Workshop 1: Hepatology/Metabolism/Life Style

O1.01
Prevalence and determinants of non-alcoholic fatty liver disease in Lifelines: a large Dutch population cohort

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Background: Non-alcoholic fatty liver disease is an increasing health issue that develops rather unnoticed with obesity, type 2 diabetes mellitus and metabolic syndrome. We investigated prevalence, determinants and associated metabolic abnormalities of non-alcoholic fatty liver disease in the largest population-based cohort to date.
Methods: Biochemical characteristics, type 2 diabetes mellitus and metabolic syndrome were determined in the Lifelines Cohort Study (N = 167,729), a population-based cohort in the North of the Netherlands. Non-alcoholic fatty liver disease was defined as Fatty Liver Index (FLI) $\geq$ 60. Exclusion criteria were age <18 years, immigrants, missing data to assess FLI and metabolic syndrome, excessive alcohol use, previous-diagnosed hepatitis or cirrhosis and non-fasting blood sampling.
Results: Out of 37,496 included participants (median age 44 years, 62·1% female), 8259 (22·0%) had a FLI $\geq$ 60. Individuals with a FLI $\geq$ 60 were more often male, older, obese, had higher levels of hemoglobinA1c, fasting glucose, liver enzymes, total cholesterol, low-density lipoprotein cholesterol, triglycerides, c-reactive protein and leukocytes and lower high-density lipoprotein cholesterol (all $P < 0{\cdot}0001$). Participants with a FLI $\geq$ 60 showed higher prevalence of type 2 diabetes mellitus (9·3% vs. 1·4%), metabolic syndrome (54·2% vs. 6·2%), impaired renal function (20·1% vs. 8·7%) and cardiovascular disease (4·6% vs. 1·6%) (all $P < 0{\cdot}0001$). Multivariable logistic analysis showed that smoking, hemoglobin, leucocytes, c-reactive protein, platelets, alanine aminotransferase, alkaline phosphatase, albumin, impaired renal function (OR 1·27, 95%CI 1·15–1·41), metabolic syndrome (OR 11·89, 95%CI 11·03–12·82) and its individual components hyperglycemia (OR 2·53, 95%CI 2·34–2·72), hypertension (OR 1·89, 95%CI 1·77–2·01) and reduced high-density lipoprotein cholesterol (OR 3·44, 95%CI 3·22–3·68) were independently associated with suspected non-alcoholic fatty liver disease (all $P < 0{\cdot}0001$).
Conclusion: Twenty-two percent of the population in the north of the Netherlands is suspected to suffer from non-alcoholic fatty liver disease, coinciding with an increased risk of type 2 diabetes mellitus, metabolic syndrome, cardiovascular disease and impaired renal function.

O1.02
Lecture: The predictors of fatty liver from birth to adulthood

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In parallel with the epidemic of obesity and associated metabolic conditions, non-alcoholic fatty liver disease (NAFLD) defined as the accumulation of fatty acid content >5% of liver weight has become the leading cause of liver damage worldwide, particularly during childhood. Environmental factors such as excessive intake of calories, processed food and a sedentary lifestyle play a key role in the predisposition to liver fat accumulation, NAFLD and progressive liver disease. In addition, inherited factors are known to contribute to the predisposition to NAFLD. Indeed, in recent years, genetic studies have identified common variants that influence hepatic fat metabolism as important determinants of NAFLD. The most validated genetic risk factors are the PNPLA3 I148M and TM6SF2 E167K mutations, which influence lipid droplet remodeling and the secretion of lipids from hepatocytes and retinol metabolism. More recently, a variant in the MBOAT7 gene has also been shown to contribute to NAFLD by affecting acyl chain remodeling of phosphatidyl inositol. Recent studies also suggest a link between NAFLD and epigenetic modifications. These are defined as stable changes in the expression of DNA unrelated to its base sequence, and can be caused by exposure to environmental risk factors in previous generations or in the intrauterine environment. However, the relative contribution of inherited vs. metabolic risk factors for NAFLD has rarely been assessed at a population level, especially prospectively and starting from early childhood.

O1.03
PNPLA3 (adiponutrin) p.I148M risk allele carriers might be at-risk of chemotherapy-associated steatohepatitis (CASH)

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Background and aim: Patients receiving systemic chemotherapy may develop liver steatosis, which increases the perioperative risks of liver resection. The pathogenesis of chemotherapy-associated steatohepatitis (CASH) is poorly understood. As shown previously, three critical gene variants, i.e. *PNPLA3* p.I148M, *TM6SF2* p.E167K and *MBOAT7* rs641738 are associated with higher hepatic fat contents. Here we investigate the association of these variants with CASH.
Methods: Prospectively we recruited 60 patients (age 31–81 years) scheduled for systemic 5-fluorouracil- or gemcitabine-based chemotherapy (68% palliative) for gastrointestinal cancer. Hepatic fat (controlled attenuation parameter, CAP) and liver stiffness (LSM) were measured by elastography before initiation of chemotherapy (CAP0) and after at least two and four cycles (CAP1 and CAP2). Patients with right lobe liver metastases were excluded. *PNPLA3*, *TM6SF2* and *MBOAT7* variants were genotyped using TaqMan assays.

Results: Included patients displayed following CAP values: CAP0-215·0 ± 55·73 dB/m, CAP1-223·3 ± 53·6 dB/m, CAP2-223·4 ± 56·7 dB/m, consistent with mild steatosis. Initial CAP correlated with BMI ($P < 0·001$) and serum triglycerides ($P = 0·026$). Before initiation of chemotherapy, none of the gene variants was associated with liver steatosis or LSM. Interestingly, at CAP1, carriers of the prosteatotic *PNPLA3* allele [148M] showed significantly ($P = 0·008$) higher CAP (248·9 ± 46·5 dB/m) as compared to patients with wild-type genotypes (209·8 ± 52·2 dB/m). On average, in carriers of the [148M] risk allele liver fat increased by 14·7 ± 23·1% between CAP0 and CAP1, whereas in carriers of the wild-type genotype the increment of liver fat was significantly ($P = 0·034$) lower (2·1 ± 22·6%). This difference disappeared at CAP2. Neither *TM6SF2* nor *MBOAT7* polymorphisms affected liver fat contents.

Conclusions: Individuals carrying the *PNPLA3* p.I148M risk allele might be prone to hepatic fat accumulation during chemotherapy. Our results imply that variant *PNPLA3* might confer more rapid remodelling of hepatic lipids under metabolic stress in this setting. The effects of liver fat deposition during chemotherapy should be investigated further in clinical trials.

O1.04
Influence of body mass index on intestinal permeability (IP): clues for a bimodal colonic absorption pattern

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Background and aim: The significance of IP as marker of intestinal barrier integrity in health and disease has not been fully elucidated yet. IP is measured non-invasively by urinary recovery of sugar probes selectively absorbed at different gastrointestinal levels: sucrose (SO) for stomach-duodenum, lactulose (LA) and mannitol (MA) for small intestine and sucralose (SA) for colon. We examined the effect of age, sex and body size on IP in healthy subjects.

Methods: A 400 mL water solution containing SO 20 g, LA 5 g, MA 1 g, SA 1 g was ingested by 40 fasting healthy volunteers (M:F = 18:22, mean age 42 yr, range 19–75 yrs) and urine collected for 6-hr. Sugar concentrations were measured by triple quadrupole mass spectrometry (Waters TQD) interfaced with HPLC (Waters Acquity UPLC) (AB Analitica, Padua, Italy). According to Body Mass Index (BMI) we studied 24 lean, 12 overweight and 4 obese subjects.

Results: Urinary concentrations of all sugars were similar in males and females and not associated with age. SO, LA, MA urinary recovery were comparable while SA excretion was higher in obese than overweight and lean subjects (2·3 ± 1·4%, mean±SD vs. 0·9 ± 0·4% vs. 0·9 ± 0·4%, respectively, $P = 0·0009$, *ANOVA*). SA excretion and BMI were negatively associated in lean subjects ($P = 0·01$, R-squared 0·24) and positively in overweight/obese subjects ($P = 0·007$, R-squared 0·41).

Conclusions: In healthy subjects IP can vary with body size, probably due to different nutritional requirements. Obese subjects show a leaky colonic barrier, which could negatively influence associated metabolic diseases. Further evaluations are needed in order to clarify pathophysiological aspects of IP in obese patients.

O1.05
Visceral fat as a marker of beneficial effects of hypocaloric mediterranean diet on metabolic patterns in obese subjects

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Background and aim: The expansion of visceral adipose tissue (VAT) is closely associated with metabolic abnormalities and increased risk of several chronic diseases. Increased VAT is also a marker of ectopic fat deposition in the liver and the heart, while decreased VAT can represent a marker of success during dietary plan. We therefore evaluated the correlation between VAT and metabolic patterns in obese subjects undergoing an hypocaloric typical-mediterranean controlled diet.

Methods: Over a 6 mo. period, 87 obese (BMI > 30 kg/m^2) subjects (53 F:34 M, 51 yrs±1·4 SE and 56·4 ± 1·1, respectively) entered an hypocaloric Mediterranean diet. The following measurements were performed at baseline and at the end of the protocol: BMI, waist circumference, bioumoral exams (i.e., insulinemia, total-, LDL- and HDL- cholesterol, triglycerides, fasting glycemia, GPT, uric acid), ultrasonographic measurements of fat (liver steatosis, subcutaneous and visceral fat) by Samsung HS70A equipment.

Results: At baseline M displayed greater metabolic abnormalities than F. Subcutaneous fat positively correlated with BMI while visceral fat correlated with BMI, fasting glycemia, insulinemia, HDL, total-C, triglycerides, and degree of liver steatosis. The 6 mo. hypocaloric mediterranean alimentary program was associated with significant improvement of anthropometric, ultrasonografic and bioumoral outcomes.

Conclusions: Visceral fat is a metabolically active tissue. VAT correlates with the metabolic profile of patients and can be used as marker in evaluating the efficacy of targeted alimentary programs in metabolically abnormal subjects.

O1.06
In vitro models of fatty liver for the study of lipid homeostasis in health and disease

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Background and aim: The liver plays a major metabolic role in controlling glucose and lipid metabolism. In the liver, the excess of triacylglycerols (TGs) are stored in cytosolic lipid droplets (LDs) acting as hubs for lipid metabolism, or secreted into the circulation. Steatosis is a condition of excess hepatic TG accumulation, and is the hallmark of non-alcoholic fatty liver disease (NAFLD), the most common liver disorder world-wide which is frequently associated with obesity, hyperlipidemia and insulin resistance. NAFLD can progress toward more severe liver damages such as steatohepatitis (NASH), fibrosis, cirrhosis, and even hepatocellular carcinoma. The systematic study of pathophysiological and therapeutic aspects of human NAFLD is hindered by the long-term progression, as well as by ethical concerns.

Methods: We recently developed *in vitro* models of simple steatosis (SS) and steatohepatitis (SH) through sequential exposure of cultured rat hepatocytes to oleate/palmitate mixture and inflammatory cytokines, The model allows the study of direct effects of pro- or anti-steatotic compounds, while minimizing the use of animal models.
Results: Steatosis is associated with increased number/size of LDs, modulation of expression/activity of the LD-associated proteins, of the peroxisome proliferator-activated receptors (PPARs), and of the lipolytic enzymes. The steatotic progression from SS to SH impairs lipid metabolism and mitochondrial structure and function, elicits oxidative stress, and induces apoptosis. The utilization of these *in vitro* models has helped screening the effects of both hepatotoxic compounds such as ethanol or xenoestrogens such as Bisphenol A and Tetrabromobisphenol, or therapeutic molecules, such as thyroid hormone derivatives and polyphenolic natural extracts.
Conclusions: We propose that our *in vitro* models resembling the most important features of NAFLD represent a reliable tool to study the regulation of hepatic lipid homeostasis by endogenous and exogenous compounds.

O1.07
The cholecystokinin-1 receptor (CCK-1R) antagonist devazepide increases cholesterol cholelithogenesis in mice

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Background: A defect in gallbladder contraction function plays a key role in the pathogenesis of gallstones. Interest in the potential role of CCK-1R in gallbladder emptying was enhanced by recognition of the important pathogenic relationship between bile stasis and gallstone formation. The CCK-1R antagonists have been extensively investigated for their therapeutic effects on gastrointestinal and metabolic diseases in animal studies and clinical trials. However, it is still unknown whether they have a potential role in gallstone formation.
Methods: To study whether devazepide, a very commonly used CCK-1R antagonists, enhances cholelithogenesis, we investigated cholesterol crystallization, gallstone formation, hepatic lipid secretion, gallbladder emptying function, and intestinal cholesterol absorption in male C57BL/6J mice treated by gavage with devazepide (4 mg/day/kg) or vehicle (as controls) twice per day and fed the lithogenic diet for 21 days.
Results: During 21 days of feeding, oral administration of devazepide significantly accelerated cholesterol crystallization and the growth and agglomeration of solid cholesterol crystals into microlithiasis, with 40% of mice forming gallstones. Whereas, only agglomerated cholesterol monohydrate crystals were found in mice receiving vehicle. Compared to the vehicle group, fasting and postprandial residual gallbladder volumes in response to the high-fat meal were significantly larger in the devazepide group during cholelithogenesis, showing reduced gallbladder emptying and bile stasis. Moreover, devazepide significantly promoted hepatic hypersecretion of biliary cholesterol, but not phospholipids or bile salts. The percentage of intestinal cholesterol absorption was higher in devazepide-treated mice, increasing bioavailability of chylomicron-derived cholesterol in the liver for biliary hypersecretion into bile. These abnormalities induced cholesterol-supersaturated bile and rapid cholesterol crystallization.
Conclusions: The potent CCK-1R antagonist devazepide increases susceptibility to gallstone formation by impairing gallbladder emptying, disrupting biliary cholesterol metabolism, and enhancing intestinal cholesterol absorption in mice. If there is a long-term administration of the CCK-1R antagonists in patients, adequate preventive measures should be taken.

O1.08
Nonalcoholic fatty liver disease and cardiovascular risk

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Nonalcoholic fatty liver disease (NAFLD) is increasingly recognized as a public health problem in the medical community. The pathogenesis of NAFLD is multifactorial, and insulin resistance (IR) plays a pivotal role in its appearance. A lot of experimental and clinical data point to NAFLD as the hepatic expression of the metabolic syndrome (MS). It is plausible that, in visceral obesity, present in the MS, the excess of portal or intra-peritoneal fat promotes the appearance and progression of NAFLD by directly increasing the flux of free fatty acids to the liver. Epidemiological studies in patients with fatty liver have suggested a higher-than-expected cardiovascular mortality. The endothelium has an important role in the atherosclerotic process. According with this, we recently reported that hypertensive patients with NAFLD show a significantly reduced endothelium-dependent vasodilation compared with hypertensives without NAFLD. Moreover, we addressed the question if the evolution in NAFLD might worsen endothelium-dependent vasodilating response in MS hypertensives. We recruited 272 Caucasian newly-diagnosed never-treated hypertensive outpatients divided into three groups according to the presence/absence of MS alone or in combination with NAFLD. MS and NAFLD were defined according to the National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATPIII) and non-invasive fatty liver index, respectively. Vascular function, as forearm blood flow (FBF), was determined through strain-gauge plethysmography after intra-arterial infusion of acetylcholine (ACh) and sodium nitroprusside. MS(+) NAFLD(+) group showed worse metabolic, inflammatory and vascular profiles compared with MS(−) NAFLD(−) and MS(+)NAFLD (−). HOMA resulted in being the strongest predictor of FBF both in the MS(+)NAFLD(−) and in the MS(+)NAFLD(+) groups, accounting for 20·5% and 33·2% of its variation, respectively. In conclusion, we demonstrated that MS+NAFLD+ hypertensives show a worse endothelium-dependent vasodilation compared with MS(+) NAFLD(−), allowing for consideration of NAFLD as an early marker of endothelial dysfunction in hypertensives.

O1.09
Behavior changes in overweight rats

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Background: Obesity is highly correlated with a sedentary lifestyle. Exercise is the strongest weapon against weight gain. Anxiety and depression are associated with metabolic syndrome, more pathways being involved. Emotional status determines behavior changes.

Objective: Our study aimed to find if experimentally induced overweight in rats will determine behavioral changes and, if so, exercise can reduce these changes.
Methods: 60 Wistar rats divided in 6 groups were given 3 types of food (standard diet, high sugar with additional daily dose of 2 mL of glucose syrup = 8 kcal, high fat with additional daily dose of 2 mL of lard gavage = 18 kcal) for 28 days. 3 groups were sedentary (one for each diet type), while 3 performed daily exercise by swimming. Open field test with spontaneous motility measurement (line crossing and rearing) was performed at the beginning and at the end of the experiment.
Results: Weight gain was obtained in high calories diets (highest in sedentary group with high fat diet – average daily weight gain of 2·28 ± 0·87 g). Overweight determined reduced spontaneous motility, as the line crossing and rearing decreased, showing an increased level of anxiety. Exploratory behavior was also lower in overweight rats. Exercise decreased the anxiety and increased spontaneous motility, s the weight gain was lower.
Conclusion: Obesity is associated with increased anxiety and low exploratory behavior. Exercise reduced the behavioral changes related to experimentally overweight in rats.

O1.10
ETN FOIE GRAS – the European network

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In Western Societies, there has been a recent surge of non-alcoholic fatty liver disease (NAFLD). Its progression to non-alcoholic steatohepatitis (NASH) is a leading risk factor for development of Type 2 diabetes, cirrhosis, and hepatocellular carcinoma. The Marie Skłodowska Curie European Training Network (ETN) FOIE GRAS is first in supporting a cohesive and synergistic intersection of complementary and interdisciplinary training skills from academic and non-academic partners. The FOIE GRAS project combines strong scientific expertise with integrated and complementary training in translational research, clinical practice, technology commercialization, and public outreach, the combination of which in targeting NAFLD is lacking in the EU. The industrial partners involved provide experience on technology commercialization alongside scientific contributions while one affiliated patient organization will contribute with important training in societal awareness topics. ESRs training will utilize network-wide workshops and secondments to foster translation of basic research to clinical applications and SME creation. This diverse yet integrated skill set enhances the employment prospects of the trained researchers in both academic and non-academic sectors. Researchers will be endowed with excellent basic scientific knowledge and timely technology transfer know-how for developing novel therapeutic approaches for reversing the burden of NAFLD, thereby advancing both health and economic well-being of European citizens and approaching NAFLD research in the EU from its counterparts in the US and Asia.

