils. The ability of human neutrophils to produce CCL23 further supports the notion of their capacity of orchestrating the recruitment of different cell types to the inflamed sites, in turn contributing to the control of the immune response.

P10.26

Investigating the role of NADPH oxidase in the regulation of intestinal homeostasis:

implication for Chronic Granulomatous Disease

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Chronic granulomatous disease (CGD) is a primary immunodeficiency characterized by defective phagocytic production of reactive oxygen species (ROS), due to mutations in the genes

encoding for different protein subunits of NADPH oxidase complex. Mutations in CYBB gene coding for the catalytic subunit gp91phox (Nox-2) give rise to X-linked CGD. Affected patients not only suffer from recurrent bacterial and fungal infections but also they develop unexplained autoinflammatory/autoimmune conditions, involving particularly the intestine. In addition to myeloid cells, the enzyme is also expressed, at lesser amount, in lymphocytes although its functional implications have been poorly investigated. Here we aim to investigate the cellular and molecular bases of intestinal inflammation in X-CGD. We found that tissue expressions of pro-inflammatory cytokines such as IL1beta, IL6, IL17 were significantly increased in the bowel of gp91phox^{-/-} mice and associated with a Th17-mediated intestinal inflammatory response. FACS analysis of intestinal T cells compartment evidenced a decreased frequency of Treg cells in knockout mice as well as reduced proportion of retinoic acid-producing CD103⁺ dendritic cells, crucially involved in the induction of tolerogenic response. Consistently, a reduced Treg differentiation of adoptively transferred CD4⁺ OTII cells was observed *in vivo* in gp91phox^{-/-} mice, upon oral OVA administration. Finally, analyzing the B cells compartment, we observed decreased IgA plasma cells differentiation in the Peyer's Patches of mutant mice. In line with this observation, we found an increased tissue expression of the antimicrobial peptide Reg3gamma produced by intestinal epithelial cells. This finding is suggestive of enhanced microbial stimulation of the intestinal epithelium in condition of Nox2 deficiency, and likely is due to defective IgA, crucial to contain gut microbes in the lumen. Our preliminary results indicate that intestinal inflammation and altered gut homeostasis in knockout mice may be due to the contribution of different immune cells type.

P10.27

A false carrier state for the c.579G > A mutation in the NCF1 gene in Ashkenazi Jews

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Objective: Mutations in the NCF1 gene that encodes p47^{phox}, a subunit of the NADPH oxidase complex, cause chronic granulomatous disease (CGD). In Kavkazi Jews, a c.579G > A (p.Trp193Ter) mutation in NCF1 is frequently found, leading to CGD. The same mutation is found in about 1% of Ashkenazi Jews, although Ashkenazi CGD patients with this mutation have never been described. In an Ashkenazi couple expecting a baby both parents were found to be heterozygotes for this mutation, as was the fetus. We investigated the chance of the fetus to be a CGD patient.

Methodology and results: Segregation analysis in the extended family was consistent with the fetus inheriting both carrier

alleles from the parents. Multiplex ligation-dependent probe amplification indicated four complete NCF1 genes in the fetus and three in each parent. Ion Torrent gene sequencing confirmed these results. Analysis of fetal leukocytes obtained by cordocentesis revealed substantial oxidase activity with three different assays, which was confirmed after birth. In six additional Ashkenazi carriers of the NCF1 c.579G > A mutation we found five individuals with three complete NCF1 genes of which one was mutated (like the parents), and one individual with in addition a fusion gene of NCF1 and a pseudogene.

Conclusions: These results point to the existence of a "false carrier" state in Ashkenazi Jews and have wide implications regarding pre-pregnancy screening for this mutation in this and other population groups.

P10.28

Src family kinases in monosodium-urate crystal-induced inflammatory responses

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Background: Deposition of monosodium urate (MSU) crystals in the joints or other tissues is a hallmark in the pathogenesis of gout, which is known to be mediated by neutrophils besides monocytes and macrophages. Although MSU crystal-mediated signal transduction is in the focus of recent investigations, the molecular mechanism is only partially characterized. In this study, we investigated the role of Src family kinases in MSU crystal induced myeloid cell activation.

Methods: Bone marrow isolated neutrophils from wild type and triple Src family kinases-deficient (Hck^{-/-}Fgr^{-/-}Lyn^{-/-}) mice were stimulated by different concentrations of MSU crystals followed by the analysis of superoxide production and cytokine release. The process of crystal phagocytosis was investigated by videomicroscopy and flow cytometry in neutrophils derived from wild type and Src family kinases-deficient mice or in human neutrophils treated by the Src-inhibitor dasatinib or vehicle control.

Results: The MSU crystal-induced superoxide release and cytokine production were dramatically impaired in Src family kinases-deficient or in dasatinib-treated neutrophils. Cytochalasin D, which is an actin polymerization blocker that can effectively inhibit phagocytosis, strongly reduced the superoxide release of neutrophils activated by urate crystals. Both in the presence of cytochalasin D or dasatinib, the crystal phagocytosis of human neutrophils was abrogated. Neutrophils with genetic deficiency of Src family kinases also failed to phagocytose the MSU crystals.

Conclusions: The Src family kinases Hck, Fgr and Lyn play an indispensable role in MSU crystal-induced superoxide and cytokine production in neutrophils. The activation of neutrophils by urate crystals require the Src kinase-dependent phagocytosis of the crystals. Identification of these novel players in urate crystal-induced intracellular signaling pathways in neutrophils leads to a better understanding of the pathogenesis of gout and may help to develop novel therapeutic strategies in MSU crystal-associated inflammatory diseases.

P10.29

The pro-inflammatory role of immunoglobulin A

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Background: Immunoglobulin A (IgA) is the most prevalent antibody at mucosal sites and a potent stimulus of neutrophils (PMN) via the IgA Fc receptor (Fc α RI). However, the function of monomeric serum IgA is still poorly understood. Serum IgA is considered an anti-inflammatory modulator by inhibiting inflammatory signals via activation of IgG receptors. This project aims to unravel the pro-inflammatory role of serum IgA by Fc α RI induced PMN activation.

Methods: Fluorescent latex beads were coated with different ratios of human pooled-serum IgA and IgG and incubated with freshly isolated human PMNs. After incubation phagocytosis, migration and chemotaxis assays were performed. Subsequently, these assays were repeated with beads that had been coated with plasma samples of healthy individuals. Levels of total IgA and IgG in these plasma samples were determined by ELISA.

Results: Beads that had been opsonized with different ratios of IgA and IgG were equally phagocytosed by PMNs. However, migration of PMNs was only observed towards IgA coated beads and not towards IgG coated beads. Similarly, only beads with a high IgA:IgG ratio induced PMN migration, which was reduced when a lower IgA:IgG ratio was present on beads. This observation was confirmed in a chemotaxis assay, since migration of PMNs was only seen towards supernatants of neutrophils that had phagocytosed IgA coated beads. Plasma levels of IgA were determined at 1–3 mg/mL and IgG at 18–21 mg/mL depending on the donor.

Conclusions: Up till now it is thought that serum IgA induces inhibitory signals that reduce activation via IgG receptors. However, phagocytosis of IgG coated beads by PMNs was not inhibited by the presence of IgA. We hypothesize that the presence of IgA may enhance IgG induced inflammatory responses, as we demonstrated that IgG neither induces PMN migration nor release of chemoattractants in contrast to IgA.

P10.30

Cross-talk of dendritic cells with neutrophils that have been activated with immunoglobulin A

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Background: Immunoglobulin A (IgA) is the most prevalent antibody at mucosal sites and a potent stimulus of neutrophils (PMN) via the IgA Fc receptor (Fc α RI). In patients with ulcerative colitis, mucosal infiltration of PMNs as well as interaction with dendritic cells (DCs) is seen. We hypothesize that mucosal pathology and immune responses are influenced by Fc α RI induced PMN activation. The aim of this study is to investigate crosstalk of IgA activated PMN with DCs.

Methods: Retinoic acid stimulated DCs (RADCs) and monocyte-derived DCs (moDCs) were derived from monocytes after being cultured for 6 days in the presence of IL-4 and GM-CSF, with or without retinoic acid (RA) respectively. Aldehyde dehydrogenase activity was measured using the ALDEFUOR kit. Fresh isolated PMNs phagocytosed IgA coated latex beads (IgA PMN) after which they were co-cultured with DCs. Subsequent, maturation marker expression on DCs was assessed. Cell-cell interactions were analyzed with ImageStream and live cell microscopy, and cytokine production in supernatants of co-cultures was measured.

Results: RA-DCs differed in morphology, expressed more CD103 and CD89, and had higher aldehyde dehydrogenase activity compared to moDCs. RA-DCs expressed less CD1c, CD80, CD83, CD86 and HLA-DR, and more CD141 and CD103 after co-culture with IgA PMN compared to moDCs. Cell interactions, bead transfer between IgA PMN and DCs and IgA PMN uptake by DCs were seen in live cell microscopy and image-stream. IL-12 was produced by moDCs after co-culture with IgA PMN, but not by RA-DCs.

Conclusions: IgA PMN induces the maturation of moDCs, but not of RADCs. DCs interacted with IgA PMN, and bead transfer from PMNs to DCs was observed. However, after co-culture with IgA PMN, RADCs did not produce IL-12. These findings suggest that moDCs become pro-inflammatory and have higher potential for antigen presentation after stimulation with IgA PMN, while RADCs induce immune tolerance.

P10.31

New possibilities in treatment of failing heart: Focus on innate immunity and inflammatory signalling pathways

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Cardiovascular diseases (CVDs), associated with chronic inflammation, are the leading cause of mortality and morbidity worldwide. Inflammation ultimately leads to poorly functioning of heart, resulting in progression of CVDs. It is becoming increasingly evident that nitration products of unsaturated fatty acids (NO₂-FA) represent an important class of endogenous biological mediators, which are generated as an adaptive response of organism to oxidative and nitrative stress. The purpose of our study was to define the role of nitro-oleic acid (NO₂-OA) in prevention and treatment of atrial fibrosis and pulmonary arterial hypertension in mice. The effect of NO₂-OA was tested in different cell types (including macrophages, neutrophils, fibroblasts, endothelial, and smooth muscle cells) both *in vivo* and *in vitro*. Interestingly, our results showed that physiologically-relevant concentrations of NO₂-OA significantly improve the heart functions and overall outcome of animals with atrial fibrosis and pulmonary arterial hypertension. These effects were associated with reduced polarization of macrophages toward pro-inflammatory and immuno-regulatory subsets, decreased activation of different signaling pathways as well as production of pro-inflammatory and pro-fibrotic mediators. Moreover, we have demonstrated that NO₂-OA effectively influenced the process of macrophage differentiation induced by growth factors and thus could regulate their activation phenotype during inflammatory processes. Additionally, NO₂-OA significantly diminished transformation of endothelial cells to the pro-fibrotic phenotype and proliferation of vascular smooth muscle cells. In aggregate, our study provided the unique results showing the protective effects of NO₂-OA in progression of cardiovascular inflammation, supporting that NO₂-FAs represent new drug candidates suitable for deployment against chronic and inflammatory diseases having a complex pathogenesis. Following main signaling pathways, we also helped to clarify molecular mechanism of nitro-lipids action. This work was supported by the Czech Science Foundation (17-08066Y), by the Ministry of Education, Youth and Sports (LD15069), and is based upon work from the COST Action BM1404 Mye-EUNITER (www.mye-euniter.eu).

Workshop 11: Hematology

P11.01

Platelet counts and lipid profile in nephropatia epidemica

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Nephropatia epidemica (NE), the mild form of hemorrhagic fever with renal syndrome (HFRS), is the most prevalent zoonosis in Russia, with average annual incidence rate of 7,200 cases. The low platelets count is characteristic laboratory finding often used to assess the risk for life-threatening bleeding. Recent studies revealed positive correlation between thrombocytopenia and low density cholesterol suggesting that lipid metabolism plays role low platelet counts. However, the association between thrombocyte counts and serum lipids remains undetermined.

In present study, 228 NE patients admitted to Republican Clinical Hospital for Infectious Disease named after Agafonov, Republic of Tatarstan were recruited. Diagnosis of NE was established based on clinical presentation and was serologically confirmed by detection of anti-hantavirus antibodies and PCR analysis. Serum samples were collected at the time of admittance and used to analyze levels of total cholesterol, triglycerides and high density cholesterol (HDCI) levels (Vector Best).

Low platelet counts was found in early NE cases as compared to controls (159.4 ± 32.1 vs 387 ± 30 ; $P < 0.01$). Bleeding and various degrees of blood coagulation disturbances were detected in 46 NE cases. Levels of triglycerides were significantly upregulated, while HDCI were decreased in NE cases when compared to controls. Total cholesterol levels remained similar to controls.

Serum lipids and thrombocyte counts were also analyzed. HDCI level was significantly increased in NE cases with low thrombocyte counts; while in NE with high thrombocyte counts HDCI level was significantly decreased. Serum level of triglycerides was higher in NE cases regardless of the thrombocyte count. Our data suggests association between high HDCI and low platelet counts in NE cases.

P11.02

HLA alleles frequency in the Republic of Tatarstan population, Russia

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Obtaining the HLA compatible organ for transplantation is one of the most challenging task. Therefore, preliminary HLA screening is essential to optimize the potential organ donor identification. To address this issue, the Charity «Rusfond», Kazan Federal University, in collaboration with «Rusfond.Register» launched the program to establish the regional register of potential hematopoietic stem cell donors in the Republic of Tatarstan (RT).

Blood samples from 176 potential bone marrow donors was collected. DNA was extracted using «PROTRANS DNA Box 500» (PROTRANS, Germany) kit according to the manufacturer's recommendations. HLA genotype was analyzed using SSP-PCR Cycloplate System «PROTRANS HLA-A*/B*/C*» and «PROTRANS HLA-DRB1*/DQB1*» according to the manufacturers protocols.