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Workshop 2: Mitochondria Biology and Medicine

O2.01
Prevention of hepatic ischemia/reperfusion damage in a surgical context: the key role of preservation of mitochondrial function

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Given that fatty livers are considerably more susceptible to acute stressors, such as ischemia/reperfusion (I/R), and knowing that the incidence of this pathology is deeply increasing, there is an urgent need to find strategies against I/R injury (I/RI) in fatty livers. In this study, we wanted to establish if an acute pre-treatment with Indirubin-3′-oxime (Ind, a GSK-3ß inhibitor) or NAD^+ (a sirtuin cofactor) prevents mitochondrial dysfunction associated with warm I/RI in fatty livers. Zucker fatty rats were subjected to 120 min of 70% warm ischemia and 12 hrs of reperfusion. In the treated groups, Ind or NAD^+ was administered in the hepatic artery 30 min before ischemia. This caused decreased serum markers of liver injury and also preserved mitochondrial cytochrome *c* content, comparatively to I/R livers, while also preventing calcium-induced mitochondrial permeability transition (mPT), the decline in mitochondrial respiratory state 3 and ATP content caused by I/R. The generation of reactive oxygen species (ROS) was also decreased in mitochondria isolated from I/R livers pre-treated with either Ind or NAD^+. Inhibition of GSK-3ß by Ind pre-treatment resulted in the prevention of cyclophilin D (CypD) phosphorylation by GSK-3ß, rendering CypD unable to bind to the adenine nucleotide translocator (ANT), thus preventing mPT induction. Furthermore, deacetylation of CypD at Lys residue causes dissociation from ANT, contributing to an increase in mPT threshold in NAD^+-pre-treated animals. This is due to a stimulation of the activity of mitochondrial SIRT3 that reduces the content in acetylated CypD in mitochondria from control and NAD^+ livers, compared to the I/R group.

O2.02
Mitochondrial toxicology: rescuing mitochondria in Wilson disease avoids acute liver failure

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Our group studies mitochondrial destruction upon diverse aspects of cell-toxic conditions. As an example we will present our latest research results concerning mitochondrial impairments in Wilson disease (WD) and related animal models.

In Wilson disease (WD) functional loss mutations in the *ATP7B* gene cause dramatic liver copper overload leading to acute liver failure. In the $LPP^{-/-}$ rat, an established animal model for WD, massive liver copper accumulation causes hepatitis that rapidly progresses to liver failure and animals' death.

In search of the lethal cause in these animals, we find that the progressive copper accumulation in the hepatocyte's mitochondrial compartment is paralleled by severe mitochondrial structural and functional impairments, similar to alterations observed in WD patients, finally culminating in mitochondrial destruction.

Importantly, reduction of the mitochondrial copper load by Methanobactin, a small peptide with an exceptionally high copper affinity, is associated with the reestablishment of normal mitochondrial structure and function, as well as with liver restoration and the avoidance of acute liver failure.

We thus conclude that depleting the devastating mitochondrial copper burden is a prime requirement for an efficient treatment strategy against acute liver failure in this WD animal model.

O2.03
Inhibition of mitochondrial complex I triggers mitophagy-dependent necroptosis and ferroptosis

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Inhibition of complex I (CI) of the mitochondrial oxidative phosphorylation (OXPHOS) system is considered a therapeutic strategy in cancer. Here we investigated the mechanism by which the CI-inhibitor BAY 87-2243 ("BAY") induces cell death in SK-MEL-28 and G361 ($BRAF^{V600E}$) melanoma cells. CI inhibition increased ROS levels, reduced glutathione levels and stimulated lipid peroxidation and opening of the mitochondrial permeability transition pore (mPTP). BAY-induced cell death was inhibited by antioxidant treatment (a-tocopherol) and upon chemical inhibition of mPTP opening (CsA), mitochondrial fission (Mdivi1), necroptosis (Nec-1, Nec-1s) and ferroptosis (Fer-1). Similarly, overexpression of GPX4 or TRAP1 or knockdown of Drp1, ATG5, PINK1, RIPK1 or MLKL inhibited BAY-induced cell death. Knockdown of GPX4 potentiated the BAY-induced loss of cell viability, whereas caspase inhibition and TRAP1 knockdown did not prevent this parameter. In BAY-treated cells, GXP4 overexpression reduced ROS levels and lipid peroxidation whereas TRAP1 overexpression reduced ROS levels and prevented mPTP opening. Knockdown of ATG5 inhibited BAY-stimulated autophagy and the BAY-induced ROS increase whereas PINK1 knockdown inhibited the BAY-induced ROS increase, DeltaPSI depolarization and mitophagy induction. Our findings suggest that CI inhibition triggers death of $BRAF^{V600E}$ melanoma cells by a mechanism requiring Drp1, GPX4, TRAP1, ATG5, PINK1, RIPK1 and MLKL. We propose a chain-of-events in

which CI inhibition stimulates lethal mitophagy and increases ROS levels, lipid peroxidation, DeltaPSI depolarization and mPTP opening, ultimately leading to combined necroptosis and ferroptosis. This mechanism is ROS-dependent and requires Drp1-mediated mitochondrial fragmentation.

O2.04
The translational potential of measuring mitochondrial health

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Emerging evidence in the last decade supports the view that loss of mitochondrial function, often referred to as "mitochondrial dysfunction" is a key mechanism in the pathological processes leading to many human diseases. Mitochondrial dysfunction has been described in many common diseases including but not limited to cardiovascular disease, diabetes and its complications, cancer, neurodegenerative diseases, septic shock, and drug/environmental induced toxicity. However, it is largely not known whether the loss of mitochondrial function is a cause or a consequence of the pathology in these diseases. In this talk I will: (a) discuss the hypothesis that damage to mitochondria in different cells and organs of the body is an early, clinically detectable event which precedes irreversible cell and organ damage; (b) Describe mechanisms by which early mitochondrial damage could lead to a vicious cycle of systemic inflammation and bioenergetic deficit leading to pathology; (c) Discuss methods which could be used in human populations to monitor mitochondrial health; and (d) describe data utilising methods which have the potential to be extended for use in translation studies to test this hypothesis. If the hypothesis that damage to mitochondria is an early event which drives mitochondrial dysfunction can be substantiated, then early detection could be of great benefit in order to detect risk of disease before cell and organ damage, and to design therapeutic strategies before irreversible damage takes place.

O2.05
Targeting mitochondria with improved dietary antioxidants

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Reactive oxygen species (ROS) formation and oxidative stress are associated with mitochondrial dysfunction and represent a critical initiating event common in most human diseases. Targeting mitochondrial oxidative stress is a very attractive strategy in the management of different pathologies, including chronic diseases or those related with aging. Although a suitable strategy for the management of mitochondrial oxidative stress involves the use of antioxidant supplements, their efficacy is limited, not only because their bioavailability is generally low, but also because there they are not specifically targeted. Because of the non-specific action of traditional antioxidants, and since ROS have recognized physiological properties at several sub-cellular levels, unwanted disturbances of the cellular redox balance may occur. We review here the development of mitochondrial-targeted antioxidants, including MitoQ and SkQ1, and their potential added value when comparing to non-mitochondrial targeted antioxidants. We follow by presenting the development of novel families of dietary antioxidants which are modified to specifically target mitochondria. Dietary phenolic acids such as hydroxycinnamic acids (HCA) are natural regulators of the cellular redox status with pharmacological interest due to their intrinsic antioxidant properties. Despite the different advantages, these molecules suffer the same drawbacks attributed to antioxidants in general. The development of mitochondriotropic HCA antioxidant derivatives can overcome the mentioned drawbacks of classic antioxidants. We demonstrated specific accumulation in mitochondria and protection against lipid peroxidation and general oxidative stress caused in different models systems. Furthermore, some of the new molecules are also inhibitors of the mitochondrial permeability transition pore (MPTP). The new families of mitochondrial-targeted antioxidants based on dietary components are a promising first step in the development of novel drug candidates.

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O2.06
The role of mitochondria in fatty liver graft preservation: an approach to preservation solutions

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Antecedents: A recent report from European Liver Transplantation Register (ELTR) suggested that Institute Georges Lopez solution (IGL-1), is a good alternative vs University Wisconsin (UW) solution, the goal one, for liver transplantation purposes. The role of mitochondrial Glycogen synthase kinase-3b ($GSK3_b$)and voltage-dependent anion channel (VDAC) in livers subjected to orthotopic liver transplantation has not fully investigated, especially when both different UW and IGL-1 preservation solutions were used.

Experimental: Livers rats were preserved in UW and IGL-1 solution enriched or not with trimetazidine (TMZ, an antiischemic drug for the treatment of angina pectoris) respectively and then subjected to OLT. Transaminases (ALT) and HMGB1 protein levels, glutamate dehydrogenase (GLDH) and oxidative stress (MDA) were measured. The AKT protein kinase and their direct substrate, GSK3-β and VDAC as well as caspase 3, 9, and cytochrome c and reticulum endoplasmic stress related protein (GRP78, p-PERK, ATF4 and CHOP) were determined by Western blot.

Results: IGL-1 + TMZ significantly reduced liver injury. We also observed a significant phosphorylation of AKT, which in turn induced the phosphorylation and inhibition of GSK3-β. In addition, TMZ has largely protected the mitochondria since we found a decrease in VDAC phosphorylation and reduced apoptosis and GLDH release in comparison with IGL-1 alone. All these results were correlated with a decreased ER stress.

Conclusion: TMZ addition to IGL-1 solution increased the tolerance of the liver graft against I/R injury through inhibition of GSK3b and VDAC contributing to ER stress diminution and cell death prevention.

O2.07
OXPHOS as a prerequisite for healthy adipocyte

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The goal of our studies is to characterize the role of lipid and mitochondrial metabolism in white adipose tissue (WAT) in relation to obesity and metabolic health. It is well established that WAT is essential for energy storage, while OXPHOS in this tissue normally contributes relatively little to total energy balance. However, in both rodents and humans, oxygen consumption (i.e. the capacity of OXPHOS) in WAT is negatively correlated with obesity and it declines with age. ATP generated by OXPHOS in white adipocytes is required for (i) lipolysis of triglycerides (TAG) and concomitant re-esterification of part of liberated fatty acids (FA) back to TAG (TAG/FA cycling); and (ii) FA synthesis (i.e. *de novo* lipogenesis, DNL). While TAG/FA cycling prevents ectopic accumulation of lipids and lipotoxicity, DNL is linked to formation of novel lipid mediators, as well as to insulin sensitivity.

Deterioration of OXPHOS and lipid metabolism in WAT in obesity could be counteracted by pharmacological and nutritional manipulations that lower inflammation in WAT. Our experiments show that a combined intervention based on omega-3 FA and very mild calorie restriction could result in additive increases of mitochondrial OXPHOS, TAG/FA cycling and anti-inflammatory effects in WAT, which could reduce dietary obesity in mice. Experiments in cold-exposed mice supported the notion that OXPHOS, TAG/FA cycling and DNL in WAT could be essential for hepatic VLDL-TAG formation and consequently for combustion of lipids in non-adipose tissues.

It is difficult to reverse obesity but it might be feasible to reduce adverse consequences of obesity by switching metabolism of white adipocytes, based on nutritional and life-style modifications, which may provide a key to metabolically healthy obesity. White adipocytes endowed with a high activity of OXPHOS, TAG/FA cycling and DNL represent healthy adipocytes that are typical for a metabolically flexible lean phenotype.

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O2.08
MitoK3, a novel mitochondriotropic menadione derivative with cytotoxic effects on cancer cells

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Background: Mitochondria are recognized as important targets for anticancer drugs. Accordingly, the use of a drug, or a combination of drugs, in which one of them targets mitochondrial metabolism in the tumor cell can be looked as a new therapeutic strategy for cancer treatment. The provitamin menadione (vitamin K_3), which contains the 2-methyl-1,4 naphthoquinone structure, was ascribed to have a relevant anticancer activity.
Materials and methods: We designed a mitochondria-targeted vitamin K_3 derivative and studied its mitochondrial targeting and toxicity, including on bioenergetics apparatus, lipid peroxidation and mitochondrial permeability transition pore. We also evaluated its cytotoxicity outline in several cell lines, including human lung adenocarcinoma A549 cells with respect to its effect on cell viability, mitochondrial membrane potential, mitochondrial morphology and caspase-dependent apoptotic induced cell death. We also tested doxorubicin (DOX) and MitoK_3 co-adjuvant treatment, in order to potentiate DOX cytotoxic effects.
Results: We successfully developed a mitochondrial-directed anticancer agent based on menadione (MitoK_3) by conjugating a TPP cation and an aliphatic lipophilic spacer to the C3 position of the naphthoquinone ring. MitoK_3, as opposed to menadione, showed a selective accumulation in mitochondria. MitoK_3 showed toxicity on the mitochondrial bioenergetic apparatus with subsequent loss of mitochondrial ATP production. MitoK_3 was ≈10-fold more selective and cytotoxic toward lung cancer cells than on lung fibroblasts. A combined strategy with currently used anti-cancer agent DOX was shown to be effective, decreasing the death-induction threshold of the latter, triggering apoptotic cell death evident by increased caspase 3/9 activities, probably through mitochondrial destabilization or by interference with mitochondrial redox processes.
Conclusions: MitoK_3 may lead to the development of potent and selective anti-cancer agent to be used in single or combined treatment with DOX or other anti-cancer agents to increase the toxicity of the later, allowing reducing its dose, with minimal non-target effects.

O2.09
miRNAs as biomarkers and regulators of liver disease

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Hepatocellular death, inflammation and fibrosis are key factors in the pathogenesis and progression of liver disease. MicroRNAs (miRNAs) are well-known regulators of disease pathogenesis and have great potential as biomarkers and therapeutic targets. In fact, deregulated miRNA profiles have been associated with disease severity and liver injury. We have recently shown that in the common bile duct ligation murine model of cholestasis, targeting of necroptosis ameliorates hepatic necroinflammation (Afonso et al., Cell Death & Dis 2016). miR-21 is oncogenic, modulates necroptosis, and is upregulated in hepatocellular carcinoma. Curiously, miR-21 ablation ameliorates liver damage, fibrosis and cholestasis in bile duct-ligated mice (Afonso et al., unpublished). miRNAs also participate in non-alcoholic fatty liver disease progression. miR-21 knockout mice display a significant decrease in steatosis severity, cell death and liver damage, inflammation and fibrosis after diet-induced steatohepatitis. miR-21 ablation is also crucial to restore metabolic pathways, and reinstate insulin sensitivity in the liver and muscle (Rodrigues et al., unpublished). Finally, miR-21 is increased in tissue samples as well as in serum of liver disease patients. These results highlight the potential of miRNAs as therapeutic targets and biomarkers in liver disease. (Supported by PTDC/BIM-MEC/0895/2014, FCT, Portugal)

O2.10

Altered metabolism and oxidative stress in skin fibroblasts from Parkinson's disease patients

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Introduction: Parkinson's Disease (PD) is a common neurodegenerative disorder, affecting more than 10 million people worldwide. Currently, PD has no cure and no early diagnostics method exists. Mitochondrial dysfunction seems to be an important component of the disease progression and it has been extensively demonstrated in PD models. To develop a personalized medicine approach to PD, one needs to identify cell proxies that can be used to test interventions aimed at improving mitochondrial function. Our hypothesis is that human fibroblasts may represent a minimally invasive tool to make an accurate diagnosis based on mitochondrial and/or metabolic alterations and to identify new strategies for treatment.

Materials and methods: Human skin fibroblasts from PD patients and their respective matched controls were cultured in high-glucose Dulbecco's-Modified Eagle Medium (DMEM). A metabolic characterization of these cell lines was performed. Cell mass was evaluated by sulforhodamine B (SRB). Cell viability was measured by resazurin assay and complemented with ATP evaluation. Assessment of bioenergetics function was performed by Seahorse XF^{e}96 Extracellular Flux Analyzer and oxidative stress was analyzed using a fluorescent dye. Several mRNA transcripts were evaluated using qPCR.

Results: Our results showed that ATP levels and glycolysis were decreased in human skin fibroblasts of PD patients, while oxidative stress was increased. However, no differences were found in oxygen consumption rate (OCR) or transcripts related with mitochondrial biogenesis, oxidative phosphorylation, mitochondrial dynamics, oxidative stress and autophagy. Metabolic viability, as measured by resorufin fluorescence was not different between groups.

Conclusions: Our data demonstrate metabolic alterations in human skin fibroblasts from PD patients, although global mitochondrial function was not altered. Human fibroblasts may represent a minimally invasive tool to study altered metabolism and interventions in PD.

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O2.11

Single nanomolar treatment with doxorubicin triggers mitochondrial adaptative responses in rat H9c2 cardiomyoblasts

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The dose-dependent and cumulative cardiotoxicity associated with doxorubicin (DOX) anti-cancer activity is a major limitation of the therapy. Our objective was to evaluate persistent effects of sub-lethal concentrations of DOX and determine whether this pre-treatment would induce mitochondrial adaptations. Rat H9c2 cardiomyoblasts were incubated with sub-lethal concentrations of DOX (10 and 25 nM) for 24 hours. Then, media was replaced and cells were kept for nine more days in culture, subcultured twice. After the nine DOX-free days in culture, cell viability, cellular morphology, cell cycle, extracellular acidification and oxygen consumption rates, and gene expression patterns were analyzed using standard methods. Proliferation rate analysis of H9c2 cells treated with 10 and 25 nM DOX showed a recovery of cell mass 9 days post-treatment. DOX treatment led to cellular and nuclear hypertrophy in H9c2 cells treated with 25 nM DOX and induced dose-dependent cell cycle arrest in G2/M. No significant changes were observed on mitochondrial membrane potential and despite a decrease in basal respiration, 25 nM DOX did not alter H9c2 spare respiratory capacity. However, glycolytic capacity and reserve were significantly decreased after DOX treatment. Transcriptionally, both DOX concentrations induced a persistent increase in mitochondrial-encoded genes, including *ND1*, *CytB*, *Co1* and *ATP8*, but those alterations were not related with changes in mitochondrial DNA copy number or with *tfam* and *polg* expression. In turn, *dnmt1* mRNA was down-regulated and protein levels were also decreased in 25 nM DOX-treated cells, suggesting the involvement of DNA methylation on the regulation of mitochondrial-encoded genes expression. Collectively, the results suggest a persistent and pre-conditioning response of H9c2 mitochondrial function to DOX nanomolar treatments, which may be further explored as a new approach for protecting progenitor cells during anticancer therapies.

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O2.12

Cytokines and SNPs in superoxide dismutase and catalase identified as markers for recurrent erysipelas infection: a case report

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A female patient aged 66 was admitted with body temperature of 38·5°C, malaise and headache. The surface of her left arm had extensive area of erythema with irregular borders and apparent swelling of the left lower leg which was moderately tender to palpation. She was diagnosed with recurrent erythematous erysipelas of the left tibia. Her anamnesis showed an erysipelas diagnosis in 2004, a recurrence 2008, and twice each year till 2016. Concomitant diseases: chronic lymphovenous insufficiency of the lower limbs and hypertension. Treatment was with antibiotics (penicillin, ceftriaxone, ciprofloxacin). Despite the systemic treatment, we observed frequently recurrence of the disease.

After her informed consent we conducted a genetic study of her SOD1g7958a, SOD2ala16Val and CATc-262t SNPs. The effects of these mutations on erysipelas had been earlier described by Emene et al. (2015) with g-allele, the val-allele and c-allele of the respective SNPs having a predisposition increasing the risk of erysipelas. The subject had all three alleles which increased the predisposition to erysipelas. We also analyzed 44 cytokines. The observed cytokine values in the acute and convalescent stages of the infection were compared to other forty-nine erysipelas patients.

Our cytokine analysis showed that IL-1β, IL-2Rα, TRAIL, IL-17, VEGFr, IL-2, IL-3, IL12 (p40) and IL-18 were higher in the subject in the acute and convalescence phase when compared to the average values of other patients. We suggest these cytokines as markers of reccurence of erysipelas. The values of CCL11, CXCL9, IL-7, IL-8, PDGF-BB, CCL4, IL-9, IL-10, IL-13 and G-CSF were higher in the subject in the acute phase than the average value in other patients but reduced to values identical to the average value of other patients during the convalescent phase and the other way round in GM-CSF and IFN-α. We suggest these cytokines as markers for the clinical symptoms of erysipelas.

O2.13

Overweight induces structural integrity damage in skeletal muscle displayed by an energy metabolism switch

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Although aging is characterized by the gradual decline in skeletal muscle mass and quality, it is increasingly clear that fat infiltration, particularly in the intramuscular and visceral compartments, play an important role in this decline. Skeletal muscle is a highly plasticity tissue that is able to response to various stimuli, including nutritional states. It is well known that aging attenuates metabolic plasticity, which exacerbates damaging effects of some chronic diseases, such as obesity. Therefore, the characterization of cellular energy homeostasis and mitochondrial profile in skeletal muscle from overweight aged population holds promise to find which are the main pathways affected in an early stage of obesity. The participants were randomly selected from the HIPA study cohort (hip fracture in elderly patients undergoing surgery in the region of Asturias (Spain)). Individuals older than 70 years (84 ± 6 years) were classified in two balance groups according to the World Health Organization body mass index (BMI): 21 normal-weight (22·4 ± 1·9) and 18 overweight (28·0 ± 1·7). The study complied with the Declaration of Helsinki. Overweight aged patients presented defective phosphagen, glycolysis and oxidative phosphorylation metabolic pathways. As a strategy to maintain ATP levels under mitochondrial dysfunction, overweight subjects exhibited a switch from oxidative to lactic acid fermentation metabolism, but without reaching aging basal levels. Moreover, lower levels of filamin C further evidence a decline in structural integrity of the muscle fibers. These findings suggest that mitochondrial dysfunction could be critical in the anticipation of the onset of sarcopenia. Exploiting mitochondrial profiles could be crucial to diagnose and face both overweight and obesity.

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O2.14

Promising new catechol O-methyltransferase (COMT) inhibitors for Parkinson's disease display lower mitochondrial toxicity

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Background: Over the last 50 years, clinical management of Parkinson's Disease has been based on a combination of dopamine agonists, carbidopa-levodopa, and MAO-B and COMT inhibitors. Nevertheless, these drugs provide only symptomatic relief and are unable to halt disease progression. Moreover, mitochondrial toxicity caused by tolcapone, the only COMT inhibitor acting in the central nervous system, is a major safety issue. Due to the mitochondrial central role in cellular energy production, calcium and redox balance, as well as cell death control, the mitochondrial toxicity must be evaluated when developing new drug candidates.
Objectives: Our objectives were: 1) to demonstrate that tolcapone displays mitochondrial toxicity and 2) to develop novel COMT inhibitors without mitochondrial liabilities and improved safety over the currently available drugs.
Methodology: We developed nitrocatechol-based derivatives of caffeic acid and caffeic acid phenethyl ester, with a pharmacological profile similar to tolcapone and entacapone. Using the Seahorse Extracellular flux-analyzer, mitochondrial and glycolytic function in human hepatoma HepG2 cells were measured with both the new and standard COMT inhibitors.
Results: The data obtained suggests that the new compounds displayed lower *in vitro* mitochondrial toxicity, observed by a lower decrease in maximal respiration and spare respiratory capacity when compared to tolcapone.
Conclusions: We suggest that the toxic effect of tolcapone on HepG2 cells may be due to an inhibition of mitochondrial respiration, possible through multiple mechanisms.
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[Correction added on 10 May 2017, after first print publication in May 2017 and corrected in the online version: Presenting author of abstract O2.14, V.A. Sardão was changed to R.F. Simões].

O2.15

Exposure of human bronchial epithelial cells to the lung carcinogen hexavalent chromium confers resistance to thermal shock and interferes with the stress response

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Although it has long been established by international regulatory agencies that, as encountered in certain industries, hexavalent chromium [Cr(VI)] compounds are carcinogenic to humans, the molecular basis underlying Cr(VI)-induced neoplastic transformation remains elusive. Nevertheless, it is generally accepted that Cr(VI) is an inducer of genotoxic and proteotoxic stress, and that metabolic and oxidative stresses likely constitute additional major stressors. As these stresses are expected to activate the stress response, it is conceivable that cells that survive Cr(VI) exposure become more resistant to further stresses, namely those encountered during neoplastic transformation.

In this study, we used acute thermal shock as a model of stress to investigate whether Cr(VI)-exposed cells were more resistant to stress than their non-exposed counterparts. In agreement with our initial hypothesis, the results obtained show that a 48 h exposure of human bronchial epithelial cells (BEAS-2B cells) to 1 μM Cr(VI) attenuates the anti-proliferative effects of both cold and heat shock. Interestingly, we also observed that the doubling time of the Cr(VI)-exposed cultures were lower than those of their non-exposed counterparts.

To gain mechanistic insight, we determined the effects of Cr (VI) on the expression of heat shock proteins 72 (Hsp72) and 90 alpha (Hsp90α) and heat shock factor 1 (HSF1), three components of the stress response with a critical role in carcinogenesis. The results show that Cr(VI) elicits a significant depletion in the mRNA levels of Hsp72 and in the protein levels of Hsp90α. These changes confirm that Cr(VI) interferes with the stress response but, by themselves, do not support a canonical induction of the response. Future experiments aimed at improving our understanding of the effect of Cr(VI) on the stress response pathway will shed light on the mechanism of Cr(VI)-induced lung cancer, potentially leading to new therapies.

O2.16

Characterisation of the mitochondrial bioenergetic, ROS and status of antioxidant defence system profiles in fibroblasts of patients showing mitochondrial abnormalities

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Very often defects in mitochondrial function are associated with several pathologies, which in turn can lead to various diseases. Improper function of the mitochondrial respiratory chain can lead to decreased ATP production, what is often considered as a main cause of the observed symptoms. However, growing evidence has suggested a direct relationship between development and progression of mitochondrial disorders and the presence of oxidative stress. Increased ROS production may lead to oxidation of DNA, lipids and proteins and thus can affect fundamental cellular processes.

The aim of our studies is complex characterisation of mitochondrial respiratory chain function and the related parameters responsible for or involved in mitochondrial defect-mediated cellular dysfunction. Our studies address mitochondrial bioenergetics and oxidative stress to elucidate how these parameters participate in the pathogenesis of different mitochondrial disorders. We want to determine which mitochondrial parameters are significant contributors to the development of mitochondrial disorder.

We characterised mitochondrial bioenergetic parameters, ROS production, and status of antioxidant defence system to generate unique multifactorial profiles of fibroblasts from controls and patients with different mutations in mtDNA (*MTND1, MTND3, MTND5 and MTATP6*) and nDNA (*SURF1; SCO2, DGUOK, PDHA1* as well as in the huntingtin gene). We obtained the unique profile of the parameters that are characteristic for healthy and patients' fibroblasts and describe detected similarities, differences and dependencies between measured parameters. Anomalies in the bioenergetic parameters, modification of the antioxidant enzymes levels as well as enhancement of intracellular ROS confirmed the occurrence of the oxidative stress in the studied fibroblasts.

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O2.17

Tracking the multiple fates of acetyl-CoA in the liver using stable isotope tracers

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In many tissues including the liver, acetyl-CoA represents the crossroads of oxidative energy metabolism and biosynthesis of endogenous lipids and other metabolites. It is therefore a crucial intermediate for observing carbon flux from ^{13}C or ^{14}C-labeled substrates and determining if these carbons will be oxidized for energy by the Krebs cycle or incorporated into biosynthetic pathways such as de-novo lipogenesis. Direct analysis of acetyl-CoA is challenging because its intracellular concentration is low, while it is also chemically labile and difficult to isolate using standard biochemical methods. Moreover, its intracellular location can determine its metabolic fate. For example, acetyl-CoA located in the mitochondrion is destined for incorporation into the Krebs cycle or ketone body synthesis, while cytosolic acetyl-CoA may be recruited for de-novo lipogenesis. Since these subcellular pools are homogenized and their identities lost when acetyl-CoA is isolated by classical destructive biochemical methods, noninvasive approaches of selectively identifying and/or sampling them need to be developed. In this lecture, the concept of selective acetyl-CoA sampling by "chemical biopsy" will be introduced and exemplified by new data from animal model studies. In addition, a novel approach for identifying acetyl-CoA that was generated from ^{13}C-glucose and subsequently recruited for de-novo lipogenesis will be illustrated with newly obtained ^{13}C NMR data from animal models.

O2.18
Monoamine oxidases in cardiovascular pathophysiology

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Oxidative stress can be generated at several sites within the mitochondria. Among these, monoamine oxidases (MAO) have been described as a prominent source. MAO are mitochondrial flavoenzymes responsible for the oxidative deamination of catecholamines, serotonin and biogenic amines, and during this process they generate H_2O_2 and aldehyde intermediates.

The role of MAO in cardiovascular pathophysiology has only recently gathered attention, since it has been demonstrated that both H_2O_2 and aldehydes may target mitochondrial function and consequently affect function and viability of the myocardium. Accordingly, MAO inhibition has been shown to afford protection against acute and chronic injury of the heart under conditions, such as ischemia/reperfusion, pressure overload and diabetes. The protective effects elicited by MAO inhibition have been extended also to muscular dystrophy.

Besides summarizing previous work I will discuss novel findings on (i) substrates utilized by MAO under pathological conditions; (ii) the role of MAO-induced ROS formation in ER stress; (iii) the activity of MAO in non-myocytes cells of the heart, such as macrophages and mast cells; (iv) the role of MAO in cardiomyocytes obtained by IPS. The data will be discussed highlighting potential physiological aspects of MAO in cell differentiation and maturation.

O2.19
The association of mitochondrial haplotypes with rheumatoid arthritis severity

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Introduction: A huge amount of studies investigates the role of nuclear genes polymorphism in rheumatoid arthritis (RA) predisposition. However, we also complicated with genetic information encoded by mitochondrial DNA (mtDNA), that heritage only by maternal line and the association of the certain mitochondrial haplotypes with several diseases (cardiovascular diseases, cancer etc.) was widely observed. It's well-known that RA 2-3 times more frequently occurs in female rather men. So, the aim of this study was to identify the association of mtDNA haplotypes with RA and its severity.

Materials and methods: mtDNA haplogroups were determined in 73 RA patient and 30 healthy donors by sequencing the D-loop region (15898-16400 bp according to CRS) and analyzing with online program (http://dna.jameslick.com/mthap/advanced.php). The study included cohorts of RA patients and healthy women being under our long-term (more than 10 years) clinical and laboratory surveillance. Clinical and laboratory parameters of RA activity (DAS28-ESR, HAQ, RF, antiCCP, ESR, CRB) were annually fixed. Statistical significance was considered at $P < 0{\cdot}05$.

Results: Almost all of the identified mtDNA haplogroups were of the European origin (H, U, J, K) and no significant difference between studied groups was observed. Haplogroup K carriers were significantly less susceptible to the trivial infections in the RA onset when compared to H, J, U haplotypes. In early RA as well as in the advanced RA stage patients with haplogroup K had significantly lower meanings of DAS28-ESR and HAQ compared to the J haplogroup carriers. What is more, in the K subgroup the percentages of RF- and antiCCP+ positive cases were significantly lower than that in the other subgroups. In the H haplotype subgroup all the RA parameters had intermediate values.

Conclusion: Thus, although the presented results are preliminary, our findings demonstrate an impact of the certain mitochondrial haplotypes on the RA severity.

O2.20
Mitochondria-targeted therapy: challenges and hurdles of drug discovery process

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The recognition of mitochondria as gatekeepers of cell life and death, as well as the perception that mitochondrial impairment underlies a diversity of pathological states, including rare inherited mitochondrial disorders, cancer, diabetes, and age-related diseases, have intensified the number of drug discovery programs. Even though the modulation of mitochondrial function is an attractive therapeutic strategy for different pathologies the development of mitochondrial-targeted drugs has been hampered by a number of challenges, with not approved therapies at the present. The clinical mismatches can be ascribed to limitations of the animal models used in pre-clinical phase, to pitfalls in clinical trial design and to druggability and drug-likeness setbacks.

The discovery of mitochondrial drug candidates is well-thought-out to be a drug discovery bottleneck as it requires at the same time target-specific affinity, i.e. drug delivery to mitochondria, and a particular safe window, related to mitochondrial toxicity. In compliance, mitochondrial drugs have strictly efficacy dosage requirements. One way to surpass the problem is the modulation of physicochemical properties (ionisation, lipophilicity, hydrogen bonding, redox potential, and others) in early drug discovery processes. The concept of drug-likeness helps to optimise pharmacokinetic and pharmaceutical properties, including solubility, chemical stability, bioavailability and distribution. Within this framework, the knowledge of the physicochemical properties of the intracellular environment is recognized to be of utmost importance.

This is certainly a daunting challenge but given the current unmet medical needs, and the possible gains, such a venture is worthwhile. Yet, the collaboration among all the stakeholders, such as research foundations, academic institutions, physicians, and pharmaceutical industry, are paramount for the success. Drug discovery

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O2.21
The gut microbiota and metabolic disorders. Will mitochondria ever be involved?

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Background and aim: The intestinal microbiota plays a key role in human health and disease, by regulating the host immune

response and energetic metabolism. The subtle and complex interplay between resident microbiota, intestinal cells and immune system involve also the modulation of mitochondrial activities.

Results: Mitochondria and bacterial members of microbiota display common features because of a probable prokaryotic origin of mitochondria. Moreover, microbiota shows a different quality and diversity in relation to mitochondrial function. The reactive oxygen species (ROS) modulate the innate immune system and inflammatory process, and are involved in the regulation of the gut epithelial barrier mediated by intestinal microbiota.

On the other hand, microbiota produces metabolites that can act on the mitochondrial respiratory chain and ATP production. While the microbial products H2S and NO inhibit the mitochondrial host respiratory chain, SCFAs positively regulate mitochondrial activities. Lastly, the presence of intestinal dysbiosis, contamination and "leaky gut", may increase the portal flow of toxic endogenous products (i.e. ethanol, LPS) which will initiate/perpetuate/worsen the metabolic hepatosteatosis/steatohepatitis, with a key role for ROS and mitochondria.

Conclusions: A strong crosstalk is present between microbiota and mitochondria, and affects the host health.

O2.22
Electron transport chain-derived reactive oxygen species in cell death

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Functional electron transport chain (ETC) is essential for initiation and progression of tumorigenesis, and ETC targeting emerges as an effective anti-cancer strategy in multiple experimental models. This is particularly relevant for cancer subtypes with enhanced ETC supercomplex assembly, and ETC-derived reactive oxygen species (ROS) play a pivotal role in this phenomenon. To understand the molecular reasons for the sensitivity to ETC blockade and the selectivity to cancer cells, we deployed a panel of ETC inhibitors in proliferating and quiescent non-transformed cells. We found that the proliferative status significantly impacts on the susceptibility to ETC inhibition. Despite upregulation of ETC activity and higher ETC supercomplex assembly in quiescent cells, these cells were protected from cell death after the ETC blockade when glucose was not limiting. This corresponded to lower ROS production in quiescent cells, which was mediated by the enhanced antioxidant defence. Functional interference with antioxidant defence sensitized to ETC-derived ROS and increased cell death. In contrast, under limiting glucose conditions, cell death was induced preferentially in quiescent cells and was correlated with intracellular ATP depletion but not with ROS. The antioxidant defence could no longer protect the quiescent cells from cell death. Hence, in quiescent cells in ample nutrient supply (the situation in healthy perfused tissues) the antioxidant defence effectively restricts ETC-blockade-induced ROS and cell death explaining specificity, while in conditions of limiting nutrients (such as in poorly perfused areas of tumors) the cells are eliminated upon ETC inhibition even in the quiescent state due to the acutely induced bioenergetics stress. This mechanism may account for some of the specificity of ETC inhibition in cancer.