Five HLA-loci were analyzed. In summary, a number of allele families included: 17 – A*, 25 – B*, 13 – C*, 13 – DRB1* and 5 – DQB1*. There were no allele families found, which are rare in Russia, such as A*69, A*74, A*80, B*59, B*67, B*73, B*78, C*18. Allele families based on frequency were: A* – A*02 (27.56%), A*24 (14.49%), A*03 (13.64%); B* – B*35 (14.49%), B*44 (10.51%), B*07 (10.23%); C* – C*07 (27.27%), C*04 (16.48%); DRB1* – DRB1*01 (15.34%), DRB1*07 (15.06%) and DQB1* – DQB1*03 (33.24%). These data advances our knowledge on the HLA allele distribution in RT and will be useful for the initial selection of potential bone marrow donors.

P11.03

VKORC1-1639G > A polymorphism and the risk of non-variceal upper gastrointestinal bleeding

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Background and aims: The purpose of the study was to examine if the VKORC1 -1639 G > A polymorphism could be a risk factor for non-variceal upper gastrointestinal bleeding (UGIB) in patients without concomitant therapy with acenocoumarol.

Methods: The study included 163 consecutive patients diagnosed with UGIB and 178 controls, without UGIB. The following data were noted: age, gender, alcohol consumption, smoking, history of UGIB, consumption of NSAIDs/low-dose aspirin. Genetic analysis included genotyping for the VKORC1 -1639 G > A mutation.

Results: History of UGIB (OR – 3.463; CI 95% 1.463–8.198, $P = 0.005$), smoking (OR – 2.498; CI 95% 1.358–4.597; $P = 0.003$), alcohol consumption (OR – 3.283; CI 95% 1.796–6.000; $P < 0.001$), use of NSAIDs (OR – 4.542; CI 95% 2.502–8.247; $P < 0.001$) or low-dose aspirin (OR – 2.390; CI 95% 1.326–4.310), and the VKORC1 -1639G > A AA genotype (OR – 1.364; CI 95% 0.998–1.863; $P = 0.05$) were associated with an increased risk of UGIB. The risk of UGIB was analyzed in patients with genotype AA who took low-dose aspirin/NSAIDs. The genotype AA was not an independent risk factor for UGIB ($P = 0.3$), although in subjects with this genotype that took NSAIDs/aspirin, the risk of UGIB was double compared to those under NSAIDs/aspirin therapy alone (OR - 7.6 vs. 3.6; $P < 0.001$).

Conclusion: Patients diagnosed with UGIB, which use of NSAIDs or low-dose aspirin, are more likely to be carriers of the VKORC1 -1639 G > A AA genotype.

P11.04

Early, norepinephrine-dependent, activation of the hematopoietic niche upon induction of experimental autoimmune encephalomyelitisT. Vigo^{*†}, N.K. de Rosbo^{*} & A. Uccelli^{*†}^{*}University of Genova, Italy; [†]AOU IRCCS San Martino-IST

In the bone marrow (BM), mesenchymal stem cells (MSC) contribute to the homeostasis of the hematopoietic niche through the production of factors which promote a quiescent hematopoietic stem cell (HSC) state. The sympathetic nervous system negatively controls the expression of these factors through the neurotransmitter norepinephrine (NE), whose interaction with β_3 -adrenergic receptors selectively expressed by MSC in the bone marrow leads to the proliferation and differentiation of HSC towards more differentiated progenitors. In the model for multiple sclerosis, experimental autoimmune encephalomyelitis (EAE), immunization with myelin antigens results in the activation of peripheral lymphoid organs where pathogenic T cells are generated. Our overall goal is to define the role of the hematopoietic niche in this process. As early as from 3 days after immunization (dpi) with the encephalitogen, we observed a significant increase in the number of immature HSC (KLS cells) in the BM, together with a reduced expression of MSC-specific genes known to be controlled by NE. Analysis of common lymphoid and myeloid progenitors showed a strong lymphoid bias of hematopoiesis at all time points. Parallel investigation indicated an early activation of the thymus in EAE, with a burst in maturation of inherent precursors from 3 dpi resulting in an elevated number of CD4⁺ T cells, and an increase in KLS cells, presumably derived from the BM, from 7 dpi. These data suggest a role for the BM hematopoietic niche in the development of EAE, and support the hypothesis that, under pathological conditions, MSC are controlled through β -adrenergic transmission to modify the peripheral immune repertoire.

P11.05

SIRT6 inhibition as a novel approach for treating Acute Myeloid Leukemia

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Currently available therapeutics against Acute Myeloid Leukemia (AML) have improved patient outcome. However, resistance develops even to novel therapies and patient overall survival remains low, especially for patients who are not eligible for allogeneic bone marrow transplantation. Therefore, there is an urgent need to overcome the biologic mechanisms underlying drug resistance in AML, to enhance the efficacy of existing treatments and to facilitate the design of novel approaches. Recently, our group has demonstrated that SIRT6, a NAD⁺-dependent histone deacetylase involved in genome maintenance, is frequently up-regulated in AML and confers poor prognosis in a series of 200 primary AML cases from our Hematology Clinic. Thus, these data suggested a role for SIRT6 in AML biology. High SIRT6 expression was typically observed in AML cell lines characterized by constitutive DNA damage and intense replicative stress. Likewise, primary AML cases exhibiting an intermediate-to-high CIN gene expression signature were also those with the highest SIRT6 expression, and worst prognosis. Subsequent studies

demonstrated that SIRT6 silencing or its chemical inhibition, as observed in Multiple Myeloma exacerbates DNA damage in response to genotoxic agents, sensitizing AML cells to cytarabine (ARA-C) and idarubicin in vitro. Overall, enhancing genotoxic stress while concomitantly blocking DNA double-strand breaks (DSBs) repair response, may represent an innovative strategy to increase chemosensitivity of AML cells. Mechanistic studies revealed that SIRT6 acts as a genome guardian in AML cells by binding DNA damage sites and activating DNA-PKcs and CtIP by deacetylation, which in turn promotes DNA repair. Overall our data suggest that genomic instability is present in all hematologic malignancies including AML. Strategies aimed to shift the balance toward high DNA damage and reduced DNA repair by SIRT6 inhibition can decrease AML growth and may benefit patients with otherwise unfavorable outcomes.

P11.06

Impact of HLA disparity on GvHD and relapse rate in haploidentical transplants followed by high dose post-transplant cyclophosphamideA.M. Raiola^{*}, N. Sacchi[‡], L. Garbarino[‡], L. Giannoni^{*}, A. Dominiotto^{*}, S. Aquino^{*}, G. Beltrami^{*}, S. Bregante^{*}, C. Di Grazia^{*}, F. Gualandi^{*}, A. Ibatici^{*}, T. Lamparelli^{*}, C. Marani^{*}, M.T. van Lint^{*}, R. Valardo^{*}, A. Bacigalupo[†] & E. Angelucci^{*}
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Background and aim: Haplo-identical donors are expected to share 4/8 HLA antigens with recipient, however casual phenotypic homozygosity in the non-shared haplotype makes the real degree of disparity usually less than 4/8. In this single center study we verified the effective number of donor/recipient disparity and its impact on outcome in patients transplanted from a haploidentical donor.

Patients and methods: We analyzed 282 consecutive haplo-transplanted patients (including diagnosis of AML, ALL, MDS, NHL, CLL or MPN) who received a myeloablative conditioning (MA) followed by unmanipulated BMT and post-transplant cyclophosphamide (PT-CY). Donors and recipients were typed for HLA A, B, C, DRB1, DQ and DPB at a high resolution level or by NGS. We evaluated cumulative incidence of grade II-IV aGVHD, moderate-severe cGVHD and relapse rate, according to the number of mismatches in the GVHD vector at loci A, B, C, DRB1.

Results: Median age was 48 years (17–74). Among 282 couples D/R the real number of mismatches in the GVHD vector was as follows: 146 (51%) 4/8, 87 (30%) 3/8, 51 (17%) < 3/8. Patients were divided into 2 groups according to the degree of antigen difference: < 3 mismatches vs \geq 3 mismatches.

With a median follow up of 562 days (range 6–2241 days), the cumulative incidence of grade II-IV aGVHD and moderate-severe cGVHD were 17% ($n = 49$) and 13% ($n = 39$), respectively. 91/282 (32%) patients relapsed. None of these parameters was significantly influenced by the number of HLA mismatches ($P = ns$). The same results are confirmed when the analysis is performed in the subset of patients transplanted in complete remission.

Conclusions: In haploidentical transplantation almost 50% of D/R pairs differ for less than 4/8 HLA antigens; however in the setting of a MA conditioning with PT-CY, HLA mismatching showed no impact on aGVHD, cGVHD and relapse rate.

Workshop 12: Cancer Clinics

P12.01

Radio sensitization of high grade gliomas

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High grade gliomas (HGG-WHO grades III and IV) are almost invariably lethal tumors due to their infiltrating nature and resistance to therapies. Their lethal character is linked to specific cell populations [glioma initiating cells (GIC)] that are refractory to radio- and chemotherapy by mechanisms elucidated in part.

Objective: The final objective is to find a new solution to HGG via an innovative radiotherapeutic approach potentially applicable to a wide set of malignant disorders.

Methodology: An holistic approach which combines i) development of novel Ataxia Telangiectasia Mutated and Ataxia Telangiectasia and Rad3 Related inhibitors (ATM/ATRi) ii) monitoring their pharmacokinetics via HPLC/MS, iii) testing therapeutic efficacy in orthotopic HGG animal models and iv) finally producing the developed drugs in Proof-of-Principle, good manufacturing practice (cGMP)-grade vials, ready for Phase I/II clinical testing is being adopted.

Results: To target HGG, we [1] and others [2, 3] have recently identified new radiotherapeutic procedures. The novel radiotherapy regimens are based on the capacity of ATM/ATRi such as KU60019, NVP-BEZ235 and AZD6738, to specifically stimulate proliferation of quiescent GIC and radiosensitize them. Increase in survival of preclinical HGG models by ATM/ATRi here pioneeringly explored, may lead to a successful clinical treatment regimen.

Conclusions: Tumor radiosensitization by ATM/ATRi may advance radiotherapy of HGG. It may further contribute to find new therapeutic approaches for a set of tumors with similar growth features (e.g. some aggressive forms of leukemia and lymphoma), thus leading to concrete impacts on health care systems, quality of life of citizens and pharmaceutical industry.

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P12.02

Self-organization of bone marrow derived mesenchymal stromal cells and neuroblastoma cells in different models of extracellular matrix in vitro

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One of the current problems in the screening of anticancer drugs is the development of test system, mimicking interaction of cancer and stromal cells. Such models will allow more accurate drug screening and study of cancer and stromal cells interplay.

In our study, neuroblastoma cells SH-SY5Y were co-cultured with mesenchymal stromal cells (MSCs) to create a model of

stromal microenvironment. MSCs have a natural tropism to tumor cells and form a metastatic niche, promote tumor growth via paracrine signaling microenvironment, immune modulation and co-evolution of the tumor and stromal compartments. Bone marrow derived MSCs expressed characteristic CD markers CD44, CD73, CD166, CD90, CD29, CD105, and did not express CD markers of hematopoietic cells (CD34, CD11b, CD19, CD45, HLA-DR). The isolated cells were capable of differentiation in osteo-, chondro- and adipogenic directions.

MSCs and SH-SY5Y were labeled with vital dyes Vybrant DiD and DiO, mixed at 1:1 ratio and cultured on Matrigel or on 0.1% gelatin coated plastic. Results were analyzed using fluorescence microscopy and flow cytometry.

48 h incubation of MSCs and SH-SY5Y on Matrigel resulted in the formation of capillary-like structures. During additional co-cultivation for 3-4 days MSCs and SH-SY5Y formed spheroids. Alternatively, when MSCs and SH-SY5Y were co-cultured on gelatin coated plastic, self-formation of cells resembled the stroma derived from the MSCs with "islands" of tumor cells.

This co-culture model can potentially be used to study interaction of tumor and stromal cells and used for screening of anti-cancer drugs. This study was supported by RFBR grant 16-34-60201.

P12.03

Antioxidant and antiproliferative effects of Green Tea (*Camellia sinensis* (L.) Kuntze) against Prostate Cancer

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Introduction: Antioxidant activity and antitumor properties of Green tea (GT) have been extensively studied. Epidemiological studies demonstrated that GT consumption decreases prostate cancer (PC) incidence. Our hypothesis is that erythrocyte oxidative stress is associated with PC and daily consumption of GT may improve the oxidative phenotype having beneficial effects on human health.

Materials and methods: An *in vitro* approach was performed to characterize the composition and antioxidant/antiproliferative activities of the commercial Chinese GT leaves of *Camellia sinensis* (L.) Kuntze plant. The total phenolic content (CHCl₃, EtOAc and *n*-BuOH) in GT extracts was measured by HPLC-TOF/MS. Green tea-antioxidant properties was measured using 1,1-

diphenyl-2-picrylhydrazyl radical (DPPH) radical-scavenging activity assay. Cell mass and inhibition of lipid peroxidation was also evaluated. Next, seventy PC patients and 120 age-matched healthy subjects participated in the study, with glutathione (GSH), malondialdehyde (MDA), and catalase activities evaluated in erythrocytes before and after GT consumption.

Results: Our results demonstrated that GT contained a high content of phenolic and flavonoid compounds, demonstrating *in vitro* antioxidant activity and significant antiproliferative effect on human prostate cancer PC-3 cell line. Moreover, GSH and catalase activities were decreased and a high level of MDA in erythrocytes from PC patients was observed. The consumption of 2-3 cups per day of GT during 6 months significantly increased GSH concentration and catalase activity and decreased MDA concentration.

Conclusions: Oxidative stress was significantly decreased by GT intake in Algerian PC patients. Regular consumption of GT for a long period may prevent men from developing PC or at least delay its progression.