O2.23
Hepatic lipotoxicity and mitochondrial dysfunction

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Mitochondrial dysfunction is often described in metabolic diseases like type 2 diabetes (T2DM) and non-alcoholic fatty liver disease (NAFLD). Among the possible causes there are the lipid and lipoprotein abnormalities that often occur in these patients. These patients often show increased plasma triglyceride (TG) concentrations, due to increased hepatic secretion and impaired clearance of VLDL, high LDL and low HDL plasma concentrations. Moreover, they are insulin resistant not only at the level of muscle and liver but also of the adipose tissue. Despite high plasma insulin levels lipolysis is not controlled resulting in abnormally high free fatty acid (FFA) release into the circulation. Circulating FFA and TG in excess tend to accumulate in non-adipose tissues or to produce lipid metabolites such as long-chain fatty acyl-CoA, diacylglycerol, and ceramides that are known to stimulate inflammatory pathways contributing to cell dysfunction and death. This phenomenon is known as lipotoxicity and in liver is associated with steatosis that can progress to non-alcoholic steatohepatitis (NASH), in pancreas with impaired insulin secretion and β cell dysfunction, in heart with cardiomyopathy and an increased risk of cardiac dysfunction, in skeletal muscle with insulin resistance and impaired glucose uptake.

Lipotoxicity triggers negative effects on multiple cellular processes including impaired insulin signaling, inflammation and mitochondrial function that is associated with reduced FFA oxidation, decreased muscle ATP synthesis, and increased oxidative stress. In hepatocytes, the increased production of reactive oxygen species (ROS) and lipid peroxidation products further impair the respiratory chain, either directly or indirectly, through oxidative damage to the mitochondrial genome. Moreover, this can accelerate the progression of NAFLD to NASH and liver cirrhosis.

O2.24
When, where and how: mitochondrial multifaceted dysfunction in Alzheimer's disease

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Alzheimer's disease (AD) is the most common age-related neurodegenerative disease with a progressive course involving synaptic dysfunction and neuronal damage in specific brain regions and circuits associated to memory and language, such as the hippocampus and brain cortex. The deposition of amyloid-beta, namely Aβ peptides 40 and 42 in senile plaques, in these brain regions, suggests that they are key players in the pathogenesis of the disease, although their link to intracellular tau hyperphosphorylation and accumulation in neurofibrillary tangles, is not yet clarified. Evidences from neuroimaging and neuropathological studies show that amyloid angiopathy develops early in AD, even in a presymptomatic stage of the disease. In vivo and in vitro studies have shown that cognitive deficits occurring in early stages of AD, are correlated to Aβ-induced glutamate receptors deregulation, endoplasmic reticulum (ER) stress, alteration of intracellular calcium homeostasis and mitochondrial dysfunction. In our studies, by using AD cybrids, that replicate the mitochondrial dysfunction observed in platelets from AD patients, an increased susceptibility to Aβ-induced ER stress leading to the activation of the ER stress-mediated apoptotic pathway was observed, further supporting an inter-organelle crosstalk during apoptosis in

AD. More recently we showed in rat brain endothelial exposed to Aβ that the failure of the adaptive unfolded protein response (UPR) led to a decrease in proteasome activity and the impairment of the autophagic flux. Simultaneously, a deregulation of calcium and redox cell homeostasis, led to endothelial cells apoptosis. In conclusion, these data may provide new strategies and therapeutic approaches targeting ER stress and mitochondrial dysfunction, not only in neurons but also in endothelial cells, aiming to prevent or delay the progression to AD.

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O2.25

The path of neurodegenerative disorders. From molecular mechanisms to biomarkers

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Neurodegenerative disorders, either in the familial or sporadic forms, are progressive and incapacitating diseases for which no cure exists, with a high socio-economic burden in western societies. The common trait of these disorders is the presence of intra or extracellular protein aggregates in specific vulnerable brain areas that have been associated with the neurodegenerative process occurring in these disorders. Although the etiology of these diseases is largely unknown, it has been suggested that multiple factors, including genetic and epigenetic components, oxidative stress, excitotoxicity, inflammation, mitochondrial dysfunction and alteration of cytoskeleton, lead to synaptic dysfunction and neuronal loss. The accuracy of the clinical diagnosis of sporadic forms of these diseases, namely in their early stages, is required for the development of putative disease modifying drugs. For this purpose, the identification of disease biomarkers that can also correlate with disease severity is mandatory. Our studies have been focused in the most prevalent neurodegenerative diseases, namely Alzheimer's and Parkinson diseases, trying to understand how initial adaptive cellular reactions lead to an irreversible alteration of brain homeostasis. In cellular and animal models of disease, we have demonstrated that mitochondrial dysfunction, intracellular calcium deregulation and changes in redox homeostasis play a crucial role in the pathogenesis of these diseases. Taking into account these results, we moved to the identification of a molecular signature of the diseases, integrating the evaluation of protein levels in CSF and/or blood, with genetic risk factors and clinical data. The added value of biomarkers in the diagnosis of the earliest stages of disease, before neurodegeneration starts, will be discussed. Supported by FCT, project ref. UID/NEU/04539/2013

O2.26

The bile acid Chenodeoxycholic acid is a powerful modulator of metabolic pathways in white adipose tissue in vitro: towards a better understanding of how bile acids decrease obesity

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Obesity has now reached epidemic levels throughout the world. Current pharmacological strategies are, in the long run, inefficient in achieving an improved healthier profile. To this goal, a novel strategy has emerged in the potential therapeutic use of bile acids (BA) to fight obesity.

Recently, BA have been found to have a hypoglycemic and hypolipidemic activity in both *in vivo* animal models and obese patients (Teodoro et al., 2011; Teodoro et al., 2014). BA are currently thought to have two major receptors that mediate these functions, the Farnesoid X Receptor (FXR) and the to the G protein-coupled receptor 5 (TGR5). Both have very different and sometimes contradicting effects, depending on type of BA used and target tissue type. Regardless, systemic BA exposure causes a total reversal of obesity and diabetic statuses, an effect at least in part caused by enhanced brown adipose tissue (BAT) thermogenesis (Teodoro et al., 2014; Watanabe et al., 2006). This is particularly interesting due to the recent discovery of functional BAT in adult humans (Ouellet et al., 2012). However, the lack of consensus about route of action and key target modulators of metabolic pathways involved in BA activity, coupled with the inherent BA cytotoxicity has rendered the development of more safe and potent BA-effect mimetic pharmacological agents difficult. We report here that the BA CDCA is a powerful reducer of *in vitro* obesity. NMR analysis demonstrates that CDCA is capable of inducing an acceleration of several metabolic pathways, including the citric acid cycle, mitochondrial oxidative phosphorylation, lipidic oxidation and a substrate-futile, energy depleting, triglyceride/glycerol+free fatty acid cycle.

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O2.27

Remodeling of cardiac mitochondrial profile in the fetal baboon by moderate global maternal nutrient reduction

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Background: Poor fetal nutrition results in intrauterine growth restriction and in sex-dependent cardiac changes, potentially predisposing the offspring to later-life cardiovascular disease (CVD). Mitochondrial bioenergetics plays a key role in cardiac energy metabolism, growth, and function. Using our well-established non-human primate model of maternal undernutrition, we addressed the hypothesis that moderate maternal nutrient reduction (MNR) impairs fetal cardiac mitochondrial function.

Methodology: Pregnant baboons ate chow *ad lib* (Control, C) or 70% of controls (MNR). Cesarean sections were performed under general anesthesia at term. Morphometric parameters and maternal and fetal blood biochemistry were measured in the C and MNR groups. Mitochondrial (mt)DNA copy number, mRNA and protein levels, enzymatic activities, and mitochondrial morphology were examined in the fetal cardiac left ventricles (LV) from both groups.
Results: Nutrient reduction decreased maternal weight gain and caused 23% reduction in placental weight. Heart weight-to-body weight ratios were similar despite decreased fetal weight. MNR increased fetal LV mtDNA content by 1·5-fold. Nutrient reduction also significantly increased mRNA for key oxidative phosphorylation (OXPHOS) mitochondrial genes, which was also confirmed by semi-quantifying protein levels by Western blotting (including NDUFB8, UQCRC1, Cyt c, and VDAC). Surprisingly, however, the OXPHOS enzymes activity was significantly decreased in MNR fetuses, together with a 78% reduction in ATP content, and increased oxidative stress in LV tissue, as evidenced by 1·4-fold increase in the lipid peroxidation marker, malondialdehyde ($P < 0·05$). Electron microscopy showed LV mitochondria with sparse and disarranged cristae in the MNR group.
Conclusions: The present study provides evidence of LV mitochondrial remodeling in the MNR fetuses. Decreased mitochondrial function has been previously linked to heart failure, and might at least partially underlie the CVD seen in the offspring of maternal undernutrition.
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O2.28
Maternal obesity (MO) in sheep decreases fetal hepatic mitochondrial respiratory chain activity and total mitochondrial lipids

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Introduction: MO has adverse effects on fetal hepatic metabolism and development. Exposure to hyperlipidemic environment can trigger changes in fetal liver metabolism, predisposing it to later-life metabolic diseases. We hypothesize that MO programs fetal sheep mitochondrial bioenergetics and metabolism, contributing to later-life altered mitochondrial capacity and susceptibility to metabolic diseases.
Methods: Ewes consumed either an obesogenic (150% of NRC requirements; MO; $n = 7$), or control diet (100% NRC; CTR; $n = 7$) from 60d prior to conception through pregnancy. Fetal livers were removed following exsanguination under general anesthesia at 0·9 gestation. Selected mitochondrial proteins were measured in liver tissue by Western Blotting. Mitochondrial respiratory chain complexes, aconitase, and citrate synthase activities in isolated fractions, and superoxide dismutase II activity in whole liver tissue, were measured by colorimetric methods. Total and specific mitochondrial phospholipids were measured in isolated fractions by colorimetric methods following chromatographic separation.
Results: Protein expression of selected respiratory chain subunits did not differ between groups. Still, decreases in Complex I ($P < 0·05$), Complex II-III ($P < 0·01$), and Complex IV ($P < 0·05$) activities were observed in MO vs. CTR. No group differences were observed in mitochondrial aconitase or citrate synthase activities. Superoxide dismutase II activity showed a 2·1 numerical increase in MO. A decrease ($P < 0·001$) in total mitochondrial phospholipid was observed in MO vs. CTR. No differences were observed in mitochondrial-specific phospholipid cardiolipin.
Conclusions: These data suggest that MO decreases fetal mitochondrial complex activity and total mitochondrial phospholipids, which might affect the normal membrane composition, possibly leading to compromised mitochondrial bioenergetics later in life.
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[Correction added on 10 May 2017, after first print publication in May 2017 and corrected in the online version: Presenting author of abstract O2.28, T.L. Serafim was changed to S.P. Pereira].

Workshop 3: Cardiology

O3.01

Serum PCSK9 reduction after ischemic stroke predict worse outcome and higher MACEs occurrence

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Background: Inflammation has shown to play a dual role in the evolution of ischemic brain injury. Among soluble mediators investigated to predict stroke outcomes, proprotein convertase subtilisin/kexin type 9 (PCSK9) might have both clinical and pathophysiological relevance.
Materials and methods: Blood from patients was sampled at admission and at day 1, 7 and 90 after acute ischemic stroke onset. Available samples (n = 72) were tested for PCSK9 by colorimetric enzyme-linked immunosorbent assay. Primary endpoint was the predictive value of early PCSK9 level variations (ΔPCSK9) from day 1 to day 7, toward a 90-day outcome by modified Rankin Scale (mRS). The association between ΔPCSK9 and the risk of major adverse cardiovascular events (MACEs) occurrence was the secondary endpoint.
Results: Poor clinical outcomes at day 90 were associated with lower level of PCSK9 in serum at days 1 and 7. ΔPCSK9 day 7-day 1 was a good predictor of poor 90-day mRS at the cut-off point identified by the ROC curve analysis: −61·28 ng/mL. Moreover, ΔPCSK9 day 7-day 1 ≤ −61·28 ng/mL was associated with an increased rate of MACEs.
Conclusion: Poor neurological outcome and MACEs occurrence at 90-day follow-up were both predicted by a decrease in PCSK9 serum levels after AIS onset. Further studies are needed to clarify the pathophysiological mechanism underlying the detrimental effects of early PCSK9 reduction in patients with AIS

O3.02

Prognostic value of the blood lactate peak in patients with acute coronary syndrome under extracorporeal cardiopulmonary resuscitation

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Background: The prognostic impact of extracorporeal membrane oxygenation (ECMO) in acute coronary syndromes (ACS) complicated by and refractory cardiogenic shock (CS) and cardio-respiratory arrest (CA) remains to be determined. We aimed at evaluating the prognostic roles of blood and procedural parameters on 30-day mortality.
Materials and methods: In this pilot study, 29 patients admitted for ACS complicated by CS and CA who underwent ECMO implantation were studied. The medical information, PPCI characteristics and hemodynamic ECMO variables during first 24 h were analyzed. The primary endpoint was to assess a relationship between the blood lactates in the first 24 h and all-cause mortality at 30 days. The secondary outcomes were to assess if the procedural feature were associated with 30-day mortality.
Results: "Survivors" and "non-survivors" had similar demographic, clinical and biochemical characteristics at admission, except for blood lactate peak in the first 24 h that was increased in non-survivors. Mortality at 30 days was 62%. The cut-off value for the lactates, determined by receiver operating characteristic (ROC) curve analysis, was found at 11 mmol/l. Procedural characteristics of percutaneous coronary intervention and ECMO were comparable in either group. The peak of blood lactate predicted 30-day mortality independently of age, sex and ECMO duration. Short extracorporeal cardiopulmonary resuscitation was independently associated with 30-day mortality.
Conclusion: The peak of blood lactate concentration in the first 24 hours was increased in non-survivors and predicted 30-day mortality in patients with ACS complicated with CS and CA.

O3.03

Stem cell therapy for myocardial ischemia: myth or reality?

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Cardiovascular disease (CD) and heart failure represent the main cause of mortality in the Western countries. CD pathogenesis is mainly related to inefficient cardioprotection, defective repair and lack of myocardial renewal following cardiac injury

and/or aging. However, recent work demonstrated that the adult mammalian heart is not completely devoid of regenerative capability, since it has an endogenous restorative program mainly based on cardiac progenitor cells (CPC) activation and resident cardiomyocyte de-differentiation and proliferation. Although these mechanisms are widely responsive during cardiogenesis and in the very early stages of post-natal life, they become quiescent and dormant in the adulthood, leaving the heart capable of limited restorative potential when facing pathological situations, such as myocardial infarction.

Therefore, a working strategy to enhance and reinstate in full the cardiac endogenous potential for both repair and regeneration will open new frontiers in cardiac medicine. In this scenario, stem cell biology has been broadly scrutinized in order to define a therapeutic approach. Despite an increasing interest toward the analysis of the cardiovascular differentiation potential of autologous or allogeneic transplanted stem cells, particular attention has been lately addressed to their paracrine modulatory influence. Indeed, the so called "paracrine effect" has been proposed as a possible working strategy to boost the endogenous mechanisms of regeneration from within the cardiac tissue.

This has led to a significant paradigm shift: from exploring the stem cell genome in terms of direct differentiation potential, to analyzing the stem cell "*secretome*", as the whole of growth factors and chemo-attractant molecules produced via paracrine secretion. Hence, the scientific community is now focusing on identifying the ideal stem cell source endowed with the most effective secretome for cardiac regeneration.

O3.04
PCSK9 and atherosclerosis: beyond LDL

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Proprotein Convertase Subtilisin Kexin type 9 (PCSK9) is a protein mainly produced by the liver, which plays a pivotal role in controlling LDL receptor (LDLR) recycling on the surface of hepatocytes .

In humans, PCSK9 loss-of-function (LoF) mutations, are associated with hypocholesterolemia and as a consequence, the reduction in cardiovascular risk in the other hand gain of function are associated with a classical familial hypercholesterolemia phenotype.

Beyond targeting the LDLR, PCSK9 i) modulates lipoprotein assembly and secretion in the intestine and in the liver [6, 7] and ii) by controlling the turnover of the very-low density lipoprotein (VLDL) receptor, the ApoE2 receptor, and the CD36 receptor impacts triglyceride rich lipoproteins (TGRLs) metabolism and fatty acids uptake in peripheral tissues. PCSK9 deficiency has been demonstrated to result in reduced postprandial lipemia and TGRLs production, while PCSK9 over expression promotes hepatic lipogenesis.

These findings raise the question whether PCSK9 deficiency might affect triglyceride metabolism both by modulating triglyceride rich lipoprotein metabolism, and by increasing fatty acids and TG accumulation in the liver as well as favoring their delivery into peripheral tissues.

O3.05
Epigenetic changes and vascular risk in cardiometabolic disturbances

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Risk of diabetic complications continues to escalate overtime despite a multifactorial intervention with glucose-lowering drugs, anti-hypertensive agents and statins. In this perspective, a mechanisms-based therapeutic approach to vascular disease in diabetes represents a major challenge. Epigenetic signatures are emerging as important determinants of vascular disease in this setting. Methylation and acetylation of DNA and histones is a reversible process leading to dysregulation of oxidant and inflammatory genes such as mitochondrial adaptor p66(Shc) and transcription factor NF-kB p65. Epigenetic modifications associated with diabetes may contribute to the early identification of high risk individuals. Ongoing epigenomic analyses will be instrumental in identifying the epigenetic variations that are specifically associated with cardiovascular disease in patients with diabetes. During my speech, I will discuss a complex scenario of epigenetic changes and their putative link with diabetic vascular disease. Pharmacological reprogramming of diabetes-induced epigenetic signatures may be a promising option to dampen oxidative stress and inflammation, and thus prevent cardiovascular complications in this setting.

O3.06
Laparoscopic sleeve gastrectomy effects on left ventricular hypertrophy in morbidly obese subjects

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Background: Obesity is associated with left ventricular hypertrophy (LVH) development. Laparoscopic sleeve gastrectomy (LSG), a relatively new bariatric procedure, showed its efficacy in obtaining sustained weight loss and a reduction of cardiovascular morbidity and mortality.
Aims: *T*he aims of our study was to evaluate if LSG has positive effects on LVH and if these effects could be mediated by weight loss, visceral adipose tissue depots reduction or metabolic profile amelioration.
Patients and methods: *We* enrolled 110 morbidly obese subjects (38 males, 72 females, mean BMI 45·5 kg/m^2) eligible for LSG. Before and about 12 months the bariatric procedure we assessed BMI, waist circumference (WC), systolic and diastolic blood pressure (SBP and DBP), fasting lipid profile, glucose and insulin levels, which were used for calculating HOMA-index, and glycosylated hemoglobin. Visceral fat area (VFA) was estimated by ultrasonography. Echocardiograms were performed for calculating left ventricular mass (LVM) has been using Devereux formula, indexed for height. LVH was defined by LVM $\geq$ 51 $g/m^{2,7}$.
Results: At baseline mean LVM was 53·6 $\pm$ 15 $g/m^{2,7}$ and LVH prevalence was 55·3%.

After surgery we observed significant reduction of BMI, WC, total cholesterol, triglycerides, glucose and insulin levels, HOMA-index, glycosylated hemoglobin, SBP, DBP, VFA and a significant increase of HDL levels. LVM and LVH prevalence significantly decreased, with LVH regression in 40% of hypertrophic subjects.

LVM reduction and LVH regression were not significantly correlated with TWL, variation of SBP, DBP, HOMA index, VFA, triglycerides, HDL and total cholesterol.
Conclusions: LSG has positive effects on weight loss and on obesity-associated comorbidities. LSG is effective in determining regression of LVH. Our data suggest that LVH regression could not be mediated by weight loss, visceral adipose tissue depots reduction and other studies are needed for better understanding the mechanism involved in LVH regression after bariatric surgery.

O3.07
Modulation of natriuretic peptide receptors in human adipose tissue: molecular mechanisms behind the "natriuretic handicap" in morbidly obese patients

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Background and aim: B-type Natriuretic Peptide (BNP) is a hormone with a crucial role in the maintenance of cardiometabolic health. Obesity is characterized by the presence of low circulating levels of BNP, a condition known as "natriuretic handicap". Recent evidences suggest an altered expression of BNP receptors — both the signaling Natriuretic Peptide Receptors (NPR)-A and the clearance NPR-C receptor — in adipose tissue (AT) as one of the putative cause of the natriuretic handicap. The present study aims at clarifying the molecular basis of the natriuretic handicap, focusing on NPRs modulation in AT of obese and control subjects.
Patients and methods: 34 morbidly obese subjects and 20 non obese controls undergoing bariatric or abdominal surgery, respectively, were enrolled in the study. The main clinical and biochemical parameters, including circulating BNP, were assessed. In visceral (VAT) and subcutaneous adipose tissue (SAT) samples, collected during surgery, the adipocytes and stromal vascular fraction (SVF) expression of NPR-A and NPR-C and the SVF secretion of IL-6 were determined.
Results: VAT and SAT from obese patients expressed a lower NPR-A/NPR-C ratio in adipocytes and the SVF secreted a higher level of IL-6, compared to the controls. Moreover, NPR A/NPR-C ratio expressed by VAT and SAT adipocytes negatively correlated with BMI, insulin, HOMA and IL-6 secreted by SVF, and the expression of the clearance receptor NPR-C, in both VAT and SAT adipocytes, showed a negative correlation with circulating BNP.
Conclusions: Overall, insulin-resistance/hyperinsulinemia and AT inflammation (i.e. high level of IL-6) are the major determinants of the lower NPR-A/NPR-C ratio in adipocytes of obese subjects. The altered expression of NPRs in obese AT could represent a relevant molecular mechanism promoting the natriuretic handicap

O3.08
Intramyocardial therapy delivery: a novel accurate and fast method to target the infarct border zone

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Background: CARTBox2 software enables the permanent demarcation of targets in late gadolinium enhanced (LGE) MRI DICOM datasets. The created datasets are fused with live fluoroscopy to enable the real time visualization of LGE-MRI defined targets during live fluoroscopic interventions without the need for an external computer. In this study we compare the clinical standard for intramyocardial injections, the NOGA system, with CARTBox2 in terms of injection accuracy to target the infarct border zone (IBZ), procedure time, fluoroscopy time and dose, and arrhythmogenicity.
Methods: In ten pigs (60–75 kg), four weeks after a 90-minute LAD occlusion, LGE-MRI scans were performed. Subsequently, 10–16 injections were delivered in the IBZ using either the NOGA system or CARTBox2. The primary endpoint was the distance of the injections to the IBZ on histology. Secondary endpoints were total procedure time, fluoroscopy time and dose, and the number of ventricular arrhythmias.
Results: The average distance of the injections to the IBZ was similar for NOGA ($-0{\cdot}7 \pm 2{\cdot}2$ mm) and CARTBox2 ($0{\cdot}5 \pm 3{\cdot}2$ mm; $P = 0{\cdot}52$). Injection procedures with CARTBox2 and NOGA required 69 ± 12 and 60 ± 17 minutes, respectively ($P = 0{\cdot}36$). NOGA procedures do however require an endocardial mapping procedure prior to injection, leading to a significantly longer total procedure time ($P < 0{\cdot}001$). Fluoroscopy time with NOGA ($18{\cdot}7 \pm 11{\cdot}0$ minutes) was significantly lower than with CARTBox2 ($43{\cdot}4 \pm 6{\cdot}5$ minutes; $P = 0{\cdot}0003$). Procedures with CARTBox2 show a trend towards causing less ventricular arrhythmias than NOGA.
Conclusion: CartBox2 is an accurate and fast vendor independent system to facilitate intramyocardial injections based on gold standard non-invasive LGE-MRI fibrosis imaging. In the present study, CARTBox2 was evaluated for the delivery of intramyocardial therapy. CARTBox2 can be used for many other applications, such endocardial biopsies or CRT-lead placement.

O3.09
Molecular mechanisms supporting ischemic postconditioning: Chaperones in cardioprotection

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Objectives: Ischemic post-conditioning (IPost-Co) has been proposed to limit infarct size by reducing ischemia and reperfusion (I/R)injury. However, the molecular mechanisms by which

IPost-Co affords cardioprotective effects remain unclear. We hypothesized that a systems biology approach could be of help to identify an integrated group of proteins with different functions involved in the cardioprotective effects of IPost-Co.
Methods: We induced myocardial ischemia (1·5 h) by balloon occlusion of the left anterior descending (LAD) in a swine model of closed-chest myocardial infarction (MI; $N = 20$). Post-conditioning was achieved by 6 cycles of 20 seconds of reperfusion and 20 seconds of re-occlusion. Benefits of IPost-Co were measured by clinical and morphological evaluation of heart function and infarct size reduction after 2·5 hours of reperfusion. Samples of the myocardium were obtained and processed for proteomic analysis. Proof-of-concept studies were run in a mice MI model ($N = 26$).
Results: Among the identified proteins in the myocardial proteome, 28% were associated to the mitochondrial-dysfunction pathway. I/R induced a decrease in 15 mitochondrial-related proteins, whereas IPost-Co rescued over 47% of those changes and induced a 10% recovery in cardiac performance. IPost-Co induced an increase in DJ-1, GRP75 and peroxiredoxin-6. As a proof-of-principle, recombinant DJ-1 was used to pre-treat mice 1 h before MI induction. Recombinant DJ-1 was then administered to mice 1 h before the induction of MI by LAD-ligation. DJ-1 administration significantly reduced infarct size (75%) by diminishing apoptosis through a multigenic response in the myocardium involving the Gαq-signaling pathway, specifically Gprc5a, and the oxidative stress-related iNOS pathway.
Conclusions: IPost-Co coordinately prevents the pro-oxidant mitochondrial-related changes that occur during I/R by increasing DJ-1, GRP75 and peroxiredoxin-6 in the myocardium. Raising DJ-1 levels may protect against myocardial injury without the need of mechanical Post-Co due to its direct cardioprotective effect.

O3.10
Automatic classification of right ventricle deformation patterns

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Introduction: Different stages of arrhythmogenic right ventricular cardiomyopathy (ARVC) can be characterized by distinct RV longitudinal deformation (strain) patterns. The criteria used for the classification are timing of onset shortening, timing of peak shortening, (systolic) peak strain and post-systolic index. Currently, classification of these patterns is performed manually. This is both time consuming and may lead to inter- and intra-observer variability, especially between different centres. The goal of this study was to design an algorithm to automatically classify RV deformation patterns, with a focus on the timing of onset shortening, the most difficult parameter to determine.
Methods: An algorithm was designed based on specific local characteristics from the strain curves to detect the timing of onset shortening. Outcome was compared with detection by an experienced operator, for a dataset containing 186 RV strain curves (3 segments per subject) obtained from 36 healthy subjects and 26 ARVC patients carrying a pathogenic plakophilin-2 (PKP2) mutation.
Results: The mean difference between the timing of onset shortening determined by the experienced operator and by the automatic detector was $6{\cdot}3 \pm 10{\cdot}7$ milliseconds. Both detection methods correlated significantly with $\rho = 0{\cdot}97$ ($P < 0{\cdot}001$).
Discussion/Conclusion: A strong correlation with small differences was observed between the experienced operator and the algorithm, indicating the algorithm is accurate and may be considered a useful alternative to manual annotation of the timing of onset shortening. The other parameters required for classification of RV deformation patterns can be obtained directly from the strain curves (e.g. peak systolic strain). Taken together, the computer algorithm seems therefore a very promising method for the automatic classification of RV deformation patterns.

O3.11
ProBNP strongly predicts future vascular events in peripheral arterial disease patients with as well as in those without the metabolic syndrome

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Aim: Pro B-type natriuretic peptide (proBNP) is an established prognostic biomarker in patients with heart failure. Its power to predict cardiovascular endpoints in peripheral arterial disease (PAD) patients with the metabolic syndrome (MetS) is unclear and is addressed in the present study.
Methods: We prospectively recorded cardiovascular events over a mean follow-up period of $4{\cdot}9 \pm 1{\cdot}7$ years in a consecutive series of 319 patients with sonographically proven PAD, including 144 subjects with the MetS and 175 without the MetS. Presence of the MetS was defined according to the current harmonized consensus definition. At baseline, proBNP did not differ significantly between PAD patients with the MetS ($n = 144$) and those who did not have the MetS (1037 ± 3386 pg/mL vs. 1027 ± 3864 pg/mL; $P = 0{\cdot}759$).
Results: During follow-up, the incidence of cardiovascular events was 57·7% among PAD patients with the MetS and 46·2% among PAD subjects without the MetS ($P = 0{\cdot}042$). Serum proBNP significantly predicted the incidence of cardiovascular events after adjustment for age, gender, BMI, smoking, systolic and diastolic blood pressure, LDL cholesterol, HDL cholesterol and the eGFR both in patients with the MetS (standardized adjusted HR 1·68 [1·30–2·17]; $P < 0{\cdot}001$) and in subjects without the MetS (HR 1·40 [1·17–1·67]; $P < 0{\cdot}001$).
Conclusion: We conclude that proBNP strongly and independently from conventional risk factors predicts future vascular events in PAD patients with the MetS as well as in PAD patients without the MetS.

O3.12
Cardioprotective effects of calcitonin gene-related peptide via redox signaling

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The calcitonin gene-related peptide (CGRP) release is associated to the signaling of Angeli's salt (AS) and the ATP-sensitive-

potassium (KATP) channel activation. Both AS and CGRP induce inotropic and vasodilator effects. AS also induces redox sensible cardioprotective effects when given as preconditioning agent. However it is unknown whether AS cardioprotective effects are mediated by CGRP and which are the mechanisms of protection. We aimed to study whether AS exerts cardioprotective effects against ischemia/reperfusion injury *via* a CGRP mechanism and analyzed the intracellular signaling pathway.

We used two different experimental models, namely the isolated rat heart and the H9c2 cell line. To assess the effects on myocardial injury, isolated hearts were pretreated with AS or AS plus CGRP8-37 (a specific CGRP-receptor antagonist) and subsequently subjected to ischemia (30-min) and reperfusion (120-min). Moreover, to analyze CGRP-protective molecular pathway we studied CGRP protection during oxidative stress (H_2O_2) and hypoxia/reoxygenation protocols in H9c2 cardiomyocytes. Cell vitality and mitochondrial membrane potential ($\Delta\Psi m$, MMP) were measured using MTT and JC-1 dyes.

Angeli's salt reduced infarct size and ameliorated post-ischemic myocardial function *via* a CGRP-dependent mechanism in isolated rat hearts. Pretreatment with CGRP increased cell survival *in vitro* upon treatment with either oxidative stress (H_2O_2) or hypoxia/reoxygenation protocol. In these conditions, pretreatment with either CGRP receptor, protein kinase C (PKC) or mitochondrial KATP antagonists, as well as pretreatment with a scavenger of mitochondrial radical oxygen species (2-mercaptopropionylglycine) blocked the cardioprotection mediated by CGRP.

Conclusion: CGRP is involved in the cardioprotective effects of Angeli's salt. In H9c2 cardiomyocytes, CGRP elicits PKC-dependent and mitochondrial-KATP-redox-dependent mechanisms. Hence, CGRP is an important factor in the redox-sensible cardioprotection of Angeli's salt.

O3.13
Ischemia and reperfusion injury mouse model: role of ageing genes in myocardium

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Introduction: Ageing is a continuously ongoing molecular process involving specific genes participating in signal transduction pathways. It is considered to be a major risk factor for acute and chronic CV diseases, such as myocardial infarction. Myocardial injury during short-term ischemia and reperfusion has become clinically important with the use of primary PCI in patients with ACS. Knowledge of the mechanisms of myocardial infarction has been improved with the use of animal models. The mitochondrial adaptor protein $p66^{Shc}$, transcription factor JunD and the TGFβ superfamily member GDF11 are a few among many ageing genes. However, not much is known about their role in the myocardium. Therefore, the goal of the present study was to examine how these proteins act during ischemia/reperfusion.

Methods: 12–14-week-old genetically modified males ($p66^{Shc-/-}$ and *cJunDTG*) together with corresponding WT controls as well as 12–14-week-old and 22–24-month-old C57BL/6 males injected daily for 30 days with either recombinant human GDF11 or vehicle were subjected to 30 min of ischemia (I) followed by 24 h of reperfusion (R). Infarct size was assessed morphologically.

Results: After I/R, $p66^{Shc-/-}$ and *cJunDTG* mice both developed markedly larger infarcts as compared to controls. Similar, both young and aged GDF11-injected mice also developed larger infarcts as compared to vehicle-treated groups. This was further associated with increased post-ischemic levels of cardiac troponin I. Finally, cardiac RISK and SAFE prosurvival pathways were less activated in both *cJunDTG* mice and $p66^{Shc-/-}$ as well as in GDF11-treated groups.

Conclusions: Thus, genetic deletion of $p66^{Shc}$ and overexpression of junD both promote increased sensitivity to I/R in the mouse heart. The same is true for the daily injections of GDF11. Such JunD-, $p66^{Shc}$- and GDF11-associated cardiac phenotypes are likely to be driven by increased cell death in the injured myocardium together with impaired function of RISK and SAFE pathways.

O3.14
Investigational therapies for cancer therapy-related cardiotoxicity

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Along with the great improvement of efficacy of cancer treatment, the importance of collateral damage due to chemotherapeutic agents has become a major issue. Indeed several chemotherapeutic agents, included modern antibody-based drugs, induce the cardiotoxicity phenomenon, impairing heart function and possibly leading to the development of heart failure, even years after treatment, in a significant proportion of the treated patients. Moreover many modern oncological treatment regimens rely on the use of multiple agents whose cardiotoxic effects could be additive or synergistic.

Find an effective treatment for cardiotoxicity is challenging as different chemotherapeutic drugs act on different target (i.e. cardiomyocytes, endothelial cells) and with different mechanisms including enhanced ROS production, DNA damage and impairing of metabolism.

For these reasons beside canonical approach to reduce incidence of cardiotoxicity, which are essentially based on the modulation of the administration of the drugs and continuous monitoring of patients after therapy, brand new approaches have been proposed by research laboratories. These approaches include exploiting protective effects of stem cell or stem cell secretome, modulation of hormonal levels and use of drugs which act on specific molecular target in cardiovascular cells.

Cutting edge technologies, as miRNA analysis on liquid biopsies, have been proposed also for the early detection of cardiotoxic effects.

Hence the lecture proposes an overview of the state of art concerning new approaches to reduce the burden of cardiotoxicity, whom will set the bases for the future therapies.

O3.15
17β-estradiol-mediated endothelium protection against apoptosis induced by TNFα requires Notch1 activation

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Women have lower risk of developing cardiovascular diseases than age matched men, but this difference disappears after menopause, suggesting that estrogens play a cardioprotective role in women. A large number of *in vitro* and *in vivo* studies highlights a role for estrogens in mediating protection from endothelial dysfunction, which is the first steps toward onset and progression of atherosclerosis and other cardiovascular diseases. Endothelial cells apoptosis is one of the hallmarks of the endothelial dysfunction and many evidence show that - inflammatory cytokines induce endothelial cells apoptosis and also inhibits the Notch pathway. We have previously demonstrated that in endothelial cells treatment with 17β-estradiol (E2) activates Notch signalling. The goal of the present work is to establish whether, under inflammatory conditions, activation of Notch is involved in E2-mediated protection of vascular endothelium.

Human umbilical vein endothelial cells (HUVECs) were treated with E2, TNFα and/or DAPT, an inhibitor of the proteolytic cut releasing the active form of Notch and the effect on apoptosis and on components of Notch pathway was investigated through flow cytometry, mRNAs and proteins studies.