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P12.04

Molecular follow-up personalization through individualized circulating tumor DNA mutation detection in patients affected by invasive breast adenocarcinoma undergoing neoadjuvant treatment with curative intent: preliminary results from a prospective, monocentric, observational, real practice study

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Aim: The present study aims to assess the clinical validity of ctDNA-based personalized follow-up of clinical practice in early invasive breast adenocarcinoma (IBC) patients.

Patients and methods: In a prospective, ongoing study involving a cohort of early IBC patients undergoing neoadjuvant treatment followed by surgery with curative intent, we are collecting a pre-treatment tumor biopsy and serial plasma samples at several time points. Massively Parallel Targeted Sequencing is performed using a commercial kit including 409 known cancer genes on DNA extracted from tumor biopsies and matched with germline DNA, in order to identify truncal, high allelic frequency somatic and putative pathogenic mutations in the primary lesion. These target mutations are then used to identify ctDNA in plasma samples by digital droplet PCR.

Results: So far, we have enrolled 48 patients, 31 patients are actively screened, and for 11 we have serial plasma samples and available pre-treatment biopsy. We identified somatic genomic alterations in 10 patients (90.9%) out of 11, and ctDNA was extracted in all plasma samples. Validation by digital droplet PCR has so far been performed in two patients, carrying somatic mutations of pathogenic significance respectively in PIK3CA and TP53. In particular, in one patient we observed the disappearance of mutated TP53 in ctDNA after surgery and its

detectability at 24 weeks, anticipating the patient's clinical relapse by 6 months. The PIK3CA mutated patient did not present ctDNA mutations at baseline, and has not relapsed at the time of the current report.

Conclusions: ctDNA analysis in IBC patients undergoing neoadjuvant treatment with curative intent appears feasible. It is premature to draw conclusions based on such case report, but the absence of detectable ctDNA in one patient at baseline may be influenced by the biology of her tumor. The completion and final analysis of the presented study are awaited.

P12.05

The role of heat shock proteins in response to stress

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According to the results of Western blot analysis, in A549 cell line expression of anti-apoptotic protein Bcl-2 elevates with depletion of the nutrient medium during 6 days, therefore the apoptosis is blocked. Autophagy activity happens to the third and sixth day that was detected by down-regulated expression of anti-autophagic kinase mTOR and expression of the main autophagy marker – LC3B protein. As for NCI-H322M cell line, minor down-regulation of mTOR kinase's expression and absence of LC3B protein's expression were noted.

During six days of cultivation macroautophagy was induced in A549 cell line that was detected by up-regulation of LC3B protein's expression, which was almost null in NCI-H322M (TP53^{mt}) cell. In A549 (TP53^{wt}) cell line on the background of macroautophagy induction HSP70 is expressed during the first day of cultivation, but down-regulated on the third and sixth day. In NCI-H322M (TP53^{mt}) cell line its down-regulation was also detected. The expression of HSP90 was down-regulated on the third and sixth day in A549 (TP53^{wt}) cell line and almost unchanged in NCI-H322M. Wherein, apoptosis is blocked in A549 cell line that confirms cytoprotective role of autophagy.

The members of HSP90 family are known to be protective agents not for cells only, but for entire organisms as well. According to studies based on genome and transcriptome sequencing of extremophiles from *Chironomidae* family, the members of HSP90 group were proved to protect them from negative effects of heat shock and desiccation. This research showed high conservatism of HSP90 group between four species of chironomids with the most stress-inducible gene coding for glycoprotein 93. Moreover, we noticed that the molecular evolution of the well-known anhydrobiotic chironomid *Polypedilum vanderplanki* led to one extra copy of gene, coding for endoplasmic precursor, which, together with its paralog, is up-regulated on the later, recovery stages of heat shock and desiccation.

P12.06

Massive parallel sequencing (MPS) in two twins affected by breast cancer: an intriguing case

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Introduction: Several gene alterations linked to carcinogenesis have been identified, but the process of breast cancer (BC) from

its origin to invasive neoplasm is unknown. Among risk factors, familiarity involves only 5% of affected patients. Here we present the exceptional case of two young monozygotic twin sisters with no familiar history of BC, simultaneously presenting with BRCA1/2 negative, HR+, Her2- BC, in the same region with identical stage: pT1c/G2/N0. We assessed their tumors and gDNA, as well as that of their parents for mutations in 409 genes involved in cancer using massive parallel sequencing (MPS) to reconstruct the digital karyotype of tumors and evaluate the presence of shared germline recessive inherited aberrations.

Methods: Specimens from bilateral total mastectomy were embedded in OCT medium, frozen and stored at -80°C. Peripheral blood was obtained from both the patients and their parents. Both germline and somatic DNA were subjected to enrichment using the Comprehensive Cancer Panel™ (Thermo Fisher Inc.). Libraries were sequenced on an Ion PGM™ Machine, Alignment was performed with Ion Reporter System™. For variant annotation we used the WANNONAR online tool. For copy number assessment, aligned BAM files were analyzed with the ONCOCNV v6.4 pipeline.

Results: We identified 25 somatic mutations, two of which were shared (FGFR2 var. N728S and MALT1 var. L339V), both with a potentially deleterious effect on the function of the encoded proteins, but unfrequently altered in BC. Surprisingly, the digital karyotypes of the patients were markedly different. Analyses on the gDNA did not pinpoint any described inheritable recessive mutation in the investigated genes. We are now investigating the complete genome of the twins and their parents, due to the biological uniqueness of the case and the potential for cancer driver discovery given by a human biological replicate such as the one presented.

P12.07

Assessment of genomic signatures associated with ageing: a pan cancer analysis

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Background: As life expectation increases, the number of individuals in Western population over age 65 is expected to raise. As cancer incidence increases exponentially with advancing age, patient age is becoming more and more a fundamental prognostic factor, to investigate clinical state and develop personalized therapeutic strategies.

Aims: The aim of the study is to evaluate the biological significance of ageing as prognostic factor, in cancer development. We investigate biology shared by cancer and ageing, identifying a panel of genes, called genomic signature. In our study, the genomic signature defines the relevance of a set of mutated genomic activities, involved in cancerogenesis in elderly patients. The second aim is to infer clinical information from extracted genomic signature, as chromosome instability, cancer aggressiveness and pathway alterations.

Patients and methods: We focused on top killer cancers: BRCA, COAD and LUAD. We collected clinical and genomic data (RNA-Sequencing) from TCGA. Clinical data include age, gender, TNM classification. We considered eligible only those patients with complete clinical and genomic data. Datasets are composed of 1099 patients for BRCA, 459 patients for COAD and 503 patients for LUAD. We performed linear regression model, adopting l1 l2 regularization method in order to avoid clinical interpretability issue produced by high amount of data.

Results: We discovered cytokine-cytokine receptor pathways are always aberrant. These pathways are deeply involved in pro-inflammatory process. In oncogeriatrics, this phenomenon is described as inflammaging, a chronic process deeply bound to ageing. Inflammaging is a highly significant risk factor for both morbidity and mortality in elderly cancer patients.

Conclusions: We identified a genomic signature that characterize cancer in elderly. The results show the alteration of cytokine pathway, which is deeply involved in inflammaging. Our results support age of a cancer patient as fundamental prognostic factor for developing biological therapeutic strategies.

P12.08

Cytochalasin B-induced membrane vesicles derived from SH-SY5Y cells stimulate angiogenesis in vivo and in vitro

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Background: Extracellular vesicles (EVs) mediate intercellular communication by cell-surface signaling and delivering of bioactive molecules (growth factors, cytokines, chemokines, mRNA, miRNAs and siRNA) into recipient cells. EVs retain the biologic activity of parent cells and are perspective instrument of cell-free therapy. Since the yield of natural EVs is limited, we obtained the cytochalasin B-induced membrane vesicles (CIMVs) and investigated their biological activity.

Materials and methods: CIMVs were prepared from SH-SY5Y cells as described by Pick et al. The CIMVs were visualized by scanning and transmission electron microscopy (EM). The biological activity was evaluated by capillary-like tube formation assay *in vitro* and by Matrigel plug angiogenesis assay *in vivo*.

Results: We revealed that more than 95% of CIMV SH-SY5Y were 100-1000 nm in size. Comparative analysis of our data with published data revealed that CIMV are similar in size to the natural microvesicles released from the surface of human cells. The biological activity of CIMVs was comparable with the activity of SH-SY5Y donor cells *in vitro* (41.3 ± 8.5 vs 43.5 ± 3.5 capillary-like network branch points, respectively). CIMVs induced blood capillaries sprouting in Matrigel *in vivo*. It should be noted that CIMVs deliver bioactive molecules and are not capable to division and tumor formation.

Conclusions: We demonstrated that CIMVs retain the properties of donor cells and stimulate angiogenesis similar to the donor cells *in vitro* and *in vivo*. Unlike natural EVs, CIMVs production can be scaled up to industrial quantities with relatively low cost. The fact that CIMVs retain biological activity of parental cells makes them a promising tool for different therapeutic approaches.

P12.09

Biobanking for clinical investigation

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Following the rapid progress in genomics research of humans and their ancestors, biomedical and health research has expanded from the study of rare monogenic diseases to common, multifactorial diseases. A sharper, biology-based definition of disease categories will enhance the development of more effective treatment, reduce undesired side effects of new treatments, improve success

in clinical trial design, and lead to new concepts of disease prevention. Elucidation of complex disease etiology is challenging because diseases are caused by a large number of small, often additive effects, representing the sum of the consequences of genetic predisposition, lifestyle and the environment.

Biological resources, such as cells, tissues or biomolecules are considered as the essential raw material for the advancement of biotechnology, human health, and for research and development in life sciences. The pan-European Biobanking and Biomolecular Resources Research Infrastructure (BBMRI-ERIC) improves the accessibility and interoperability of the existing comprehensive collections, either population-based or clinical-oriented, of biological samples from different (sub) populations of Europe. These collections include data on factors such as health status, nutrition, lifestyle, and environmental exposure of the study subjects. Combined with the expertise of the clinicians, pathologists, bio-informaticians, and molecular biologists involved, a globally unmatched, Europe-wide platform for translational medical research will be described, with the aim to develop personalised medicine and disease prevention. BBMRI ensures sustainable access to key resources required for science-based responses to several of the health-related grand challenges, such as sustainable health care for ageing population, new pandemics, and security threats. Patient communities are also involved, to achieve standards and guidelines that properly balance individual values, with facilitated access to progress in health care and disease prevention.

The need for synergy between the infrastructure and clinical investigation will be highlighted, as biobanking represents a vital component of clinical research for the production of health data.

P12.10 Role of blood marker HE4 (Human Epididymis Protein 4) in gynecological neoplastic diseases and clinical-pathological correlations

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The Human Epididymis Protein 4 (HE4) is a new serum tumor antigen proposed as biomarker for ovarian and endometrial cancer.

We retrospectively reviewed patients referred to the Department of Obstetrics and Gynecology, IRCCS San Martino – IST from February 2015 to 2016 with available preoperative HE4 and CA 125 plasma assay and subsequent histopathological examination.

Among endometrial diseases, we observed 25 cases of malignant tumors (73.53%), 6 cases of benign conditions (17.65%) and 1 case of atypical hyperplasia (2.94%). 22 out of 25 malignancies (64.71%) were FIGO I-II and 3 cases (8.82%) were FIGO III. Among ovarian diseases, we observed 19 cases of malignant tumors (38.78%), 3 cases of borderline tumors (6.12%) and 27 cases of benign conditions (55.10%). 6 malignancies (31.58%) were FIGO I and 13 (68.42%) were FIGO III.

Globally, a highly significant ($P < 0.001$), fairly strong ($\rho = 0.52$) correlation between HE4 and CA 125 was observed.

In endometrial diseases, the correlation appear weaker ($P = 0.029$; $\rho = 0.37$). HE4 was higher in patients with endometrial malignancies compared to those with benign conditions ($138,14 \pm 117,93$ Vs $57,6 \pm 18,67$ pmol/L; $P = 0.02$); no significant differences were observed between FIGO I-II and FIGO III. Similar correlation was not observed for CA 125.

In ovarian diseases, the correlation was highly significant ($P < 0.001$) and very strong ($\rho = 0.68$). Both HE4 and CA 125 were higher in patients with malignancies compared to those with benign conditions ($349,39 \pm 510,44$ Vs $66,44 \pm 48$ pmol/L; $P < 0.001$). Both markers were also higher in FIGO stage III compared to FIGO stage I ($448,65 \pm 595,88$ Vs $134,33 \pm 68,64$ pmol/L; $P = 0.043$).

HE4 seems an effective neoplastic marker increasing in endometrial and ovarian malignancies, with a good correlation with CA 125, stronger in the ovary than in uterus, where CA 125 fails to reliably distinguish between benign and malignant diseases.

P12.11 Correlation between cell-free DNA and survival in patients receiving Nivolumab for advanced non-small cell lung cancer

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Background: Nivolumab is an immune checkpoint inhibitor approved for previously treated advanced non-small cell lung cancer (NSCLC). Liquid biopsy is a non-invasive blood test that detects cell-free DNA (cfDNA) shed from the tumor into the bloodstream. Monitoring cfDNA in patients with NSCLC under treatment with Nivolumab might be helpful to assess efficacy of the therapy and may be related with patients' survival.

Methods: Peripheral blood samples were obtained from 74 patients with pretreated advanced NSCLC at the start of treatment with nivolumab and during subsequent cycles. All the patients underwent CT-scan every 4 cycles and responses were classified according to immune-related Response Criteria. Cell-free DNA was extracted from each plasma sample and quantified. The associations between cfDNA levels and clinical outcomes in terms of response and survival were explored.

Results: Globally, 72 patients were evaluable for cfDNA analysis; 14 experienced early death, 25 progressive disease (PD), nine partial response (PR), 19 stable disease (SD) and five were not evaluable for response. 27 out of the 28 responsive patients (PR+SD) were still alive at the time of analysis. In 25 patients with PD after the first radiological evaluation, median cfDNA < 786 ng/mL was significantly associated with improved median overall survival (OS) as compared to cfDNA ≥ 786 ng/mL (295 vs 96 days respectively, HR = 0.09290, 95% CI 0.019987–0.4322, P -value: 0.0052). Analyzing the OS of the 72 evaluable patients, median survival of those with cfDNA < 786 ng/mL is still undetermined, while it is equal to 181 days for those with cfDNA ≥ 786 ng/mL (HR 0.3559, 95% CI 0.1674–0.75680-1674–0.7568, P -value 0.0035).