We report that TNFα-induced apoptosis is counteracted by E2, but when Notch1 is inhibited by siRNA or DAPT, no protection is observed. In addition, Notch1 ectopic overexpression diminishes TNFα-induced apoptosis. Furthermore, TNFα reduces Notch1 activation while E2 partially restores Notch1 levels. Additionally, we show that TNFα-mediated Akt phosphorylation is Notch1-dependent and E2 enhances this effect. Moreover, siRNA against estrogen receptor β (ERβ), but not ERα, abolishes E2 effect on apoptosis and on Notch1 activation. In summary, our data show that E2-mediated endothelial protection is dependent on ERβ and it requires active Notch1.

These findings indicate that in subjects with an impaired Notch1 signalling, E2-based hormone therapy might not be able to prevent endothelial dysfunctions and, therefore, reduce the progression of cardiovascular diseases.

O3.16
Abscisic acid: a new player in cardiomyocyte protection from hypoxia

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The plant hormone abscisic acid (ABA) lies at the interface between abiotic stress and metabolic signaling through several signaling pathways.ABA is present and active also in mammals, where it stimulates innate immune cell function, including NO production, and regulates glucose disposal, through its receptor LANCL2. Plasma ABA increases after glucose load in humans and stimulates insulin secretion and glucose uptake by skeletal muscle cells and adipocytes. The ABA response to hyperglycemia is impaired in diabetes mellitus (DM) and in gestational diabetes and intake of exogenous ABA improves glucose tolerance in rats and in humans.

DM predisposes to ischemic heart disease, not only because it is associated with traditional risk factors for coronary atherosclerosis, but also via specific mechanisms, as yet poorly understood.

We hypothesized that endogenous ABA could play a physiological role in the protection of myocardial tissue via NO production.

Exogenous micromolar ABA to H9c2 cardiomyoblasts cultured under hypoxia for 12 hours significantly increased cell survival compared with untreated cultures. Endogenous ABA was released by H9c2 cells subjected to 30 min of nitrogen-flushed hypoxia (<2% O_2) hypoxia-induced ABA release in turn stimulated NO production from H9c2 and also from endothelial HUVEC cells. Addition of L-NAME, abrogated the pro-survival effect of ABA on hypoxic H9c2 cells, indicating a causal role of NO in the cardioprotective effect of ABA. ABA also increased glucose uptake by H9c2, NADPH and GSH levels, these metabolic effects contributing to cell energy production and defense against oxidant damage. Stimulation of NO release and increased NADPH levels in response to ABA were also observed in primary neonatal ventricular cardiomyocytes isolated from wild-type, but not from LANCL2-knockout mice.

These in vitro results indicate a role for endogenous ABA, through its receptor LANCL2, in cardiomyocyte protection against hypoxia, through the stimulation of NO production, and suggest that defective endogenous ABA production in DM may play a role in diabetic cardiomyopathy.

O3.17
Phospholamban immunostaining is a highly sensitive and specific method for diagnosing phospholamban p.Arg14del cardiomyopathy

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Background: The pathogenic phospholamban (PLN) p.Arg14-del mutation causes dilated and/or arrhythmogenic cardiomyopathy and is associated with an increased risk of ventricular arrhythmias and heart failure. Recently we showed that PLN p.Arg14del cardiomyopathy can be diagnosed by PLN immunohistochemistry (IHC) which allowed microscopic detection of

PLN-containing aggregates that were concentrated in cardiomyocytes in dense perinuclear aggresomes.
Objective: The purpose of this study was to determine the sensitivity and specificity of PLN IHC in apical left ventricular myocardial specimens, harvested during left ventricular assist device (LVAD) implantation, to diagnose PLN p.Arg14del cardiomyopathy.
Methods: Included were myocardial LVAD specimens from 30 diverse genetic cardiomyopathy cases with known gene mutations (9 PLN p.Arg14del cases and 21 cases with other pathogenic cardiomyopathy mutations). For the IHC analysis, monoclonal phospholamban antibody 2D12 was used to visualize PLN protein aggregation. Blinded histological assessment of PLN aggregates in LVAD samples was performed by 2 independent observers (AJHS and WPtR).
Results: Immunohistochemical analysis revealed typical dense perinuclear globular PLN-positive aggregates (representing aggresomes) in all 9 PLN p.Arg14del cases. In 20 non-PLN cases no PLN staining was found. In one non-PLN case one of us misinterpreted PLN staining of heavily wrinkled nuclear membranes in cross-sectioned cardiomyocytes as perinuclear PLN aggregates. Thus, in this LVAD cohort, PLN immunostaining had a very high sensitivity (100%) and specificity (95%) for demonstration of PLN protein aggregates in PLN p.Arg14del cardiomyopathy.
Conclusions: In this genetic cardiomyopathy cohort, PLN IHC analysis in LVAD biopsies was found to be a highly sensitive and specific method for demonstration of PLN protein aggregates in PLN p.Arg14del cardiomyopathy. In clinical practice PLN IHC analysis of LVAD specimens can be of incremental value in the diagnostic workup of this cardiomyopathy, even more so if genetic analysis cannot be performed.

O3.18
The scaffold-like protein NF2 drives fatty acid-induced endothelial damage: implications in obesity and insulin resistance

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Introduction: High levels of free fatty acids are key underpinnings of vascular disease in obese individuals, but the underlying mechanisms are incompletely understood.NF2 is a scaffold-like protein implicated in various cellular processes, namely growth, differentiation and survival. NF2 is inactivated by Akt-dependent phosphorylation at Ser518, whereas its dephosphorylation by the myosin phosphatase target subunit 1 (MYPT-1) leads to an active conformation. The role of NF2 in obesity-related alterations of endothelial phenotype remains elusive. We sought to determine whether NF2 participates to endothelial damage in this setting.
Methods: Human aortic endothelial cells (HAECs, passages 5-6) - cultured in endothelial growth medium-2 (EGM-2) supplemented with 2% fetal bovine serum (FBS) - were exposed to palmitic acid (PA, 200 uM) for 48 hours. Gene silencing of NF2 was performed by small interfering RNA (siRNA). Gene and protein expression were assessed by real time PCR and Western blot, respectively. Cellular apoptosis was assessed by caspase-3 activity assay, whereas 3-nitrotyrosine (3-NT) was employed to measure oxidative stress levels. Interaction of NF2 with endothelial proteins was investigated by co- immunoprecipitation.
Results: Exposure of HAECs to PA significantly decreased NF2 phosphorylation at Ser518. This finding was explained by a reduction of Akt phosphorylation at Ser473 and a concomitant increase of MYPT-1 phosphorylation at Thr696. We next asked whether PA-induced NF2 activation was involved in the modulation of proteins relevant to endothelial homeostasis. Pull-down experiments revealed that NF2 binds and activates Caveolin-1 (Cav-1), a pivotal repressor of endothelial nitric oxide synthase (eNOS) catalytic activity. Interestingly, NF2 knockdown in PA-treated HAECs prevented eNOS–caveolin-1 interaction, thus preserving eNOS functionality. Consistently, gene silencing of NF2 blunted PA-induced endothelial apoptosis and oxidative stress, as assessed by caspase-3 and 3-NT levels.
Conclusions: Targeting NF2 may contribute to rescue endothelial damage in the setting of obesity and insulin resistance.

O3.19
Current treatment of cancer therapy-related cardiotoxicity

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Cancer drug therapy has significantly improved survival of patients, but has also increased morbidity due to cardiotoxic represented by heart failure, coronary and peripheral artery disease, vein thrombosis and pulmonary embolism, hypertension, arrhythmias, pulmonary hypertension, myocarditis and pericarditis. Since the risk of developing cardiotoxicity mainly depends on the drug doses and pre-existing cardiac disorders or risk factors, actions up today designed to prevent cardiac side effects focus on selection of patients and limitation of the doses. As a result of this approach, several patients are excluded from the best anticancer therapies. It has been recently introduced the concept of actionable cardiotoxicity, which proposes: first, to evaluate case by case the benefit/risk ratio between anticancer efficacy and cardiac outcome; second, to make clinical decisions, which must not be confined to dose reduction or withdrawal of therapy, but must also apply to a series of interventions that range from: (1) establishing pre-treatments with cardioprotective agents; (2) monitoring patients to detect preclinical signs of toxicity so that introduce cardiovascular drugs at their first appearance; (3) treating cardiac side effects while maintaining oncological therapy in cases in which patient's survival and quality of life depend more on cancer outcome than cardiac side effects.

Several models of actionable cardiotoxicity have been proposed. While there is a general consensus on how-to-manage recent issues, such as anti-HER2-related left ventricular dysfunction or the occurrence of hypertension during anti-VEGF therapy, paradoxically there are still differences of opinion about old drugs such as anthracyclines or fluoropyrimidines whose toxicity has been well-known for a long time. Pros and cons of doable means of prevention of cardiotoxicity of these agents will be discussed.

O3.20
Clinical profile and therapeutic management of patients with atrial fibrillation in a tertiary care hospital

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Background: Atrial fibrillation (AF) is the most common cardiac arrhythmia with an increasing prevalence and incidence worldwide. The prevalence of AF increases with age and, in

the elderly population, cardiovascular (CV) risk factors and comorbidities are common.
Objectives: The aim of this study was to determine the clinical profile and therapeutic management of patients with different types of AF.
Materials and methods: A total of 405 patients, average age 57·23 years, 40·7% female, were admitted to Cardiology Department between January 2016 and December 2016. Patients with documented AF were classified into three subgroups: paroxysmal, persistent or permanent according to the latest ESC guidelines for AF. Statistical analyses of the collected data were performed.
Results: During the study period AF was paroxysmal, persistent, and permanent in 33·3%, 53·3%, and 13·4% respectively. Hypertension was the most common underlying CV condition (56·7%), followed by coronary artery disease (39·4%), heart failure (24·7%), diabetes (14·7%), and previous stroke (14%). The commonest presenting complaint was dyspnea (32·3%) followed by palpitation (23%). The median CHADS2, CHA2DS2VASc and HAS-BLED scores were $1{\cdot}8 \pm 1{\cdot}2$, $2{\cdot}7 \pm 1{\cdot}2$ and $1{\cdot}2 \pm 0{\cdot}8$ respectively. After excluding mechanical valve prosthesis, antithrombotic therapy had been prescribed in 90·1% of patients at baseline, including antiplatelets in 13·4%, vitamin K antagonists (VKA) in 66·1% and 10·6% direct oral anticoagulants. In patients under therapy with VKAs, INR values were within therapeutic range in 62% .
Conclusion: Patients with AF have higher risk profile for thromboembolism while antithrombotic therapy remains suboptimal. Better recognition of the clinical profile and therapeutic management of AF are needed to develop improved methods for AF prevention and management.

O3.21
The association between inflammation and myocardial injury following cardiac surgery

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Objectives: The primary study aim was to investigate the association between the intensity of the inflammatory response and the extent of myocardial injury after cardiac surgery. The second study aim was to evaluate the effect of dexamethasone treatment on postoperative myocardial injury.
Methods: We performed a post-hoc analysis of laboratory data in 985 patients undergoing cardiac surgery and who were randomized to dexamethasone or placebo treatment in the DExamethasone for Cardiac Surgery (DECS) trial. The relationship between postoperative peak C-reactive protein (CRP) and creatinine kinase isoenzyme MB (CK-MB) was investigated in the placebo group using multivariable linear regression analysis. Furthermore the extent of myocardial injury (measured in CK-MB) and the CRP response were compared between dexamethasone and placebo treated patients.
Results: There was no significant association between peak CRP and peak CK-MB (beta 0·06, 95% CI −0·01 to 0·13, $P = 0{\cdot}12$). The median peak CK-MB value was 36 U/l (IQR 25–65) in the dexamethasone group vs. 36 U/l (IQR 26–59) in the placebo group ($P = 0{\cdot}84$). The median peak CRP value was 84 mg/l (IQR 49–145) in the dexamethasone group vs. 167 mg/l (IQR 87–237) in the placebo group ($P < 0{\cdot}001$).
Conclusions: The intensity of the inflammatory response after cardiac surgery was not associated with the extent of postoperative myocardial injury. Furthermore, dexamethasone treatment effectively reduced postoperative CRP, however had no effect on myocardial injury.

O3.22
Diagnostic and therapeutic challenges in patients with coexistent chronic obstructive pulmonary disease and chronic heart failure

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Studies have indicated that 10–40% of HF patients have concurrent COPD, which is an independent predictor of rehospitalisation and mortality. The diagnosis of COPD is based on respiratory symptoms, exposure to tobacco smoke or other noxious agents, and evidence of airflow obstruction. But a major lung function abnormality in COPD is lung hyperinflation, which is of great importance for the understanding of heart-to-lung interaction. In COPD, impaired left ventricular filling associated with a reduced preload was observed by echocardiography and lung hyperinflation is likely to contribute to this abnormality. In addition, hyperinflation is associated to reduced intrathoracic blood volume and pulmonary vein dimensions, which likely contribute to decrease left ventricular volume and stroke volume. Specific cardiovascular phenotypes have been reported to be associated with loss in lung function in apparently healthy middle-age individuals. Decline in $FEV_{1\cdot0}$/ FVC was associated with underfilling of the left heart and low cardiac output, whereas decline in FVC with preserved $FEV_{1\cdot0}$/FVC was associated with left ventricular hypertrophy and diastolic dysfunction. A still debated problem is the use of beta-blockers in patients with coexisting COPD and cardiovascular disorders. Although recent reports showed an increase in survival and a decrease of exacerbations in COPD patients on beta-blocker therapy, there is still reluctance to prescribe them, perhaps because some of these patients may also have asthma. The first-line drugs for COPD treatment are bronchodilators, i.e., long-acting muscarinic antagonists (LAMA) and long-acting $beta_2$-agonists (LABA). Because of their pharmacological properties both may have cardiac side effects. Only recently, a large clinical trial in 16,485 COPD patients with heightened cardiovascular risk showed that 3-yr treatment with LABA alone or in combination with inhaled corticosteroid did not increase the occurrence of cardiac adverse events. A similar safety profile in patients with cardiovascular risk had been reported also for LAMA monotherapy.

O3.23
Intermittent fasting in heart failure: a pilot study

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Background: The effects of Calorie Restriction (CR) on cardiovascular system have been deeply studied, with an overall beneficial impact on systemic inflammation, traditional risk factors, vascular oxidative stress and endothelial function. Also, studies conducted on laboratory rodents and primates have shown that CR ameliorates the age-associated cardiovascular impairment of left ventricular diastolic function and arterial elasticity.

However, sustained CR is hard to follow for the majority of people and it is associated with side effects (i.e. reduced bone mineral density).

The term "Intermittent Fasting" (IF) encompasses different paradigms involving alternating between periods of unrestricted

feeding and periods of dietary restriction. Data from preclinical experiments in animals suggest that IF leads to improved cardiovascular risk profile and it can be easier to follow.

Heart Failure (HF) is increasingly recognized as a multisystem disease with important metabolic comorbidities. Inflammation, endothelial dysfunction and oxidative stress are key pathophysiologic elements.

Aims: 40 overweight/slightly obese HF outpatients (NYHA I-II) will be randomly assigned to IF or control group for six months. IF scheme is a '5:2-diet' (ad libitum food for 5 days during the week and fasting -i.e. a maximum of 500 Kcal intake- for two nonconsecutive days).

Primary objective of this study is to determine whether IF reduces the level of chronic inflammation (decrease in high sensitive C-reactive protein level).

The secondary objective is to determine whether IF mimics the metabolic and cardiovascular effects of CR, evaluating different outcomes:

- body weight, waist circumference and body composition modifications measured by DEXA
- lipid profile, glucose tolerance
- oxidative stress markers
- blood pressure
- arterial stiffness measured by Pulse Wave Velocity and Augmentation Index
- arterial endothelial function measured with the flow dependent vasodilation technique
- heart stiffness measured by transthoracic echocardiogram

Serum samples will be collected for future analysis (hormones, adipokines).

Samples collection regarding oral microbiota will be held too.

O3.24

Chemotherapy-related cardiac dysfunction – a systematic review of genetic variants modulating individual risk

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Background: Chemotherapy-related cardiac dysfunction (CTRCD) is a prognostic significant side effect of anticancer treatment. Known treatment- and patient related risk factors do not adequately explain individual CTRCD risk, indicating the presence of other determinants. Genetic variation may contribute considerably to individual CTRCD risk.

Objective: The aim of this systematic review was to provide a comprehensive overview of all genetic variants that have been implicated to influence susceptibility to CTRCD and asses their added value in CTRCD risk stratification.

Methods: We conducted a systematic literature search in PubMed and Embase, to identify studies investigating genetic risk factors for CTRCD. The validity of identified studies was assessed on the basis of ten criteria including amongst others the assessment of population stratification, statistical methodology and replication of findings in and independent cohort.

Results: In total, 33 studies met our inclusion criteria: 27 studies explored genetic risk factors for anthracycline-induced cardiotoxicity and 6 articles covered trastuzumab-associated cardiac damage. The majority (82%) of studies had a candidate-gene approach, whereas three GWAS have been performed. We identified 25 genetic variants in 20 genes that were reported significant in at least one study. The overall validity of the identified studies was limited, with small cohorts, failure to accurately assess population ancestry and lack of replication. Identified genes support the hypothesis that the genetic basis of CTRCD is multifactorial. SNPs with the most robust evidence up to this point are *CELF4* rs1786814 (sarcomere structure and function), *RARG* rs2229774 (topoisomerase-2ß expression), *SLC28A3* rs7853758 (drug transport), *UGT1A6* rs17863783 (drug metabolism) and one intergenic variant (rs28714259).

Conclusion: Existing evidence supports the hypothesis that genetic variation contributes to CTRCD susceptibility. Although a number of variants identified by this systematic review show potential to improve risk stratification, future studies are necessary for thorough validation and assessment of their value in a diagnostic setting.

O3.25

Real time fusion of MRI derived infarct zone and latest contracting segments with 3D fluoroscopy during cardiac resynchronization therapy implantation

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Background: Previous studies have shown that targeted left ventricular (LV) lead placement can improve response rate of cardiac resynchronisation therapy (CRT). Targeted LV lead placement can be performed using pre-implantation derived MRI images fused with X-ray images obtained during CRT implantation.