Conclusion: Our data show improved survival with Nivolumab for NSCLC patients having cfDNA < 786 ng/mL compared to those with higher cfDNA; the correlation with OS is observed in patients experiencing early PD.

P12.12

Concordance among different response evaluation criteria during treatment with nivolumab For advanced non-small cell lung cancer

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Background: Immune check-point inhibitors have changed the management of advanced non-small cell lung cancer (NSCLC); however, their mechanism of action creates concerns on the most appropriate method to determine radiological responses. Our aim is to compare different evaluation criteria for patients receiving nivolumab for advanced NSCLC.

Methods: Patients with pre-treated advanced NSCLC received nivolumab in a single-institutional translational research study; computed tomography (CT) was performed at baseline and after every 4 administrations. Assessments were performed using Immune-related response criteria (irRC), response evaluation criteria in solid tumors (RECIST 1.1), World Health Organization (WHO), and immune-related RECIST (irRECIST), which are based on the original RECIST with the following differences: 1) new lesions do not automatically define progressive disease (PD), but are added to the target lesions count; 2) PD needs confirmation after 2 additional cycles. The concordance among the different criteria was determined with Cohen's kappa coefficient (K).

Results: Fifty-two patients were eligible. At the first assessment, responses were reported as it follows; RECIST 1.1: partial response (PR) = 7.7%, stable disease (SD) = 36.5%, PD = 55.8%; irRC: P = 5.8%, SD = 44.2%, PD = 50.0%; WHO: PR = 5.8%, SD = 38.5%, PD = 55.8%; irRECIST: PR = 7.6%, SD = 46.2%, PD = 46.2%. The best response was reported as it follows; RECIST 1.1: PR = 17.3%, SD = 26.9%, PD = 55.8%; irRC: PR = 15.4%, SD = 36.5%, PD = 48.1%; WHO: PR = 13.5%, SD = 32.7%, PD = 53.8%; irRECIST: PR = 21.2%, SD = 34.6%, PD = 44.2%.

First evaluation and best response were generally concordant for all the criteria (K range: 0.783-0.839); the concordance between irRECIST and irRC was high (K = 0.828) and RECIST 1.1 had good concordance with irRC (K = 0.734), irRECIST (K = 0.767), and WHO (0.766).

Conclusion: The assessment methods were generally concordant. Since assessment is simpler with irRECIST than irRC, the former might be proposed as an appropriate method of response evaluation.

P12.13

The relevance of CEA and CYFRA21-1 as predictive factors in nivolumab treated advanced non-small cell lung cancer (NSCLC) patients

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Background: CEA, CYFRA21-1 and NSE are tumor markers acknowledged as useful predictors of response to chemotherapy for advanced adenocarcinoma, squamous and small-cell lung cancer, respectively. However, their role in cancer immunotherapy needs to be investigated.

Methods: We treated 74 advanced NSCLC patients with nivolumab (3 mg/kg) every 14 days within a single-institutional translational research study. Blood samples were collected at baseline and at each cycle up to 5 cycles, and then every two cycles. All patients underwent a CT-scan every 4 cycles and responses were classified according to RECIST and Immune-Related Response Criteria (irRC). The serum level of CEA was measured with a Chemiluminescent Microparticle Immunoassay while CYFRA21-1 and NSE with an Immuno Radiometric Assay. The performance of tumor markers in predicting ORR and PFS was analyzed by ROC analysis and a reduction $\geq 20\%$ over baseline was used as cut-off level.

Results: Fifty-one patients were evaluable for the analysis. Significant correlations were observed between CEA and CYFRA21-1 decrease and responses with both criteria: with RECIST, a reduction $\geq 20\%$ of CEA was achieved in 43.5% of responders and in 11.5% of non-responders ($P = 0.021$), while a reduction $\geq 20\%$ of CYFRA21-1 occurred in 62.5% of responders and in 7.7% of non-responders ($P < 0.001$); with irRC, a reduction $\geq 20\%$ of CEA was achieved in 44.4% of responders and in 8.7% of non-responders ($P = 0.010$), while a reduction $\geq 20\%$ of CYFRA21-1 occurred in 53.6% of responders and in 13% of non-responders ($P = 0.003$). A CEA and CYFRA21-1 reduction $\geq 20\%$ constituted a positive prognostic factor for PFS with both response criteria. Multivariate analysis confirmed the ability of CYFRA21-1 reduction $\geq 20\%$ to predict ORR (RECIST, $P = 0.002$; irRC, $P = 0.024$) and PFS (RECIST, $P < 0.001$; irRC, $P = 0.016$).

Conclusion: The reduction in serum level of CEA and CYFRA21-1 might be a reliable biomarker to predict immunotherapy efficacy in NSCLC patients.

P12.14

Endocrinological side-effects of nivolumab in advanced non-small cell lung cancer

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Background: Immune check-point inhibitors (ICPIs) are considered well-tolerated drugs. Previous experiences with

ipilimumab in advanced melanoma have shown possible endocrine toxicities, while less data have been collected about nivolumab. ICPIs act by blocking inhibitory signaling and, therefore, enhancing T-cell activity against tumor cells. This mechanism might result in impaired self-tolerance with subsequent development of immune-related adverse events (irAEs), with particular regard to endocrine toxicities.

Methods: From May 2015 to April 2016, 74 patients with advanced pretreated NSCLC (52 Male, 22 Female, mean age: 64 years) received at least one dose of nivolumab. Blood samples were collected at baseline and at each cycle in order to dose TSH, ACTH, cortisol, Prolactin [PRL], testosterone and autoantibodies (ATG, ATPO and anti-TSH). Thyroid morphology was evaluated by ultrasonography at baseline, eventually repeated if TSH anomaly was observed.

Results: Thyroid function was assessed in all 74 patients. During treatment, 4 patients developed transient thyrotoxicosis evolving to hypothyroidism in 75% of cases. All patients with transient thyrotoxicosis had increased thyroid autoantibodies; 8 patients developed hypothyroidism, with negative thyroid autoimmunity. Adrenocortical axis was evaluable in 55 patients, of which 14 receiving corticosteroids (equivalent of 10 mg/day of prednisone). Among the remaining 31 patients, 7 showed significant cortisol alterations (2 elevated, 5 reduced). Gonadal axis was evaluated in 38 male patients, and no significant change was observed. PRL was assessed in 56 patients; among these, 10 were treated with drugs known to increase PRL levels. 20 patients had at least one elevated PRL value, only 8 showed significantly increased values (3 occurred during therapy).

Conclusion: Thyroid function abnormalities seem the major endocrine adverse event related to nivolumab. With respect to other hormonal axes, further conclusions might be drawn after a longer follow-up, due to the heterogeneity of available results and the presence of interfering factors.

P12.15

Comparison between CT scan evaluation criteria and PERCIST for evaluation of immune check-point inhibitors response

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Background: Immune check-point inhibitors (ICPIs) block inhibitory signaling and consequently enhance T-cell activity against tumor cells; This peculiar mechanism of action presents many difficulties in evaluating clinical response with the usual CT imaging. The aim of this study was to assess the role of fluorodeoxyglucose positron emission tomography (FDG-PET) in supporting clinical decision based on CT scan.

Methods: From May 2015 to April 2016, 74 patients with advanced pretreated non-small cell lung cancer (NSCLC) were treated with nivolumab. Among these, 58 patients were evaluable for response assessment. The patients underwent CT scan and FDG-PET every four cycles and, in case of progressive disease (PD), an additional evaluation was performed after two further cycles in order to confirm it. We evaluated the response to

treatment by CT scan with RECIST 1-1, Immuno-related Response Criteria (irRC), while the metabolic response has been assessed with PERCIST. Finally, we computed the concordance between CT evaluation criteria and PERCIST with Cohen's kappa value.

Results: We observed low concordance of CT scan evaluation criteria to PERCIST. In fact, 46% and 55% of patients, defined in PD with CT evaluation criteria, were defined in stable metabolic disease (SMD) by PERCIST; among these, only 55% of patients considered in PD by RECIST are still alive at 6 months versus 100% of patients considered in SD by RECIST. Finally, patients with PD by CT evaluation criteria, but partial response by PERCIST had a similar overall survival to those achieving partial response with both RECIST and PERCIST (> 9 months).

Conclusion: FDG-PET evaluation by PERCIST could not be helpful when SMD was reported. PERCIST seems to underestimate PD, in fact patients with RECIST PD maintain a poor prognosis compared to RECIST SD. Conversely, PERCIST PMR could be informative, especially when PD is reported by RECIST.

P12.16

Administration of nivolumab beyond early disease progression in advanced non-small cell lung cancer

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Background: The mechanism of action of immune check-point inhibitors implies the possibility to treat patients beyond progressive disease (PD) on the basis of parameters such as clinical benefit or mild progression; however, guidelines for managing early PD during cancer immunotherapy have not been defined yet. Our aim is to evaluate the approaches to patients experiencing early PD during treatment with nivolumab for advanced non-small cell lung cancer (NSCLC).

Methods: Patients receiving nivolumab for advanced NSCLC were considered eligible in case of PD at their first response assessment (after 4 cycles), assessed by response evaluation criteria in solid tumors (RECIST) and immune-related response criteria (irRC). Since irRC require the confirmation of PD after 2 further cycles, a cut-off of 6 cycles was set to define the patients who continued nivolumab beyond progression.

Results: Globally, 31 patients were eligible; 25 patients had early PD with both criteria, while 4 had PD only with RECIST and 2 had PD only with irRC. The decision of continuing nivolumab beyond progression was based on reported clinical benefit, on the observation of a very limited progression, or on discordance between response criteria. With RECIST, 36% of the patients received nivolumab beyond progression; at the time of the analysis, the median overall survival (OS) of these patients was not reached (11.7 + months), compared to 4.3 months for the patients who discontinued nivolumab at PD. With irRC, 33% of the patients received nivolumab beyond progression; at the time of the analysis, the median OS of these patients was not reached (12.2 + months), compared to 4.3 months for the patients who discontinued nivolumab at PD.

Conclusion: Administering nivolumab beyond progression might influence the outcomes of a population of NSCLC patients selected by clinical judgment. Additional parameters for defining which patients will benefit from nivolumab continuation are being investigated.

P12.17

The potential role of serum-based pretreatment tests in patients with non-small cell lung cancer treated with nivolumab

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Background: Anti-PD1 inhibitors are becoming the treatment of choice for 2nd line non-small cell lung cancer (NSCLC). While PDL-1 expression may correlate with benefit from nivolumab, current data do not support its determination. We evaluated the utility of a serum-based pre-treatment test, initially developed to identify patients benefitting from anti-PD1 therapy in metastatic melanoma, in a prospective cohort of patients with NSCLC. These results were compared to the data obtained by applying the established VeriStrat test to the same samples.

Methods: 60 patients treated with nivolumab for advanced NSCLC were included in this prospective study. Pretreatment serum samples were classified using the mass spectrometry-based multivariate tests BDX008 and VeriStrat. BDX008 generates a binary classification of positive (BDX008 +, good outcomes) or negative (BDX008-, poor outcomes), while VeriStrat classifies samples as Good or Poor. The association of test classifications with overall survival (OS), progression-free survival (PFS), and time to treatment failure (TTF) were assessed using Kaplan-Meier method, Cox proportional hazards model, and log-rank *P* values.

Results: 37% of patients were classified as BDX008 + and 63% as BDX008-; 62% were classified as VeriStrat Good and 38% as Poor. Median OS was significantly stratified according to BDX008 status (BDX008 + : not reached; BDX008-: 5.5 months; *P* = 0.0026) and VeriStrat status (Good: not reached; Poor: 4.1 months; *P* = 0.0186), and remained significant in multivariate analyses (*P* = 0.0167 and 0.0184, for BDX008 and VeriStrat, respectively). On the contrary, neither of the two tests was correlated with PFS or TTF.

Conclusions: The serum protein test developed for immunotherapy of patients with melanoma can be applied to pretreatment NSCLC patient samples, and shows a substantial separation for these. Clinical utility of this test needs to be further evaluated. VeriStrat is also prognostic for the same patients; however, its numerical effect on OS separation is smaller than BDX008.

Workshop 13: Rheumatology

P13.01

Improvement of skin blood perfusion and clinical symptoms in Raynaud's phenomenon patients treated with aminaphtone: a six-month study

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Objectives: The aim of this study was to evaluate skin blood perfusion variation and clinical symptom changes during aminaphtone treatment in patients with both primary and secondary Raynaud's phenomenon (RP), during a six-month follow-up.

Methods: Forty-six patients with active RP were enrolled during routine clinical assessment (11 primary RP and 35 secondary RP to systemic sclerosis). Aminaphtone was administered 75 mg twice daily (off label) in addition to current treatments, which remained unmodified during the follow-up. Blood perfusion was measured by laser speckle contrast analysis (LASCA) at the level of fingertips, periungual areas, dorsum and palm of hands, and face, at baseline (T0), after one (T1), four (T4), twelve (T12) and twenty-four (T24) weeks of treatment. Raynaud's condition score (RCS) and both frequency and duration of Raynaud's attacks were assessed at the same time. Forty-six patients with RP (9 primary RP and 37 secondary RP to systemic sclerosis) not treated with aminaphtone were also enrolled as a control group and evaluated at T0 and T24.

Results: A progressive statistically significant increase of blood perfusion was observed from T0 to T12 in all skin areas ($P < 0.001$). From T12 to T24 any further increase of blood perfusion was not observed. The patients reported the improvement of their clinical condition. A progressive statistically significant decrease of RCS ($P < 0.0001$), frequency of Raynaud attacks/day ($P < 0.0001$) and Raynaud duration ($P < 0.0001$) was also recorded from T0 to T12. The results were similar in both primary and secondary RP patients ($P = 0.40$). Aminaphtone administration had to be stopped in 2 patients due to headache, and one patient was lost during follow-up. Any statistically significant variation of blood perfusion was not observed in the control group.

Conclusions: This study demonstrates that aminaphtone treatment increases skin blood perfusion and improves RP symptoms, even in patients affected by systemic sclerosis.

P13.02

Evaluation by laser speckle contrast analysis of peripheral blood perfusion in patients with systemic lupus erythematosus not complaining Raynaud's phenomenon clinical symptoms

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Objectives: Peripheral blood perfusion (BP) is reduced in patients with primary Raynaud's phenomenon (RP). However, reports investigating skin BP in systemic lupus erythematosus (SLE) are scanty. The aim of this study was to investigate peripheral BP in different skin areas of the hands and whole face in patients with SLE without clinical symptoms of RP.

Methods: A total of 14 SLE patients without clinical symptoms of RP (ACR criteria), 14 primary RP patients (LeRoy criteria) and 14 healthy subjects (CNT), of comparable age, were enrolled during the winter period, after informed consent. BP was assessed by laser speckle contrast analysis (LASCA) at the level of fingertips, periungual areas, dorsum and palm of both hands, and face, an recorded as perfusion units (PU). All subjects were not taking vasodilator drugs.

Results: Both SLE and primary RP patients showed a statistically significant lower BP than CNT at the level of fingertips (median 113, 84, 187 PU, respectively; $P < 0.0001$ for both), periungual (median 102, 72, 143 PU, respectively, $P = 0.05$ SLE vs CNT, $P = 0.0002$ PRP vs CNT), dorsal (median 73, 60, 122 PU, respectively, $P = 0.001$ SLE vs CNT, $P = 0.0001$ PRP vs CNT), and palm areas of hands (median 93, 74, 117 PU, respectively, $P < 0.0001$ for both). Conversely, SLE, primary RP and CNT groups showed similar BP values at the level of the face (median 145, 147, 125 PU, respectively, $P = n.s.$). Primary RP patients showed lower BP values than SLE patients in all hand areas (fingertip $P = 0.03$, periungual $P = 0.006$, palm $P = 0.02$, dorsum $P = 0.05$), but not at the level of the face ($P = 0.90$).

Conclusions: This study demonstrates that SLE patients not complaining clinical symptoms of Raynaud's phenomenon show a reduced peripheral BP at the level of the hands when compared with healthy subjects. The clinical value of this new finding is undergoing further analysis.

P13.03

Identification of subclinical skin involvement by two ultrasound transducers with different frequency in limited cutaneous systemic sclerosis patients

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Objectives: The modified Rodnan skin score (mRSS) is the validated method to assess dermal thickness (DT) in systemic

sclerosis (SSc) and to classify patients as affected by either limited cutaneous (lcSSc) or diffuse cutaneous (dcSSc) skin involvement. Also skin high frequency ultrasound (US) is a reproducible technique to measure DT in patients with SSc. The aim of this study was to compare the values of DT obtained by using two ultrasound transducers with different frequency (18 and 22 MHz) in lcSSc patients, in comparison with healthy subjects (CNT).

Methods: Thirty-seven lcSSc patients and 37 sex and age-matched CNT were enrolled. DT was evaluated by both US (Esaote, Genova) and mRSS in all subjects in the usual seventeen areas of the skin (zygoma, fingers, dorsum of hands, forearms, arms, chest, abdomen, thighs, legs, feet).

Results: DT evaluated with the 22 MHz probe was found significantly higher in all body areas in comparison with the 18 MHz transducer, both in lcSSc patients ($P < 0.01$) and in CNT ($P = 0.05$). Of interest, DT evaluated by 18 MHz transducer was recognized significantly higher in lcSSc patients than in CNT ($P < 0.001$) also in four out of six skin areas where the mRSS was found normal (score = 0) (upper-arms, chest and abdomen), with exclusion of thighs ($P = 0.08$), in contrast with the classification of lcSSc. However, by using the 22 MHz transducer a statistically significantly higher median DT was found in all skin areas of lcSSc patients, included thighs ($P < 0.01$). Finally, a positive statistically significant correlation was observed between the two transducers in the evaluation of DT ($P < 0.0001$).

Conclusions: This study suggests that subclinical dermal involvement may be detectable by skin high frequency US already in patients with lcSSc. The employment of US probes with higher frequency, may contribute to avoid the risk of DT underestimation.

P13.04

Modified Rodnan skin score, high frequency skin ultrasound, and plicometer skin test: three methods to assess dermal thickness in systemic sclerosis patients with different capillaroscopic patterns of nailfold microangiopathy

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Objectives: The modified Rodnan skin score (mRss) is the validated method to assess the severity of skin involvement in systemic sclerosis (SSc) patients (1). High frequency skin ultrasound (US) has the capability to quantize the severity of skin damage (2,3), and the plicometer skin test (PST) is a further method to evaluate cutaneous involvement (4). The aim of this study was to identify possible correlations between mRss, US and PST to evaluate dermal thickness (DT) in SSc patients with different capillaroscopic patterns of nailfold microangiopathy.

Methods: Sixty-three SSc patients (mean disease duration 7 ± 6 years, mean age 64 ± 11 years) were enrolled. All subjects were assessed by mRss, US and PST to evaluate the DT in the seventeen skin areas of the body usually evaluated by mRss (zygoma, fingers, hands, dorsum of hands, forearms, arms, chest, abdomen, thighs, legs, feet). Nailfold videocapillaroscopy (NVC) was performed to assess the proper pattern of microangiopathy ("Early", "Active" or "Late") and to calculate the microangiopathy evolution score (MES) (7-8). Statistical evaluation was performed by non-parametric tests.

Results: A positive correlation was observed in SSc patients between the three methods to evaluate DT (PST vs mRss $r = 0.98$, $P < 0.0001$; PST vs US $r = 0.53$, $P < 0.0001$; US vs mRss $r = 0.53$, $P < 0.0001$). All methods demonstrated a progressively higher DT in patients with "Early", vs "Active" and vs "Late" pattern of nailfold microangiopathy ($P < 0.005$), and a positive correlation was also observed with MES ($r = 0.71$ $P < 0.001$).

Conclusions: This study demonstrates a relationship between different methods to assess DT (mRss, US and PST) in SSc patients and a relationship between skin damage extent and nailfold microangiopathy degree.

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P13.05

Presence of abnormal capillary dilations in primary Raynaud phenomenon subjects might anticipate transition to the capillaroscopic "Early" scleroderma pattern: a case control study

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Background: Nailfold Videocapillaroscopy (NVC) is a reliable method for differentiation of Primary (PRP) from Secondary Raynaud Phenomenon (SRP), allowing early diagnosis of systemic sclerosis (SSc) associated "Early" scleroderma pattern [1,2]. Quantitative evaluation by NVC was demonstrated to be of value for capillary parameters analysis [2].

Objective: To investigate on presence and localization of diameter abnormalities (dilations $> 20 \mu\text{m}$), during NVC follow up of PRP subjects, before their transition to SRP.

Methods: 6112 NVC images from 191 patients were analyzed at baseline and after a follow up of 42.77 ± 35.80 months on average. 48 patients affected by SRP and 143 matched controls confirmed with PRP were selected. The diameter of the most dilated limb (arterial, venous, and apical) was measured in 16 images per subject. Statistical analysis was performed using nonparametric tests.

Results: Mean capillary diameter values were significantly different for arterial, venous, and average diameter (mean value of arterial, venous, and apical) between patients with PRP and SRP ($P < 0.0001$). The cited alterations were found to be independent predictors for disease development ($P = 0.015$). A threshold value of $30 \mu\text{m}$ (area under the curve = 0.802, sensitivity/specificity = 0.85/0.63) was identified for average, arterial, and venous diameters, with a shortening effect on time to disease development.

Conclusion: The study showed that capillary diameter is an independent predictor for development of SSc-associated SRP. Progression to SRP is unlikely for subjects affected by RP when average capillary diameter is under $30 \mu\text{m}$. Therefore, we suggest that the execution of the qualitative/quantitative integrated analysis should be part of the NVC follow up of RP subjects.

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P13.06

Long-term integrated treatment with bosentan and iloprost in systemic sclerosis patients: increase of nailfold absolute capillary number, fingertip blood perfusion and improvement of clinical status

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Background: Iloprost (ILO) and bosentan (BOSE) integrated therapy was demonstrated to improve microvascular structure and function in systemic sclerosis (SSc) patients, in two long-term follow up studies [1, 2].

Objective: Since alterations in capillary number seem to predict the incidence of clinical SSc complications [3], we aimed to quantify the effects, in SSc patients, of long-term integrated therapy with BOSE and ILO on absolute capillary number (by nailfold videocapillaroscopy (NVC), fingertip blood perfusion (FBP) by laser Doppler flowmetry (LDF) and laser speckled contrast analysis (LASCA). Results were correlated with clinical outcomes.

Methods: Thirty SSc patients attending the clinic and already receiving intravenous ILO (80 µg/day), for 5 continuous days, every 3 months, were recruited. Fifteen patients continued such treatment (ILO), while in 15 patients BOSE (125 mg twice/day) was added (ILO+BOSE), after onset of pulmonary arterial hypertension or digital ulcers (DUs). The follow-up was of 4 years. Every year NVC, LDF, DUs incidence, diffusing capacity of the lung for carbon monoxide (DLCO), systolic pulmonary arterial pressure (sPAP), renal arterial resistive index (RI) and other biomarkers were evaluated. From T2 to T4 LASCA was further performed. Non-parametric tests were used for statistical analysis.

Results: Limited to the ILO+BOSE group, capillaries absolute number and FBP (LDF and LASCA) showed an independent progressive increase ($P = 0.01$ and $P < 0.0001$, respectively). In addition, during follow up, there was a significant reduction (80%) in the incidence of DUs ($P = 0.002$), whereas DLCO and sPAP did not worsen ($P = 0.46$, $P = 0.8$).

Conclusion: The study shows, in SSc patients, in four years of integrated therapy with ERAs and intravenous prostanoids, a progressive significant improvement in microvasculature structure and function and an organ involvement stabilization, independently from disease severity.

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P13.07

Low serum vitamin D concentrations in systemic sclerosis patients from two European centers: clinical correlations and treatment perspectives

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Background: Immune responses are influenced by vitamin D (25(OH)D) in physiology and pathology. Systemic

sclerosis (SSc) patients show low 25(OH)D serum concentrations.

Objective: To evaluate in SSc, correlations between 25(OH)D serum concentrations and clinical parameters, seasonality and effects of standard oral supplementation.

Methods: 154 SSc patients (131 females and 21 males, age 59 ± 15 years, diffuse 24.7% form and limited form 75.3%) were enrolled, at any time of the year. LIAISON 25-OH vitamin D assay (Diasorin, Italy) was used (< 20 ng/mL = deficiency, 20–30 ng/mL = insufficiency). Pulmonary function test, chest x-ray, lung CT scan, electrocardiography, Doppler echocardiography, renal artery resistive index by eco color Doppler, Dual X-ray absorptiometry, nailfold videocapillaroscopy (NVC), Medsger disease severity scale (DSS), were performed at the time of sample collection. Drug assumption was recorded. Non-parametric tests were used for statistical analysis.

Results: Average 25(OH)D serum concentrations were 18.7 ± 9 ng/mL. A significant difference in 25(OH)D concentrations was observed among seasons (winter: 14.6 ± 7.8 ng/mL, spring: 17.2 ± 7.9 ng/mL, summer 21.43 ± 10 ng/mL, autumn 20.2 ± 10 ; $P = 0.032$). A significant correlation was found between 25(OH)D serum concentrations and presence/absence of bi-basal fibrotic changes at lung CT scan (16.1 ± 8 ng/mL and 20 ± 10 ng/mL respectively, $P = 0.04$). Peripheral vascular ($P = 0.03$), kidney ($P = 0.02$), gastrointestinal ($P = 0.05$) DSS parameters correlated with serum 25(OH)D. No correlation was observed with digital ulcers incidence, strictly related to patterns of microangiopathy observed at NVC ($P < 0.0001$). Interestingly, no influence of treatment with vitamin D analogues (1,000 UI daily) was found: 18.8 ± 10 ng/mL in treated and 18.7 ± 9 ng/mL in not treated patients ($P = 0.81$).

Conclusion: Low serum 25(OH)D concentrations characterize SSc patients, not influenced by treatment. Correlations with lung involvement, peripheral vascular, kidney and gastrointestinal DSS parameters and seasonality are evident. Therefore, supra-physiological oral vitamin D3 doses or programmed UVB light exposure should be considered in SSc.

P13.08

RRI age-related cut-offs better than RRI fixed cut-off describe systemic involvement in systemic sclerosis

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Background: Renal resistive index (RRI) reflects changes in both renal vascular and tubular-interstitial compartments and in systemic vascular compliance related to physiological (age) and pathological conditions among which hypertension, diabetes, hyperuricemia and chronic kidney disease are reported in literature. In systemic sclerosis (SSc), RRI was related with disease duration, creatinine clearance and nailfold-videocapillaroscopy pattern, although tested on small samples. The role of age-related RRI changes in sclerodermic kidney was never investigated.

Methods/Objective: In our SSc population we studied the relationship between the pattern of a fixed age-independent RRI cut-off (0.70) or of SSc-specific age-adjusted pathologic cut-offs (obtained by dividing whole population in quartiles and considering pathologic RRI cut-offs those values above the 75th percentile for each quartiles) with different organ damages.