Purpose: To investigate the feasibility of real-time image fusion during CRT implantation.

Methods: Fifteen patients eligible for CRT will be included in this feasibility study, undergoing cardiac MRI prior to CRT implantation. MRI late gadolinium enhancement is used to determine areas of infarction and areas of latest contraction are determined with endocardial time-displacement curves using CINE-MRI. The MRI images are pre-processed into DICOM images using CARTBox software (CART-Tech, Utrecht, The Netherlands) CARTBox' derived DICOM images are fused with a 3D rotational X-ray obtained during CRT implantation. Real-time image fusion offers visualization of the infarcted (white) and latest contracting segments (green) on live X-ray, as shown in an anteroposterior projection. Thereby, LV lead placement can be guided to a tributary of the coronary sinus, away from infarct regions and towards the latest contracting area.

Results: The first patient was included recently. Fusion of MRI determined area of infarction and area of latest contraction was successful. A quadripolar LV lead was implanted in an inferolateral side branch of the coronary sinus, towards the basal part of the LV. There was no phrenic nerve stimulation (>10 mV) and pacing threshold was low (0·7–3·8 mV). Mean distance of the four quadripolar electrodes to the infarct region of the electrodes was 3·3 ± 0·8 mm. Lead position was adjacent to the latest activated region. Local electrical delay (QLV) was 113 ± 12 ms.

Conclusions: LV lead placement during CRT implantation can be guided by fusion of pre-implantation derived MRI images with 3D rotational X-ray images. Fourteen more patients will be included in the following months.

O3.26
Decreased kidney function is associated with intraplaque hemorrhage and fibrous-atheromatous content in patients undergoing carotid endarterectomy

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Objective: The aim of this study was to investigate the association between kidney function and atherosclerotic plaque composition in patients undergoing carotid endarterectomy (CEA).
Background: Chronic kidney disease (CKD) is associated with an increased risk of cardiovascular events (CVE). Acceleration of vascular inflammatory responses is considered to be causally involved in progression of atherogenesis in CKD patients. Although CKD has been associated with risk of CVE in CEA patients, the association between kidney function and plaque composition has not been thoroughly investigated yet.
Methods: Atherosclerotic plaques were harvested from 1826 patients who underwent CEA. After immunohistochemical staining, presence of macrophages, smooth muscle cells, calcifications, collagen, microvessels, lipid core size and intraplaque hemorrhage were examined. Cytokine levels were measured in a subgroup of randomly selected 552-1176 carotid plaques and plasma samples. Endpoint analysis was performed for moderate kidney failure and adverse outcome during follow-up after CEA.
Results: No consistent associations of decreased kidney function with inflammatory plaque or plasma markers was found, indicating that decreased kidney function was not associated with a more inflammatory plaque or systemic phenotype. Decreased kidney function was associated with increased odds ratio of intraplaque hemorrhage, OR 1·15 (95% CI: 1·02–1·29 (P = 0·024)) and increased odds ratio of fibrous-atheromatous plaques (plaques with lipid core presenting more than 10% of total plaque surface) OR 1·21 (95% CI: 1·07–1·38 (P = 0·003)) per decrease of 20 points in eGFR. End point analysis showed that all-cause mortality associated with the moderately impaired kidney function group with a HR 1·85 (95% CI 1·05–3·26 P = 0·035)
Conclusion: Decreased kidney function was associated with plaque hemorrhage and a fibrous-atheromatous plaque but not associated with inflammatory histological plaque characteristics. The current data suggests that intraplaque hemorrhage is involved in subsequent poor outcome in patients with decreased kidney function and severe atherosclerotic disease.

O3.27
New opportunities to tackle an old problem: hospitalizations in chronic heart failure

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Hospitalizations are a key feature of many chronic diseases, including heart failure. The talk will discuss their frequency, predictors and prognostic impact, and try to underline potential new opportunities and solutions.

O3.28
Study-outline – Deformation imaging to assess regional effects of cardiac regenerative therapy – a systematic review

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Background: Currently, left ventricular ejection fraction (LVEF) is the most commonly used endpoint in cardiovascular stem cell therapy research. This technique is effective in detecting global function changes within the heart, and has been shown to be a predictor of cardiovascular adverse events and mortality. A recent meta-analysis enrolling 2602 patients with ischemic heart disease has shown bone-marrow derived mononuclear stem cells to increase LVEF with 2·92%. Considering high inter- and intra-operator variability, we hypothesize that LVEF is unsuitable for detecting regional functional changes induced by regenerative therapy, while deformation imaging is more effective in detecting regional functional improvements by cardiac regenerative therapy.
Objective: The aim of this systematic review is to provide a comprehensive overview of the current literature on the added value of deformation imaging in cardiac regenerative therapy.
Methods: Following the PRISMA guidelines, we will perform a systematic review of current literature available on PubMed, Embase, Cochrane databases regarding animal models and patient studies in which deformation imaging was used to study cardiac stem cell therapy efficacy. After critical appraisal we aim to summarize outcomes of the included studies regarding study design, type of cell therapy, procedural characteristics, outcome measure, method for measuring strain and efficacy of regenerative therapy on both LVEF and deformation parameters. This will lead to a conclusion regarding the value of strain analysis in cardiac regenerative therapy.
Conclusion: Strain analysis might be superior to LVEF in the assessment of efficacy of cardiac regenerative therapy.

Workshop 4: Cancer Immunoregulation and Immunotherapy

O4.01
Adoptive immunotherapy with haploidentical KIR-ligand mismatched NK cells for acute myeloblastic leukemia patients

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Natural killer (NK) cells, which are defined by CD56 or CD16 expression and absence of CD3, have been shown to play a role in immune control of tumor development and growth. NK cells express activating and, more importantly, inhibitory receptors which recognize MHC class I alleles, termed "Killer cell Immunoglobulin-like Receptors" (KIRs). In the transplantation setting, preclinical and clinical data from haploidentical T-cell–depleted SCT have demonstrated that alloreactive KIR-Ligand (L)–mismatched NK cells play a major role as antileukemia effector cells and protect AML patients against leukemia relapse. We and others described the safety and feasibility of infusing allogeneic NK cells into AML patients outside the transplantation setting. Specifically, donor-versus-recipient alloreactive NK cells have been demonstrated in vivo by the detection of donor derived NK clones and adoptively transferred NK cells were alloreactive against recipient's leukemic cells (Blood 2011). Recently, we extended our previous experience by reporting the clinical and the correlative biologic results of KIR-L–mismatched NK cell adoptive immunotherapy in 17 elderly AML patients in CR (Clin Cancer Res 2016). Our results confirm that NK cell–based therapy is feasible and has a potential clinical benefit for elderly patients with poor prognosis AML. Moreover, the positive correlation between the frequency of donor alloreactive NK cell repertoires and the clinical outcome strongly indicates the therapeutic effect of the whole procedure relies on the immunologic activity of alloreactive NK cells included in the graft rather than on the chemotherapy. Thus, NK cell infusion has the potential to prevent disease relapse in patients with AML. These results have paved the way for designing a new generation of clinical studies aiming to prove the clinical efficacy of NK cell-based immunotherapy in the clinical management of AML patients (Exp Hematol 2016).

O4.02
Anti-cancer telomerase vaccines are entering the age of maturity

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Telomerase, the enzyme synthesizing the telomeric regions of chromosomes, is considered a universal tumor associated antigen because expressed by the majority of cancers. The studies performed in the last 15 years have progressively swept away the doubts initially related to its immunogenicity. In particular, the immunogenicity concerns have been now dispelled by demonstrations that: (1) telomerase is presented by tumor and antigen presenting cells; (2) ex vivo generated telomerase-specific CTL kill efficiently telomerase-expressing tumors; (3) circulating telomerase-specific T cells are present in 90% of cancer patients and, surprisingly, in 100% of healthy individuals. These findings boost the search for a new generation of telomerase vaccines able to overcome the limits of their first generation. In this effort, our group recently completed a phase I/II trial in prostate and renal cancer patients with GX301, a new generation cancer vaccine. This multi-peptidic vaccine includes four telomerase peptides, which bind promiscuously several HLA class I and II alleles allowing the coverage of the majority of HLA haplotypes and the induction of both helper and cytotoxic T cell responses. It also contains two adjuvants with complementary activities, making it able to efficiently activate both innate and adoptive immune responses. The results of a phase I/II trial, showing a 100% rate of telomerase-specific immune responses associated with evidences of clinical responses, suggest that telomerase vaccination is entering the "age of maturity".

O4.03
Immunoregulation in leukemia: the role of Ido

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The cancer cell death induced by some chemotherapeutic agents, especially anthracyclines, such as daunorubicin (DNR), is highly immunogenic and results in the efficient cross-priming of tumor antigens via dendritic cells (DCs). Such process, named immunogenic cell death (ICD), is characterized by intracellular and pericellular modifications, including the translocation of calreticulin (CRT) from nucleus to cell surface as well as the extracellular release of high mobility group box 1 (HMGB1) and adenosine triphosphate (ATP). Recent reports indicate that anticancer drugs, while triggering ICD, induce expansion of regulatory T cells (Tregs). DCs are key regulators of adaptive immunity, promoting or suppressing T-cell responses, the latter occurring through indoleamine 2,3-dioxygense (IDO), which is a potent inducer of Tregs.

Here, we report that ATP released from dying acute myeloid leukemia (AML) cells during ICD concomitantly activates antileukemia immune response and induces Tregs accumulation through IDO1 up-regulation in DCs. *Ex vivo* analysis of AML patients, undergoing DNR-based chemotherapy, revealed a concomitant induction of leukemia-specific CTLs and Tregs. Similarly, in a mouse AML model, DNR treatment increased plasma levels of both activatory (IFN-γ, IL-1β, TNF-α, IL-12) and tolerogenic (IL-10) cytokines. Interestingly, tumor-infiltrating T cells after DNR treatment showed increased Tregs. DNR treatment promoted ATP release from AML cells *in vitro* and *in vivo*. In DNR-treated mice a significant increase of $CD11c^+$ mature DCs which express IDO1 in tumor infiltrate was observed. *In vitro*, loading of DNR-treated AML cells into DCs induced full maturation, but also IDO1 upregulation.

Interestingly, extracellular ATP was directly involved in DCs maturation and IDO1 expression via purinergic receptor P2Y11. Overall, these data suggest that combining chemotherapy with IDO1 inhibitors may fully exploit the immunogenic potential of conventional antineoplastic agents.

O4.04
CAR-T cells in oncology: challenges and opportunities

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T cells genetically engineered with chimeric antigen receptors are on the verge to revolutionize the way cancer is managed and finally cured. In order to fulfill this promise, however, a number of hurdles need to be successfully addressed. These include the identification of target antigens different from CD19, the implementation of strategies to control toxicities and, finally, the democratization of manufacturing outside clinical trials and highly specialized centers. My talk will focus on these issues and propose smart solutions in order to transform challenges in exciting opportunities.

Workshop 5: Lipidology and Atherosclerosis

O5.01
Autoimmunity against HDL/apoA-1: an emergent marker and mediator of CV risk?

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Since the initial discovery in 1998 of autoantibodies against high-density lipoprotein cholesterol (anti-HDL IgG) and its major protein fraction apolipoprotein A-1 (anti-apoA-1 IgG) in the serum of patients suffering from systemic lupus erythematosous and anti-phospholipid syndrome, a cumulating body of evidence point to humoral autoimmunity against HDL/apoA-1 as a cardiovascular-relevant molecule. To summarize, most clinical studies have demonstrated that anti-apoA-1/HDL IgG are associated with prevalent and incident CHD in subjects with and without autoimmune diseases, as well as in the general population, independently of established cardiovascular risk factors (CVRFs). Nevertheless, currently unpublished data derived from a genome-wide association study population-based cohort indicate that taking individual genetic susceptibility may be required for accurate anti-apoA-1 IgG-related CV risk prediction. These results also provide initial pieces of understanding regarding the determinants anti-apoA-1 IgG/HDL existence as being influenced both by autoimmunity-predisposing genes and environmental factors, such as HDL-raising therapeutics. In parallel, anti-apoA-1/HDL IgG were shown to play i) a direct pathogenic role as pro-inflammatory and pro-arrhythmogenic mediators acting through specific innate immune receptor and cellular pathways, promoting atherogenesis, myocardial necrosis and mortality in mice, and ii) an indirect pathogenic role by promoting HDL dysfunction. Furthermore, it was also demonstrated that the human anti-apoA-1 IgG response was biased toward de c-terminal part of apoA-1, allowing the utilization of an optimized apoA-1 mimetic peptide to be used both for the detection and the neutralization of anti-apoA-1 IgG. In this translational review, we will cover the main results obtained in this field, covering clinical, in vitro, and in vivo studies to appraise the role anti-HDL/ApoA-1 antibodies as of emergent markers and mediators of CV risk. As such, autoimmunity to HDL/ apoA-1 could represent an emerging therapeutic target allowing the identification of high CV-risk individuals that could benefit from immunomodulation in top of standard of care.

O5.02
PCPE2 promotes adipocyte maturation via regulation of SR-BI activity

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Procollagen C-endopeptidase enhancer protein 2 (PCPE2) is a protein that enhances the cleavage of the C-termini of procollagen by bone morphogenetic protein 1. But the function of PCPE2 is not limited to collagen maturation. Recently our lab reported that PCPE2 increased HDL-associated cholesteryl ester uptake via scavenger receptor class B type 1 (SR-BI), an HDL receptor highly expressed in adipose tissue. Interestingly, TwinsUK study in humans provided data showing that PCPE2 mRNA is highly correlated with adipose tissue distribution. Our lab observed a reduced size of visceral fat pads in PCPE2 deficient mice despite no difference in body weight compared to wild type. To study the molecular and cellular mechanisms of how PCPE2 regulates SR-BI function and how it affects adipose tissue formation, we generated a PCPE2 knockout ($PCPE2^{-/-}$) line from 3T3-L1 cells using CRISPR-Cas9. Induction of 3T3-L1 cells to differentiate into mature adipocyte increases the expression of both PCPE2 and SR-BI around 3 fold, which parallels the process of lipid droplet formation. Immunofluorescence studies showed that PCPE2 is distributed all over the cell in mature adipocyte, while SR-BI is distributed along the cytoplasmic and lipid droplet membranes. in addition to the perinuclear region. More interestingly, $PCPE2^{-/-}$ 3T3-L1 cell shows an impairment in lipid droplet formation during the induction of differentiation, with <10% of cells generating lipid droplets, and the size of lipid droplet being only about 50% of that in wild type. Although $PCPE2^{-/-}$ 3T3-L1 cell expresses SR-BI at a higher level compared with wild type cell, nearly 50% of the SR-BI in wild type cell are in homodimer structure while $PCPE2^{-/-}$ 3T3-L1 cell has less SR-BI multimer, indicating that SR-BI in $PCPE2^{-/-}$ 3T3-L1 cell is inactive. We conclude that PCPE2 plays a critical role in maintaining SR-BI conformation, which, in turn, controls adipocyte maturation.

O5.03
HDL functionality as a new possible clinical biomarker

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Several epidemiological studies define HDL as the most powerful plasmatic factor with atheroprotective activity in humans. Some post hoc analyses from randomized controlled trials also suggest that raising HDL-C beneficially affects the risk of CVD. However, the clinical efficacy of raising plasma HDL-C levels to achieve cardiovascular risk reduction has been difficult to prove. Published outcome trials involving the addition of niacin or dalcetrapib to standard low-density lipoprotein cholesterol reduction therapy failed to demonstrate clinical benefit despite increases in HDL-C concentration. Furthermore, genetic variants associated with increased HDL-C, thus conferring lifelong exposure to higher circulating levels, are not consistently associated with improved vascular outcomes. These findings have reinforced the idea that changes in HDL-C levels are an inadequate surrogate for therapeutic use. Therefore, an emerging concept is that of quality of HDL, which are heterogeneous in terms of size, charge and lipid content and display functional differences, such as cell cholesterol efflux promotion. Cholesterol efflux from peripheral tissues is a key function of HDL particles, and the first step of reverse cholesterol transport to the liver for biliary secretion. Recent studies have shown that cholesterol efflux capacity (CEC), as a measure of HDL functionality in humans, is inversely associated with prevalent ASCVD and incident cardiovascular events in a population-based cohort without cardiovascular disease at baseline. These associations were independent of HDL-C and traditional cardiovascular risk factors, which suggested that the HDL-C level is only a modest

biomarker of HDL function, and that CEC may be more closely correlated with cardiovascular outcomes. This review of different published results could help to better understand that HDL-CEC might provide an alternative mechanism for therapeutic modulation of the HDL pathway beyond HDL cholesterol concentration to help reduce risk of coronary heart disease.

O5.04
HDL from chronic kidney disease exhibits an increase in sphingosine-1-phosphate content and superior oxidative stress protection in vitro

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Background: Chronic kidney disease (CKD) exacerbates the risk of death due to cardiovascular disease (CVD). Modifications to blood lipid metabolism which manifest as increases in circulating triglycerides and reductions of high density lipoprotein (HDL)-cholesterol are thought to contribute to increased risk. In CKD patients, higher HDL-cholesterol levels were not associated with reduced mortality risk. Recent research has revealed numerous mechanisms by which HDL could favourably influence CVD risk. In this study, we compared plasma levels of sphingosine-1-phosphate (S1P), HDL-associated S1P (HDL-S1P) and HDL-mediated protection against oxidative stress between CKD and control patients.
Methods: HDL was individually isolated from 20 CKD patients and 20 controls. Plasma S1P, apolipoprotein M (apoM) concentrations, HDL-S1P content and the capacity of HDL to protect cardiomyocytes against doxorubicin-induced oxidative stress *in vitro* were measured.
Results: CKD patients showed a typical profile with significant reductions in plasma HDL-cholesterol and albumin and an increase in triglycerides and pro-inflammatory cytokines (TNF-alpha and IL-6). Unexpectedly HDL-S1P content ($P = 0{\cdot}001$) and HDL cardioprotective capacity ($P = 0{\cdot}034$) were increased significantly in CKD patients. Linear regression analysis of which factors could influence HDL-S1P content showed an independent, negative and positive association with plasma albumin and apoM levels, respectively.
Discussion: The novel and unexpected observation in this study is that uremic HDL is more effective than control HDL for protecting cardiomyocytes against oxidative stress. It is explained by its higher S1P content which we previously demonstrated to be the determinant of HDL-mediated cardioprotective capacity. Interestingly, lower concentrations of albumin in CKD associated with higher HDL-S1P.