Results: 190 patients (56 ± 15 years, disease duration 6 ± 8 years, 66 with hypertension, 5 with DM, 13 with hyperuricemia) were enrolled. In the whole SSc population RRI was positively related with age ($P < 0.001$), hypertension ($P < 0.001$), DM ($P = 0.044$) and hyperuricemia ($P = 0.006$). Only renal function, DLCO and skin involvement were significantly related to the 0.70 RRI, used as fixed pathologic cut-off. On the contrary age-related cut-offs were not related with the reduction in renal function, but were significantly associated with SSc fibrotic [interstitial lung disease ($P = 0.015$), tendon friction rubs ($P = 0.032$), skin fibrosis vs no skin involvement ($P < 0.001$), higher mRSS ($P = 0.001$)] and vascular damages [late scleroderma pattern ($P = 0.002$), digital ulcers ($P = 0.006$)]. **Conclusion:** Age-related pathological RRI cut-offs better than a fixed age-independent RRI cut-off identify SSc patients with fibrotic and vascular extra-renal damages.

P13.09

RRI changes as predictor of clinical worsening: experience from a single center

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Background and methods: In systemic sclerosis (SSc) increased renal resistive index (RRI) values correlate with disease duration, lower creatinine clearance, more advanced nailfold-video-capillaroscopy pattern (VCP) and digital ulcers (DU).

In SSc patients we investigated the relationship between RRI pattern at baseline or at follow-up (FU) and the worsening patterns of systemic disease or of skin, peripheral vessels (DU and VCP pattern), pulmonary (chest HRCT or FVC and DLCO decline), cardiac (PAH or ventricular arrhythmias) and renal (scleroderma renal crisis or reduction of GFR ≤ 30 mL/min) organ-damages. Mortality was also registered.

Objectives: To test the prognostic value of baseline RRI values analyzed as absolute fixed cut-off or age-adjusted cut-offs or RRI delta change at 3-6 ± 2-6 years of follow-up (FU) in predicting general organ-specific worsening in SSc patients.

Results: 190 SSc patients (age 56 ± 15 years, 170 women, disease duration 6 ± 8 years) had been enrolled. At follow-up, 89 (46.8%) patients showed systemic worsening; skin, peripheral vascular, cardiac, pulmonary and renal worsening were found in 14(7.4%), 40(21%), 32(16.8%), 38(20%) and 11(5.8%) pts, respectively; 10(5.2%) pts died and 43(22.6%) showed multiple organ worsening. The baseline pattern of RRI values or of > 0.70 cut-off RRI values were not associated with pattern of systemic or organ-specific worsening; baseline SSc- age-adjusted pathologic RRI cut-offs were slightly associated with cardiac worsening ($P = 0.065$). At the opposite a worse Δ RRI at follow-up was associated with systemic ($P = 0.029$) and cardiac worsening ($P = 0.006$) and these associations were highly significant when the delta in RRI values at FU was calculated in comparison with baseline SSc age-adjusted RRI values ($P = 0.017$ and $P < 0.001$, respectively).

Conclusion: the delta change in SSc age-adjusted RRI values at FU rather than RRI values at enrollment should be used as a marker for systemic and cardiac damage in SSc patients.

P13.10

Chondroprotector "Drastop" suppression of inflammatory responses in monosodium iodoacetate-induced osteoarthritis rats

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Background: Osteoarthritis (OA) is characterized by progressive structural-metabolic changes in joint tissues and inflammation. Chondroitin sulfate (CS) is recommended as a therapeutic intervention in the multimodal approach of OA management. But mechanism of action of CS in management of OA is still not clear.

Materials and methods: The studies were performed on 30 white Wistar rats weighing 180–260 g which were divided into 3 groups of 10 animals which. Animals of the 1st group were control. In rats of 2nd group we induced OA with monosodium iodoacetate (MIA) by intra-articular injection. Rats of 3rd group after induced OA were injected chondroprotector "Drastop" (chondroitin sulphate sodium) manufacturing «World Medicine» in therapeutic dose 3 mg/kg every day for 28 days. In blood serum we determined the level of prostaglandin E₂ and concentration of pro- and anti-inflammatory cytokines.

Results: Histological analysis confirmed the decrease of degenerative changes in the joints of rats after 28 days administration of "Drastop". It was established that "Drastop" exerts its anti-inflammatory effect in OA by suppressing of pro-inflammatory cytokines release (interleukin (IL)-1 β by 24 % ($P \leq 0.05$) and tumor necrosis factor- α by 31 % ($P \leq 0.05$)) and by activation of anti-inflammatory cytokine (IL-10 by 22% ($P \leq 0.05$)) release. But concentration of IL-4 and IL-12B p40 didn't change. Also it was established normalization of prostaglandin E₂ level in blood serum.

Conclusions: The evaluation of anti-inflammatory action of "Drastop" in MIA-induced OA rats is associated with a decrease in the intensity of pro-inflammatory cytokines release and by activation of anti-inflammatory cytokine release, normalization synthesis of prostaglandin E₂. Our results were confirmed morphologically.

P13.11

Impact of PGE2 on cholinergic anti-inflammatory pathway; potential for treatment of chronic inflammatory diseases

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Inflammation, the immune response to infection or injury, synchronizes host defense and tissue repair, but also has the capacity to damage the tissues in chronic state when left unresolved. On the other hand, vagus nerve stimulation (VNS) has been shown to be effective against chronic diseases such as depression and epilepsy. Recently, it has been proven to combat chronic inflammation through a pathway, called cholinergic anti-inflammatory pathway (CAP). The CAP is the anchor of neuro-immunoregulation and therefore, VNS is now on clinical trial for rheumatoid arthritis.

Our group has previously shown that another key regulator of inflammation, prostaglandin E₂ (PGE₂) or its synthesizing enzyme, microsomal prostaglandin E synthase-1 (mPGES-1), alters the response of VNS *in vivo*. We aimed to investigate the

underlying mechanism of PGE₂ involvement in connection with acetylcholine and cytokine release, using wild type and mPGES-1 knockout mouse spleen cells.

Single spleen cells from both the mice were isolated, cultured and stimulated by norepinephrine and nicotine in order to mimic the VNS effects *in vitro*. Cholinergic function was then assessed by quantification of acetylcholine (and free choline), expression of acetylcholine producing enzyme and downstream by cytokine measurement. Moreover, T-lymphocytes were sorted and specifically targeted to investigate as well as to compare the same readouts. As expected, anti-inflammatory effects such as increased cholinergic response and reduced cytokine release were observed in wild type cells. In contrast, the knockout cells failed to exert these responses to the same extent.

Our data show that mice deficient in mPGES-1 have an altered cholinergic system, manifested by decreased acetylcholine release in the spleen. Thus, mPGES-1 and consequently PGE₂ might play a protective role in the CAP. However, these data need further confirmation with more sensitive methods. Eventually PGE₂ might lead to potential therapeutic applications to treat chronic inflammatory diseases, especially rheumatoid arthritis.

P13.12

Effects of chondroprotector "Drastop" on oxidative/antioxidative status in serum and cartilage on carrageenan simulation of acute inflammation in hind limb of rats

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Introduction: The emergence of new chondroprotectors "Drastop" (World Medicine) based on chondroitin sulfate requires study of its effectiveness and toxicity. The chondroitin sulfate polysaccharide has a straight no fixed length and molecular weight; different molecular weight can vary bioavailability, cumulative properties, withdrawal.

Method: We developed carrageenan model of inflammation in non-linear white rats by subplantar injection of 0.1 mL of 1% carrageenan solution in right rear limb of animal. The treatment group received therapeutic dose of drug "Drastop" (3 mg / kg, intramuscularly). The control group of rats got an equivalent amount of saline. Histological analysis visualized inflammation of edematous tissue of rats. Determination of biochemical parameters performed on blood serum and cartilage homogenates. We checked content of hydrogen peroxide, products of protein and lipid oxidation, antiradical enzyme activity.

Result: After injection of carrageenan we observed considerable swelling in the rats feet. Swelling of "Drastop" group were less considerable. Histological images showed leukocytes and histiocytes infiltration. Tissues of therapy animals were also swollen, but the leukocyte infiltration was less intense. Carrageenan inflammation caused increasing of hydrogen peroxide and products of protein and lipid oxidation. Inflammation reduces superoxide dismutase activity in serum and cartilage, while catalase activity increases dramatically. Chondroprotector injection decreased level of hydrogen peroxide and products of protein and lipid oxidation. Samples of therapeutic group showed partial return to normal enzymes activity of antiradical system.

Conclusion: Chondroprotector "Drastop" contributes to the restoration of oxidation-antioxidant balance in the blood serum and knee cartilage, indicating anti-inflammatory and antioxidant properties of the investigational drug. Protective efficacy of

"Drastop" requires further research to establish the molecular mechanisms of its effects on the body in the development of inflammation musculoskeletal system.

P13.13

Correlation between microangiopathy damage extent and organ involvement in systemic sclerosis patients with "late" nailfold videocapillaroscopic pattern

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Objectives: To investigate the possible correlations between microangiopathy damage extent and internal organ involvement in systemic sclerosis (SSc) patients with the "Late" nailfold videocapillaroscopy (NVC) pattern at baseline, during a follow-up of 5 years.

Methods: Twenty-three SSc patients with the "Late" capillaroscopic pattern of microangiopathy at baseline were evaluated. The microangiopathy evolution score (MES) and the absolute nailfold capillary number (CN) per linear millimetre were detected by NVC. SSc organ involvement was detected by oesophageal manometry, lung volume tests, diffusing capacity for carbon monoxide (DLCO), lung computed tomography, Doppler echocardiography and renal echography with artery resistive indexes (RI) assessment, history of active or past digital ulcers (DU).

Results: The decrease of CN correlated with worsening of forced vital capacity (FVC) ($r = 1$, $P = 0.02$) and DLCO ($r = 0.9$, $P = 0.03$) values. The decrease of CN correlated with the increase of renal arteries RI values ($r = -0.9$, $P = 0.03$), DU number ($r = -1$, $P = 0.02$), pulmonary arterial pressure values ($r = -0.9$, $P = 0.03$) and MES ($r = -0.8$, $P = 0.05$). At baseline 17 patients (74%) had esophageal dysmotility, 11 (48%) interstitial lung disease (ILD), 9 (39%) DU, 21 (91%) limited cutaneous SSc (lcSSc) and 2 (9%) diffuse cutaneous SSc (dcSSc); no patients had pulmonary arterial hypertension (PAH). After five years of follow-up, 21 patients (91%) displayed esophageal dysmotility, 22 (96%) ILD, 11 (48%) DU, 2 (9%) PAH and 8 (35%) dcSSc. A progressive statistically significant decrease of CN, FVC and DLCO values was observed from T0 to T5 ($P \leq 0.0001$ for all). Also a progressive statistically significant increase of renal artery RI, DU number ($P < 0.0001$ for both) and MES values ($P = 0.01$) was observed from T0 to T5. No statistically significant variation of mean pulmonary arterial pressure was observed ($P = 0.1$).

Conclusions: Nailfold capillary damage extent and progressive reduction of nailfold capillary density are associated with progressive organ involvement in SSc.

P13.14

Role of postpartum long-term microchimerism in rheumatoid arthritis triggering

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Introduction: Microchimerism defines as the presence in the human body less than 1% of all genetically foreign cells. Two-

way exchange of transplacental blood cells and cell-free substances from mother to fetus and vice versa routinely occurs during normal pregnancy. It's been shown that microchimerism can persist for decades and long-term experience of chimeric cells may affect immune system, causing the development of autoimmune diseases. So, the aim of this study to investigate the role of postpartum long-term microchimerism (PLTM) in rheumatoid arthritis (RA) triggering and its severity.

Materials and methods: PLTM related to RA was assessed in women by genotyping the HLA-DRB1 region. We applied a qRT-PCR in early and advanced RA patients ($n = 70$), their children ($N = 96$) and their mothers ($N = 56$), healthy women without autoimmune diseases in family history, their children and their mothers (controls, $N = 54$).

Results: A higher frequency of PLTM was detected among RA patients and their daughters compared to a healthy donors (28.6% vs 12.5%, $P < 0.03$). Also we found that the source of the chimeric alleles was of maternal origin (100% in donors and 62.5-75% in RA patients and their daughters).

In the RA patients with PLTM the earlier disease onset and a more pronounced joints disability on the early stage of the disease were revealed with a tendency to lower the activity of the inflammatory process however there was no difference in the RA severity on the advanced stage of the disease. In the group of the daughters of the RA patients – the carriers of the chimeric alleles – a variety of subclinical articular were more frequent than that in the PLTM negative group, and only PLTM- positive persons developed RA during the observation.

Conclusion: In patients with rheumatoid arthritis, long-term microchimerism detected more frequently than in the population and it can modify the severity of the disease.

P13.15

Evaluation of bone quality using the new trabecular bone score (TBS) tool in rheumatoid arthritis patients

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Background: Patients affected by Rheumatoid Arthritis (RA) show an increased risk of low bone mass, as a result of multi-systemic disorders including toxic drug, low vitamin D levels and physical inactivity. Trabecular Bone Score (TBS), is an index extracted from the dual-energy X-ray absorptiometry (DXA) images, that provides an indirect measurement (Score) of bone axial microarchitecture and allows to get information about bone quality (1,2).

Objectives: The aim of this investigation was to evaluate bone quality in AR patients (high risk population) receiving vitamin D supplementation from at least 3 month, using the TBS.

Methods: 108 female patients (mean age 61 ± 8 years) affected by RA and 60 age- matched controls (CNT) (mean age 64 ± 11 years) were enrolled. Bone Mineral Density (BMD, g/cm^2) of the lumbar spine (L1-L4) was analyzed using a DXA scan (GE, Lunar Prodigy). Lumbar spine TBS (TBS iNsight Med-imp) was derived for each spine DXA examination. All patients were evaluated for serum 25 hydroxyvitamin D (25 (OH)D) concentrations.

Results: 78 RA patients (80%) presented a bone loss that was significantly lower when compared with control group ($P < 0.001$). Likewise, lumbar spine TBS score was found significantly lower in RA patients compared with CNT ($P < 0.001$). Finally, RA patients showed lower 25(OH)D concentrations (18.4 ± 1.3 ng/mL) than CNT (26.2 ± 0.9 ng/mL; $P < 0.04$).

Conclusion: This study shows in RA patients a reduction of TBS values that seem placed side by side with reduced BMD values and 25(OH)D serum concentrations. Therefore, TBS could become a new and safe diagnostic tool for the quantification of the bone quality and related osteoporosis, in chronic systemic inflammatory rheumatic diseases, such as RA.

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P13.16

The complement system in patients with active rheumatoid arthritis and insufficient response to traditional disease-modifying antirheumatic drugs

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The complement system (CS) plays a crucial role in the innate immunity against environmental pathogens, but it is also involved in the adaptive immune response of host.

Recently, it has been shown that its dysregulation may result in an abnormal production of C5a, a potent anaphylatoxin that binds its specific receptor (C5aR) on the membrane of several cell populations, causing many systemic autoimmune diseases.

We decided to explore the role of CS in rheumatoid arthritis (RA), since poor data are available for this specific inflammatory condition.

We enrolled patients with active RA eligible for biological treatment with Abatacept, drug that acts by binding to CD80/CD86 on antigen-presenting cells and preventing the co-stimulatory signals needed for T-cell activation.

We evaluate complement anaphylatoxin C5a plasma levels and the expression of C5a receptor (CD88), CD86 and CD80 on inflammatory cells before and after pharmacological treatment.

At baseline and after three months of biological treatment, C5a plasma levels were higher in RA patients compared to controls.

CD88 was at similar levels both at baseline and after three months of treatment on monocytes of RA patients, but higher compared to healthy donors.

Interestingly, CD86 was found highly expressed on monocytes of RA patients at baseline, showing a drop reaching almost controls' levels after three months of treatment.

These preliminary data suggest that complement anaphylatoxin C5a might have a role in maintaining the inflammatory response in RA patients despite the results on CD86, that give a confirmation on the drug interference in the second signal of T cell activation.

The study is still ongoing to better understand the peculiar role of CS in the modulation of immune cell response.

P13.17

Endothelin-1 promotes a profibrotic alternatively activated macrophage phenotype in circulating human monocytes of systemic sclerosis patients and healthy subjects

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Objective: Alternative activated (M2) macrophages are observed in both blood and damaged tissues of systemic sclerosis (SSc) patients and they contribute to the fibrotic process by the release of profibrotic molecules (i.e. transforming growth factor- β 1, TGF β 1).

Endothelin-1 (ET1) is involved in SSc fibrosis by inducing the transition of endothelial cells and fibroblasts into profibrotic myofibroblasts.

The study investigated the ability of ET-1 to promote the polarization of human monocytes isolated from SSc patients and healthy subjects (HS) into M2 macrophages.

Methods: Human monocytes were isolated from 4 SSc patients and 6 HS, characterized as CD14⁺ cells, and then treated for 6 days with ET1 (100 nM) or treated for 1 hr with ET1 receptor antagonist (ET_{A/B}RA, 10 μ M) before stimulation with ET1. Cultured cells maintained in growth medium were used as untreated cells.

Gene and protein expressions of M2 phenotype markers - mannose receptor (CD206), scavenger receptors (CD204 and CD163), macrophage-derived chemokine CCL22- and TGF β 1 were evaluated by qRT-PCR and Western blotting.

Results: In cultured SSc and HS monocytes, ET1 upregulated the gene and protein expressions of all M2 markers and TGF β 1 compared to untreated cells. This ET1-mediated upregulation was antagonized by ET_{A/B}RA treatment.

Conclusion: ET1 seems to induce the transition of cultured human monocytes into profibrotic M2 macrophages, which might contribute to the fibrotic process. ET_{A/B}RA treatment partially contrasts the ET1 mediated polarization of cultured human macrophages into a profibrotic M2 phenotype.

P13.18

Multicenter, randomized, active-controlled phase 3 pilot study on efficacy and safety of modified release prednisone in patients with polymyalgia rheumatica

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Background: Modified release prednisone (PNMR) formulation adapts glucocorticoid (GC) release to endogenous cortisol rhythms, and contrasts the night inflammation and related morning clinical symptoms. We assessed the efficacy and safety of PNMR compared to PN immediate release (PNIR) in patients with newly diagnosed polymyalgia rheumatica (PMR) previously untreated with GCs.

Methods: Patients meeting the 2012 EULAR/ACR criteria for PMR were randomized to double-blind PNMR or PNIR 15 mg/day for 4 Ws. PNMR/placebo was taken at approximately 10 pm and PNIR/placebo was taken between 5am and 9am. Patients recorded duration of morning stiffness and symptoms of PMR, global pain, shoulder pain and fatigue on visual analog scales, CRP, ESR and IL-6 were measured at study visits. The primary efficacy endpoint was the % of complete responders (CRs, defined as \geq 70% reduction in PMR VAS, duration of morning stiffness and CRP [or CRP < 2 \times ULN]) at W4, and analyzed by a logistic regression model. Non-inferiority was concluded if the lower limit of the 95% CI for the treatment comparison (PNMR vs. PNIR) was above -15%.

Results: The study randomized 62 patients; 66% female, mean age 69 yrs. The % of CRs at W4 was 54% for PNMR and 41% for PNIR (53% and 33%, respectively, in the full analysis population [FAP]). Non-inferiority of PNMR versus IR was not almost proved in the primary analysis on the protocol population ($N = 48$; treatment difference: 12.22% in favour of PNMR; 95% CI: -15.82%, 40.25%) as the lower 95% CI was less than -15%, but sensitivity analysis on the FAP was in favour of PNMR ($N = 62$; treatment difference: 15.56%; 95% CI: -9.16%, 40.28%). PNMR showed a larger efficacy in reducing IL-6 levels.

Conclusion: The results of the study show a beneficial clinical effect of PNMR over PNIR in patients with PMR, with improvements observed as early as W1.

P13.19

Modified-release prednisone as treatment option in moderate activity systemic lupus erythematosus during pregnancy: an implemented case-control study

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Systemic lupus erythematosus (SLE) primarily affects women during childbearing age. Prednisone is a fundamental option in SLE management and is safely used during pregnancy, if maintained at lowest doses (< 7.5 mg daily). Modified-release prednisone (MRP) has the advantage of respecting of the physiological cortisol circadian secretion. Despite FDA approved MRP use during pregnancy, no data are available regarding SLE. The aim of the study was to establish if MRP is effective and safe during SLE pregnancy, as compared to the immediate release prednisone (IRP). We retrospectively enrolled 14 SLE female patients consulting our Center in a 4-years range, all of them experiencing a successful pregnancy. Cases were SLE women taking low-dose MRP (5 to 7.5 mg/daily) at baseline, from at least 6 months. Controls were 14 SLE patients with similar age, diseases duration and prednisone regimen, but in the IR formulation. Subjects age and duration of diseases (months); overall pregnancy outcomes; SLE disease activity (during pregnancy, SLEPDAI) and at baseline+post-partum (SLEDAI); patient's VAS (mm); need of treatment changes throughout pregnancy and at postpartum (%) were assessed. SLEDAI at baseline was 1.5 \pm 0.5 among MRP and 1.4 \pm 0.8 among IR; SLEPDAI, 1.9 \pm 0.9 and 2 \pm 0.6 ($P = ns$). SLEDAI at postpartum was 2.6 \pm 0.8 in MRP and 4.1 \pm 1.4 in IR ($P < 0.05$). VAS (MRP vs IR) was 21 \pm 6 and 40 \pm 9 during pregnancy ($P < 0.05$) and 33 \pm 3 and 49 \pm 9 at postpartum ($P < 0.05$). Rates of treatment changes were 0.07 in MRP 0.5 in IR

($P < 0.001$). The two sub-populations were homogeneous for age and disease duration. Activity (SLEDAI) was significantly higher at postpartum (requiring treatment escalation) in IR patients, in comparison to the MRP. VAS was significantly

higher among IR, both during pregnancy and postpartum. Despite the limited number of subjects, MRP treatment seems to be as safe, but more effective, than the standard IR one, during pregnancy of SLE women.

Workshop 14: Flow Cytometry Advancements in Clinical Investigation

P14.01

Benefits and limitations of extracellular vesicles detection and characterization methods

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Background: Quantifying the amount of extracellular vesicles (EVs) circulating in human body fluids is used for detecting and monitoring different diseases, for example, cancer, myocardial infarction etc. However, there are varying data on the amount of EVs. The reason is that researchers for EVs quantification use methods with different resolutions that leads to detecting only a part of the EVs. We compared the medium size of EVs, determined by the most common methods used for EVs detection and characterization: flow cytometry, dynamic light scattering (DLS), and transmission (TEM) and scanning electron microscopy (SEM).

Materials and methods: We used cytochalasin B treatment of donor cell (HEK293FT) and mechanical action for stimulating of membrane vesicles pinch off (cytochalasin B-induced membrane vesicles - CIMV). The CIMVs were characterized by flow cytometry using FACS Aria III, DLS - ZetasizerNano ZS, TEM - Jeol 1200, SEM - Merlin (Carl Zeiss).

Results: Electron microscopy is a recognized standard for measuring the size of micro- and nanostructures. According to the TEM and SEM, the size of CIMV ranges from < 100-1800 nm with 100-600 nm peak. According to DLS data CIMV size range from 164.2 to 3580 nm, with the peak in the range of 164.2-712.4 nm. Flow cytometry results indicate that CIMV size ranging from > 1340 to 5000 nm.

Conclusion: Electron microscopy is the most reliable method for EVs analysis. The results obtained by TEM and SEM, were taken as a positive control. However electron microscopy is not suitable for clinical application. DLS method has high resolution (0.3 nm – 10 μm). DLS can be recommended as a quick and easy way of counting and characterizing EVs taking into account that DLS detects proteins and EVs aggregates also. Flow cytometry with enhanced FSC detector will increase the resolution of the instrument and accuracy of the EVs quantification.

P14.02

Phenotypic alterations of treg populations in systemic sclerosis

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Systemic sclerosis (SSc) is a connective tissue disease characterized by tissue fibrosis, vasculopathy and autoimmunity. Although the exact pathogenetic mechanisms behind SSc remain to be fully elucidated, a great deal of evidence suggests the existence of an unbalanced ratio between the inflammatory and regulatory arms of the immune system. With regard to the Treg compartment, we observed that both CD4 + and CD8 + Treg

subsets showed quantitative and functional alteration in the peripheral blood of SSc patients. Concerning CD4 + CD25 + highCD127low Treg, we observed a reduced frequency of these cells in the circulation of SSc patients with respect to controls. In addition, CD4 + CD25 + displayed decreased levels of suppressive activity in patients with diffuse and active disease with respect to healthy controls. Also non-antigen specific CD8 + CD28- Treg subset displays functional defects in SSc-affected patients. In vitro generated CD8 + Treg showed significantly reduced suppressive activity in patients affected with SSc. Since CD127 down-modulation and CD39 up-regulation characterize both CD4 + and CD8 + Treg lymphocytes, the phenotypic expression of these molecules was analyzed in SSc patients respect to healthy controls. In vitro generated CD8 + Tregs obtained from SSc patients displayed reduced expression of the CD39 molecule as compared to controls. Moreover, the percentage of CD127 + cells was significantly higher in vitro generated CD8 + Tregs from SSc patients compared to CD8 + Tregs obtained from healthy donors. Concerning CD4 + Tregs, these cells display a significantly reduced expression of the CD39 as compared to healthy controls. Taken together, these findings indicate an impairment of Treg cells in SSc patients. This impairment involves phenotypic abnormalities that are mainly characterized by an altered expression of CD39 and/or CD127 that are essential for the regulatory activity.

P14.03

The GIC-GPMI working group: proposals for the standardization of clinical reports of flow cytometry immunophenotyping

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Background: The Italian Cytometry Society (GIC) in collaboration with the GPMI (Gruppo Policentrico Marcatori Immunologici) has supported among the activities for professional accreditation in flow cytometry a preliminary project for the standardization of clinical flow cytometry reporting.

Aim: To standardize reporting criteria among different flow cytometry laboratories and to obtain a shared standard core template for flow cytometry reporting.

Methods: 41 professionals, belonging to 34 different flow cytometry laboratories recruited from all over the national territory, agreed to join the project. In order to estimate the level of agreement on the core aspects and minimal requirements of the clinical flow cytometry report a questionnaire was prepared. A total of 27 questions, composed by 17 main queries some divided in sub questions, were devised. Four main topics were identified: 1) patient's general information (personal data and patient identification); 2) request specificities (sample characteristics, qualitative and quantitative analysis results); 3) criteria on how to draw the final interpretations; 4) responsibility. Any "YES" answer was given a score of 1 point, while any answer

"DIFFERENT THAN YES" was given 0 points. The degree of agreement was measured by calculating the % of "YES" out of the total answers received.

Results: In 14/27 questions an agreement of $\geq 75\%$ was found; in 6/27 the agreement was $\geq 90\%$; in 7/27 the agreement was between 61% and 75%; in 5/27 was between 40% and 61%; for one of the questions the level of agreement was deemed as not assessable.

The results of this project suggest an initial consensus on what should compose the core template of a clinical flow cytometry immunophenotyping report, although some aspects are still under debate and need further discussion. The proposals emerged from this query should therefore be considered as only preliminary and only in regards of the core template of the clinical report.

Workshop 15: Vaccination and Autoimmune Disease: From Theory to Practice

P15.01

Influenza and pneumococcal vaccinations of patients with systemic lupus erythematosus: current evidences upon safety and immunogenicity

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Objective: Systemic lupus erythematosus (SLE) is a chronic immune-mediated inflammatory multisystem disease. Viral and bacterial infections, such influenza and *Streptococcus pneumoniae* diseases, may favor the exacerbation of the disease, amplify autoimmune processes and contribute to mortality and morbidity. In this narrative review, we aimed to summarize the current recommendation for the use of influenza and pneumococcal vaccinations in SLE patients and to report the evidences upon the efficacy and safety of these vaccines within this group of population.

Methods: A narrative review was conducted upon studies published in the international literature regarding immunogenicity and safety of influenza and pneumococcal vaccines in SLE patients.

Results: Current evidences showed that influenza and pneumococcal vaccinations in SLE patients are recommended to reduce the risk of development of these infections. In particular, these vaccinations are strongly suggested in elderly subjects and in those receiving high dose immunosuppressive treatments. Influenza and pneumococcal vaccines have been demonstrated to be efficacious, even if specific immune responses may be lower than in the general population, as generally the humoral response fulfills the criteria for vaccine immunogenicity. Finally, these vaccines are safe in inactive disease and do not seem to favor SLE flares, although they may favor a transient increase in autoantibody levels.

Conclusions: The current available evidences suggest the need to vaccinate patients with SLE to reduce the risk of the development of influenza and pneumococcal infections. Indeed the risk-to-benefit ratio of vaccinations can be considered favorable for patients with chronic inflammatory autoimmune diseases such as SLE, allowing to limit the burden of influenza and *Streptococcus pneumoniae*-related diseases within this group of patients.

P15.02

A possible role for nerve glial antigen 2 in dendritic cell activation

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In adult central nervous system, nerve/glial-antigen 2 (NG2) is expressed by oligodendrocyte progenitor cells (OPCs) and is

an early marker of pericyte activation in neuroinflammation. NG2 could therefore play a role in experimental autoimmune encephalomyelitis (EAE), a disease associated with increased blood-brain barrier (BBB) permeability, inflammatory infiltrates and demyelination. We show that induction of EAE with myelin oligodendrocyte glycoprotein (MOG) in NG2 knock-out (NG2KO) mice results in milder EAE than in wild-type (WT) mice with less intense neuropathology. In addition to macrophages, we found that NG2 was also expressed in WT mice by most T cells and 40-50% dendritic cells (DCs). To assess the possibility that NG2 could play a role in the immune response in EAE, we induced EAE in bone-marrow chimeric mice, generated with WT recipients of NG2KO bone-marrow cells and vice versa. Regardless of their original phenotype, mice receiving NG2KO bone marrow developed milder EAE than those receiving WT bone marrow. While no inherent defect could be associated with NG2KO T cells, assessment of recall T-cell responses to MOG showed that, while WT and NG2KO T cells proliferated equally, NG2KO T cells were skewed towards a less inflammatory Th2-type response. Analysis of WT and NG2KO MOG-primed lymph node cells for intracellular expression of IL-12 indicated that the proportion of IL-12-expressing DCs was significantly lower in NG2KO mice, and that the proportion of IL-12-expressing cells was significantly lower in CD11c⁺ NG2⁻ cells than in CD11c⁺ NG2⁺ cells in WT mice, suggesting a role for NG2 in DC activation. Our preliminary experiments to understand if the expression of NG2 in DCs is constitutive or induced support this hypothesis, showing a switch of sorted NG2-negative to NG2-positive DCs upon activation. Our data suggest that NG2 plays a role in DC activation and could be an important target of inflammation.

P15.03

Analysis of the genetic characteristics of N-acetyltransferase (NAT) detoxification system in patients with erysipelas

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Aim: To identify associations between NAT2 Gly286Glu (857G/A) gene polymorphism, detoxification of xenobiotics and clinical characteristics of erysipelas.

Materials and methods: The study group comprised of 71 patients with various forms of erysipelas (erythematous 33 (46%) and erythematous-bullous 38 (54%) patients), aged 55 to 78. The disease was significantly higher in women (69%) than men (31%). Primary erysipelas was diagnosed in 38 (53%), recurrent in 33 (47%) patients. The control group comprised of 71 healthy subjects. Venous blood was collected from each

participant, lysed, hydrolyzed with proteinase K, DNA purification performed with phenol/chloroform and precipitated with ethanol. Genotyping of polymorphic loci was performed by PCR. All procedures were carried out in accordance to standard procedure.

Results: Statistical comparison of polymorphic variants of NAT2 Gly286Glu gene (857G/A) in the study and control groups showed that the frequency of NAT2 genotypes in patients with erysipelas was significantly different from the control group ($\chi^2 = 17.32$, $P < 0.05$). The disease occurred in its severe form in 9 (12.2%) patients and moderate form in 65 (87.8%). Erythematous form of erysipelas was diagnosed in 34 (46%) patients and erythematous-bullous form in 40 (54%) patients.

The A-allele caused a higher rate of toxic metabolites formation in patients (OR = 2.702; 95% CI 1.132 to 6.445, $P = 0.0251$) with the GA genotype. A significant difference in recurrence of primary erysipelas (89.5%) in patients of the different genotypes and also in patients with the erythema-bullous form 82.4% ($\chi^2 = 7.016$, $P < 0.05$) was observed. A connection between the genotypes and the development of erysipelas in the women ($P < 0.05$) was established.

Conclusion: The GA genotype is associated with the risk of the primary erysipelas, erythematous form and severity of the infection. Thus, NAT2 Gly286Glu (857G/A) polymorphism may be a risk for erysipelas.

Workshop 16: Hypertension, Uric Acid and Cardiovascular Risk

P16.01

Exercise training and antioxidant supplementation effect on hypertension

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Background: Obesity is increasing its prevalence. Metabolic related disorders are highly correlated with a sedentary lifestyle. Hypertension is a leading cause of cardiovascular mortality. Hypertensive response to exercise (HRE) is caused by endothelial dysfunction and arterial stiffness, also associated with metabolic syndrome.

Objective: We studied how chronic exercise and antioxidant supplementation can change the blood pressure and hypertensive response to exercise in experimentally induced metabolic syndrome in rats.

Methods: 12 groups of 10 wistar male rats were given 3 types of food (standard, high sugar, high fat) for 4 weeks. For each diet, one group was sedentary, one group exercised daily by swimming 1 h, and two other groups (sedentary and trained) were supplemented with coenzyme Q10. Mean arterial pressure (MAP) was measured before and after one bout of exercise (swimming), at the beginning and at the end of the 28 days of the experiment, using a Biopac MP150 system.

Results: Sedentary groups had higher basal MAP and a more frequent and more important HRE. Chronic exercise reduced MAP in high calories diets, while antioxidant supplementation managed to reduce HRE in high fat group. Basal heart rate was reduced by chronic exercise in high calories diet. No significant changes were induced by antioxidant supplementation on heart rate.

Conclusion: Chronic exercise reduces basal MAP, basal heart rate and HRE. Antioxidant supplementation can reduce HRE in high calories diet.

P16.02

Antibodies against HSP60 and cardiac myosin at essential hypertension

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Elevated antibody production may be one of the pathogenetic mechanism of at least some cases of essential hypertension (EH). Foreign or pathogen-derived antigens may trigger an

immune response against "self" peptides of similar homology. Antigenic mimicry between human Hsp60 (hHsp60) and microbial Hsp60 as well as between hHsp60 and myosin is well established.

Aim: Analyze the peculiarities of serum immunoreactivity to Hsp60 and human cardiac myosin at EH.

Materials and methods: We studied 27 patients with EH. Recombinant proteins GroEL *Escherichia coli* and human Hsp60 (hHsp60) and cardiac myosin obtained from heart affected by dilated cardiomyopathy were used as antigens. The donor's sera with low reactivity to the antigens were used as control.

Results: High levels of anti-GroEL, anti-hHsp60 and anti-myosin antibodies were revealed in 62.96% and 55.55% and 18.0% of sera respectively. It was found that sera of patients who had the risk of a stroke by clinical parameters had recognized hHsp60 in Western blotting despite they were anti-hHsp60 negative in 50% cases according to ELISA results. High serum immunoreactivity to hHsp60 observed in Western blotting may be mediated not only by anti-hHsp60 itself but also by the cross-reactivity of anti-GroEL and anti-myosin antibodies presented in sera. Patients bearing the elevated levels of anti-Hsp60 antibodies have had damages in target organs in anamnesis. IgG anti-Hsp60 antibodies affinity purified from patients' sera recognized prokaryotic Hsp60 (GroEL) and hHsp60 and also cardiac myosin by Western blotting. Possible common antigenic determinants of GroEL, hHsp60 and myosin were prognosticated by bioinformatics methods for prediction of linear b-cell epitopes.

Conclusion: Obtained results showed the development of autoimmune processes in patients with hypertension.

P16.03

Engagement of miRNA-1/IGF 1 in AGE-related changes of structure and function of spontaneously hypertensive rat heart

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The miRNA-1 is known as a inhibitor of cardiac hypertrophy and insulin-like growth factor 1 (IGF1) which, in turn, activate hypertension. Left ventricular hypertrophy is an independent risk factor for the development of heart failure. However, the role of such regulatory mechanism in ageing-related or arterial hypertension is not clear. The aim of the study was to evaluate the miRNA-1 and IGF1 expression in model of genetically determined hypertension and ageing.

We used male Wistar rats and SHR both of 6 and 18 months old. In all groups, cardiohemodynamic parameters were monitored with ultra-small catheter 2F ("Millar Instruments", USA). The samples of LV heart tissue were collected and assayed using morphological methods. The levels miRNA-1 and IGF1 expression were estimated by real-time PCR analysis. The decrease

of the following cardiohemodynamic parameters in 18-mo SHR was observed: stroke volume 2 times, ejection fraction 1,8 times, stroke work 5 times, end-diastolic volume on 17 %, end-systolic volume 1,3 times, also increase of arterial stiffness 1,4 times. The morphological changes were revealed: fibrosis compiled 18,1 % in the left ventricle area (comparing with 1,8 % in Wistar rats), however, adaptive angiogenesis, providing myocardial blood supply, was detected. The expression of miRNA-1 was also changed showing the reciprocal regulation with IGF1. SHR demonstrated a lower levels of IGF1 mRNA than Wistar rats that

indicates the presence of inhibitory effect of miRNA-1. In old rats, the decrease of miRNA-1 expression levels was found.

Thus, significant changes in miRNA-1 expression occur in heart due to pressure overloading and senescence. Dynamic of these changes during LV hypertrophy development confirms the presence of phase regulation of IGF1 by miRNA-1 at the translation level. In ageing, reduction of miRNA-1 level and its inhibitory effect on IGF1 may be a factor of the progression of heart failure, especially in SHR.

Workshop 17: New Therapeutic Approaches to Epilepsy

P17.01

Seizure vulnerability linked to astrocytes: a model mouse of deregulated polyamine metabolism

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Standard epilepsy models are unsatisfactory in the search for new antiepileptic drugs; new models are needed to identify molecular mechanisms that contribute to epileptogenesis, to discover new targets for novel drugs which may interfere with the processes underlying epilepsy in susceptible individuals.

Here we report that a mouse model of chronic polyamine catabolism activation (a transgenic mouse conditionally overexpressing spermine oxidase in neocortical neurons; Dach-SMOX mouse) exhibits increased vulnerability to kainate or pentylenetetrazol-induced epileptic seizures. We investigated the mechanisms for increased vulnerability to chemical-induced seizures with electrophysiological, immunocytochemical, biochemical and neurochemical approaches. An *in vitro* model of epileptic-like activity in combined hippocampus-neocortex slices, recorded with a multi-electrode array device, confirmed increased susceptibility to kainate-evoked cortical epileptogenic activity in Dach-SMOX mice, and indicated that it was dependent on astrocyte function. In the cerebral cortex of Dach-SMOX mice we observed reactive astrogliosis. The cerebrocortical astrocyte processes from Dach-SMOX mice overexpressed the cystine/glutamate antiporter system xc⁻, and expressed Ca²⁺-permeable GluA2-lacking AMPA receptors coupled to release of glutamate. The capability of kainate to evoke release of glutamate from the Dach-SMOX astrocyte processes depended on activation of these AMPA receptors. Antioxidant systems including the enzymatic scavenger's superoxide dismutase and catalase, and non-enzymatic factors (metallothioneins), appeared stimulated as sign of oxidative stress condition in Dach-SMOX cerebral cortex.

In conclusion, our findings suggest that in Dach-SMOX mice reactive astrocyte activation, with astrocyte processes

overexpressing xc⁻ system and expressing functional Ca²⁺-permeable AMPA receptors, might contribute to a secondary cascade of glutamate release, which, worsened by increased reactive oxygen species production, could increase brain vulnerability to epileptic seizures and excitotoxic/oxidative insult. This model of chronic dysregulation of glutamatergic transmission in neuron-astrocyte networks could help in the search for models of vulnerability to epileptic seizures, also possibly contributing to understand the processes underlying epilepsy in susceptible individuals.

P17.02

Ictal high frequency oscillations in drug resistant epilepsy: a spectral power analysis

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Interictal and ictal high frequency oscillations (HFOs) have been shown previously to correctly delineate the seizure's onset zone and possibly the epileptogenic zone. Our main hypothesis was that resection of the brain area under subdural contacts with maximum increase in spectral power in the ripples frequency range would correlate with seizure's freedom.

A series of 7 patients with focal seizures who have been diagnosed with epilepsy intractable to medical treatment were included in this study. They underwent implantation of intracranial electrodes and the positions of the electrodes were determined based on the pre-surgical evaluation. Brainstorm was used for registration of postoperative CT with preoperative MRI to obtain the locations of the intracranial electrodes. Ictal epochs were defined as epochs where first electroencephalographic changes were noticed and followed by clinical seizures.

In 4 out of the 7 patients, the contacts with maximum increase (> 50% compared to baseline) in spectral power were included in the resection procedure. The other three patients could not have the area under the contact with maximum increase in power resected either due to inaccessibility or because of incompatibility with motor and language brain mapping results. The follow-up period of the 7 patients was 6 months in average. While the 4 patients who had the contacts with maximum spectral power removed were seizure free, the other three patients were not.

In conclusion, our results show that spectral power analysis of ictal HFOs is useful in identifying the contacts with maximum ictal HFOs activity. The inclusion of areas under these contacts in the resection procedure seems to correlate with better seizure freedom in the short-term.