O5.05
Homozygous FH: updates of prevalence, genetics and therapeutical approaches

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Homozygous Familial Hypercholesterolemia (HoFH) is a rare autosomal codominant disorder caused by – in order of decreasing prevalence mutations in the genes encoding the LDL receptor (LDLR; OMIM#606945), apolipoprotein B (ApoB; OMIM#107730), and/or proprotein convertase subtilisin-kexin type 9 (PCSK9; OMIM #607786). Loss-of-function mutations in LDLR and ApoB cause familial hypercholesterolemia (FH1) and familial defective apolipoprotein B (FDB or FH2), respectively. Even the rare gain-of-function mutations in PCSK9 (FH3) have also been shown to cause an HoFH phenotype. As a consequence of impaired LDL-R function, untreated total plasma cholesterol levels are typically >13 mmol/l, resulting in premature and progressive atherosclerosis often leading to cardiovascular disease (CVD) before age 20 years, and death before age 30 years. Early initiation of aggressive treatment for these patients is, therefore, essential. The prevalence of HoFH has been frequently discussed and ranged in particular from 1 in 1000 000 in the early reports in the 1970s to a prevalence of 1:30 000 to 1:275 000 in more recent studies. Patients with HoFH respond inadequately to conventional drug therapies (resins, statins, ezetimibe), which generally reduce LDL cholesterol (LDL_C) through upregulation of hepatic LDLRs. Therefore, the current standard of care for FH includes Lipoprotein Apheresis (LA), which transiently reduces LDL_C by more than 60% and can delay the onset and progression of atherosclerosis. However, even with the combined use of available drug therapies and LA, these patients still have substantially elevated levels of LDL_C and persistently high risk of CVD. In recent years, alternative therapeutic approaches have been developed that target either ApoB synthesis, the production of VLDL, the precursor of LDL (Microsomal Triglyceride Transfer Protein - MTP) and PCSK9, an enzyme encoded by the PCSK9 gene in humans on chromosome 1, which binds to the receptor for low-density lipoprotein particles (LDL).

O5.06
Anti-Apolipoprotein A1 autoantibodies disrupt the cholesterol pathway via Srebp-2 and decrease circulating miR-33a in hypercholesterolaemic children

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Humoral autoimmunity has recently been shown to represent an important modulator of atherogenesis and cardiovascular diseases through different cellular pathways. Furthermore, autoantibodies were recently shown to modulate the production of

microRNA (miRNA) involved in atherothrombosis, indicating that autoantibodies could modulate atherosclerosis through miRNA synthesis. Anti-ApolipoproteinA-1 antibodies (anti-ApoA-1IgG) were recently shown to promote inflammation and atherogenesis through toll-like receptor (TLR) 2/4 complex signalling. Nevertheless, given their specificity, knowing whether anti-ApoA-1 IgG could also modulate cholesterol homeostasis by interfering with miRNA synthesis is unknown. Therefore, we explored the possible association between anti-ApoA-1 IgG and miR-33a, a key lipid homeostasis regulator, in hypercholesterolaemic children ($n = 29$), and we evaluated the impact of anti-ApoA-1 IgG on the production of miR-33a, and crucial proteins involved in cholesterol homeostasis (CH) using human monocyte-derived macrophages (HMDM).

Prevalence of high anti-ApoA-1 IgG levels was 24% and unrelated to familial hypercholesterolemia. Children with high anti-ApoA1IgG levels had lower levels of circulating *miR-33a* than children tested negative for those autoantibodies (9·59 vs 7·92, $P = 0·02$ respectively, values represent the median ΔCt of miR-33a normalized to cel-miR-39a, so higher ΔCt correspond to lower miR-33a level) and a negative Spearman correlation between miR-33a and anti-ApoA-1 IgG was observed ($r = -0·3$, $P < 0·05$). On HMDM, anti-ApoA-1 IgG lead to a decrease of miRNA-33a synthesis (0·6 vs 1·3 $P = 0·041$) as quantified by real-time PCR, but induced a TLR/NFkB/AP1-dependent increase of SREBP-2, LDL-R, HMGCR, SCAP and ABCA1 according to western blot analyses. Control antibodies were devoid of any effects.

In conclusion, we report for the first time that high levels of anti-ApoA-1IgG exist in children with hypercholesterolemia where they are associated with lower *miR-33a* levels. Our HMDM model highlights a new role for anti-ApoA-1IgG as modulator of CH through a complex interplay involving miR-33a, TLR2-4, and key proteins/enzymes of lipid metabolism which awaits further investigations.

O5.07
Gene expression analysis of scavenger receptors class B in pathogenesis of atherosclerosis

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Introduction: It is well known that after the oxidation the low density lipoproteins (oLDL) must have to be utilized. They quite easily recognized by scavenger receptors located mainly in the monocytes/macrophages cell membranes but some of them such as CD36, SCARB1 and SCARB2 that belong to class B of scavenger receptors do not selectively bind to oLDL. Anyway, they can play a certain role in atherogenesis but it's still not so clear. So, the aim of this study was to determine the *CD36*, *SCARB1* and *SCARB2* genes expression in peripheral blood cells and plaques in patients with different atherosclerosis lesions.

Materials and methods: Gene expression analysis was carried out on venous samples blood and atherosclerotic plaques (for IPA patients only) from 46 patients with acute coronary syndrome (ACS), 48 patients with confirmed multifocal atherosclerosis (IPA) and 16 healthy donors. The relative gene expression level (RQ) evaluated by the 2deltaCt method and Student t-test ($P = 0·05$).

Results: Gene expression analysis for *CD36*, which triggers a cascade of inflammatory reactions and foam cells formation showed a significantly increased activity (RQ 26·12, $P = 0·047$) in atherosclerotic plaque and at the same time decreasing expression level in peripheral blood in patients with IPA (RQ 1·19). However, the *SCARB2* gene analysis showed the dramatically decreasing level of gene activity in both groups (RQ 0·0035 and 0·031, $P < 0·001$ for IPA and ACS respectively) and no gene activity was detected in atherosclerotic plaques. Also we found no gene expression for *SCARB1* gene in atherosclerotic patients with different atherosclerosis lesions.

Conclusion: Our findings indicate that the class B scavenger receptors genes expressed differently in patients with atherosclerotic lesions and it can be served as a marker for progression of atherosclerotic plaque formation.

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O5.08
Clinical management of Heterozygous Familial Hypercholesterolemia (FH)

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HeFH is the most common monogenic cause of raised serum cholesterol, affecting about 1 in 500 people at least. New data from different countries suggest a higher prevalence. It is dominantly inherited. Affected family members have LDL cholesterol (LDL-C) levels typically double those of unaffected first degree relatives. Serum cholesterol is thus commonly 9–12 mmol/l in affected adults. LDL cholesterol is high since birth. It is higher from birth and HeFH can be diagnosed in childhood. Untreated it results in tendon xanthomata typically in the Achilles tendons and extensor tendons on the dorsum of the hands. Subperiosteal xanthoma is also sometimes present on the tibial tuberosities. CVD occurs with increasing frequency from the third decade so that without medical intervention over half of affected men and 15% of affected women die before the age of 60 years. The clinical syndrome of HeFH results from defective LDL catabolism. Most cases are due to mutation of the LDL-receptor. A smaller proportion are a due to mutations of the apoB100 gene (Familial Defective ApoB), which interferes with its binding to the LDL-receptor. The most common of these is apoB3500, but it is only a minority of heterozygotes with this who express hypercholesterolemia of such severity as to cause HeFH. On the other hand, gain-of-function mutations of proprotein convertase subtilisin-kexin9 (PCSK9), an uncommon cause of HeFH. PCSK9 is involved in the degradation of hepatic LDL receptors. There has been a dramatic reduction in premature mortality coincident with the introduction of statin therapy. Early treatment removes modifies excess cardiovascular risk. Case finding and family cascade family screening is indicated to prevent premature cardiovascular morbidity and mortality. New therapies like PCSK9 monoclonal antibodies are also highly effective in lowering LDL-C allowing the achievement of normal LDL-C levels even in cases with severe heterozygous Familial Hypercholesterolemia.

O5.09

Circulating miR-200c is up-regulated in pediatric patients with familial hypercholesterolemia and correlates with miR-33a and miR-33b levels

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Hypercholesterolemia is a major risk factor for atherosclerosis and the development of cardiovascular disease. miRNAs play a major role in the low-grade inflammatory process induced by hypercholesterolemia and may have a pro-atherogenic action. miR-33a/b modulate cholesterol homoeostasis, high-density lipoprotein-cholesterol (HDL-C) formation, fatty acid oxidation and insulin signaling. We previously showed that the circulating miR-33a/b expression levels are up-regulated in children with familial hypercholesterolemia (FH). miR-33b has been recently showed to target directly the transcription factor ZEB1. ZEB1 inhibits miR-200 family (miR-200s) expression and miR-200s, in turn, inhibit ZEB1 protein expression. Therefore, an increase of miR-33, downregulating ZEB1, could enhance miR-200s. Our previous studies showed that miR-200 family is induced by oxidative stress and miR-200c is the member of the family most up-regulated in endothelial cells (ECs). Moreover, we showed that miR-200c is responsible for ECs apoptosis and senescence.

We aimed to verify whether circulating miR-200c is induced in FH in pediatric age, and whether a correlation exists between miR-33a/b and miR-200c.

28 FH children were compared with 25 age-matched healthy subjects (HS) .Total RNA was extracted from plasma and miR-200c levels were measured by quantitative real-time PCR.

We found that miR-200c was significantly up-regulated in FH compared to HS (4·00 ± 0·48-fold increase, $P < 0·05$) and exhibited a significant positive correlation with miR-33a and miR-33b (Rs = 0·68, $P \leq 0·0001$; Rs = 0·66, $P \leq 0·0001$, respectively). Interestingly, miR-200c did not correlate with Total Cholesterol (C), LDL-C, HDL-C, ApoB or Triglycerides. In contrast, miR-200c, correlated with CRP plasma levels (Rs = 0·39 $P < 0·01$) and glycemia (Rs = 0·51 $P < 0·01$), although these children are not insulin resistant and display a normal glycemia.

In conclusion circulating miR-200c is up-regulated in FH; this increase is directly related to CRP and glycemia and unrelated to plasma lipids. This modulation might be ascribed to an increase of oxidative stress and inflammation associated to FH and a miR-33a/b-dependent ZEB1 down-modulation.

O5.10

New therapeutical strategies in statin intolerant He-Fh patients

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Introduction: This is a clinical report of six patients suffering from genetically characterized Heterozygous Familial Hypercholesterolemia (He-FH), statin intolerant, treated with Alirocumab or Evolocumab (the new anti-PCSK9 monoclonal antibodies).

Methods: One patient (male) was in secondary prevention, five patients (two females and three males) were in primary prevention. At baseline three patients were free from any oral therapy, three patients assumed Ezetimibe 10 mg/die, in one case in association to a nutraceutical pill with red rice, berberine and policosanols (RR/BBR/P), in one case in association to Fenofibrate 145 mg/die. Patients were treated with Alirocumab 75 mg or 150 every 14 days or Evolocumab 140 mg every 14 days.

Results: At the first LDL–C control, after four weeks of administration of monoclonal antibody at the maximum dose, in the three patients without oral therapy, the LDL-C was reduced of 25-35% respect to baseline: adding plant sterols 1·6 g/die and yeast red rice with monacoline K 5-10 mg, well tolerated by patients, after four weeks, we obtained a significant reduction of LDL-C from the baseline (40–55%). The same results were obtained in the two patients previous treated with ezetimibe alone or plus RR/BBR/P. In the patient in therapy with Ezetimibe plus Fenofibrate, the administration of Alirocumab 75 mg, after eight weeks, determinated LDL-C reduction of 48%.

Conclusion: The clinical report of these six cases demonstrated the efficacy of PCSK9 inhibitors, but, in genetically characterized He-FH patients, with very high LDL-C baseline levels, they are not sufficient to reach LDL-C target when administrated alone. Inhibition of HMG-CoA reductase with monacolin K and/or of cholesterol absorption with plant sterols or Ezetimibe could help physician to obtain LDL-C target.

O5.11

Elevated circulating PCSK9 concentrations predict subclinical atherosclerotic and metabolic changes in low risk obese and non-obese patients

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Background: PCSK9, as well as adipose tissue is closely related to the development of CV diseases. Aim of this study was to investigate the relationship between circulating PCSK9 levels, subclinical vascular changes and liver steatosis in obese and non-obese patients with low CV risk.

Methods: 120 healthy patients, without CV diseases, DM, lipid-lowering therapy, were divided into three groups based on BMI: normal weight ($N = 50$), overweight ($N = 30$) and obese ($N = 40$). Lipid and non-lipid parameters and PCSK9 levels were quantified. Vascular changes were detected by carotid ultrasound (cIMT) and by echo-tracking method for detection of arterial stiffness parameters (PWV, AI, Beta). Liver steatosis quantification was based on the calculation of hepatorenal index (HRI).

Results: Significant increase in plasma levels of PCSK9 in obese vs. overweight and vs. normal weight group was detected. PCSK9 levels significantly correlated with values of BMI. In addition to increases in laboratory parameters, significant increase in HRI, cIMT and parameters of functional and structural vascular changes (Beta, AI, PWV) were detected in the groups with elevated BMI. Significant positive linear correlation of PCSK9 concentrations and cIMT, PWV and Beta was found. In multivariable regression analysis after adjusting for sex, age, BMI, LDL, impact of PCSK9 on cIMT remained significant; as well as for Beta, and PWV. Patients with higher BMI had a higher prevalence of fatty liver, but not in all cases associated with subclinical vascular changes. Presence of vascular changes in patients with steatosis was accompanied by significant elevation of PCSK9 concentration in comparison with the subgroup with steatosis without vascular changes.

Conclusions: Plasma levels of PCSK9 significantly correlated with subclinical vascular changes. Lower plasmatic levels of PCSK9 were the predictor of "safety" steatosis without the vascular changes and inflammatory profile. We assume that PCSK9 could be an important mediator as well as an early marker of vascular and cardiometabolic changes in obese, as well as non-obese patients.

Workshop 6: Hematology

O6.01
Integration of somatic mutations in diagnosis and risk assessment in myelodysplastic syndromes (MDS)

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The combination of overt marrow dysplasia and clonal cytogenetic abnormality allows a conclusive diagnosis of MDS, but this is found in only a portion of patients. Major efforts have been made to identify novel diagnostic tools that may increase the accuracy of the diagnosis of MDS. Recently, genes encoding for spliceosome components (SF3B1, SRSF2, and others) were identified in a high proportion of patients with MDS. A major step forward in genotype-phenotype correlation in MDS has been represented by the identification of somatically acquired mutations in SF3B1 in patients with ring sideroblasts; this finding may lay the foundation stone for a molecular classification of MDS. Although most of mutated genes in MDS can be detected in different myeloid neoplasms and are not specific for MDS, they may be of value to provide evidence for a clonal disorder in patients with suspected MDS.

MDS are a heterogeneous group of disorders, and risk-adapted treatment strategy is mandatory for conditions showing a so highly variable clinical course. In 1997, Greenberg and coworkers developed the International Prognostic Scoring System (IPSS). The IPSS proved to be useful for predicting survival and risk of leukemic evolution in patients with MDS, and has been the reference for clinical decision-making. Recently, IPSS was revised based on a large multicenter cohort of untreated patients. Recently, a comprehensive analysis in 439 MDS patients identified mutations in five genes - ASXL1, RUNX1, TP53, EZH2, and ETV6 - that were significant predictors of poor survival, after adjustment for IPSS risk groups. Moreover, SF3B1 mutations were found to be independent predictors of favourable clinical outcome.

Taken together, these evidences suggest that the integration of somatic mutations in next-generation prognostic scoring systems is resulting in more accurate stratification of individual patient risk and further refine clinical decision making in MDS.

O6.02
Lower risk myelodysplastic syndromes

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Myelodysplastic syndromes (MDS) are clonal stem cell disorders characterized by unproductive hematopoiesis leading to peripheral blood cytopenias, and by a high incidence of progression to acute myeloid leukemia. The pathophysiology of MDS is a multistep process including genetic changes or smaller anomalies demonstrable only by more sophisticated methods. Somatic mutations can involve genes encoding signaling molecules (NRAS, KRAS, CBL, JAK2, FLT3), epigenetic regulators (TET2, ASXL1, EZH2, UTX, IDH1, IDH2, DNMT3A, SETBP1), splicing factors (SF3B1, SRSF2, ZRSF2, U2AF1), and transcription regulators (RUNX1, NPM1 and TP53). Widespread gene hyper-methylation, on the other hand, is a major finding during progression of MDS.

Lower-risk MDS are defined as having low or intermediate 1 risk by the International Prognostic Scoring System (IPSS) and are characterized mainly by anemia in most cases.

Supportive care—primarily red blood cell transfusions—remains the cornerstone of their treatment, but exposes patients to inadequate correction of anemia, alloimmunization, and organ iron overload.

Treatment aimed at preventing anemia recurrence should therefore be used whenever possible. Erythropoiesis stimulating agents remain the first-line treatment of anemia in most lower-risk MDS without del(5q), whereas anemia of low-risk MDS with del 5q responds to lenalidomide in approximately 60% of the cases. A new recombinant fusion protein consisting of a modified form of the extracellular domain of the human activin receptor type IIB linked to the human immunoglobin G 1 (IgG1) Fc domain luspatercept (now in advanced phase III study) is able to provide transfusion independence in a significant number of patients.

Some patients, while remaining at a "lower risk" MDS level, have severe cytopenias and/or poor prognostic factors, found using newer prognostic parameters, or resistance to treatment, making them suitable candidates for more intensive approaches, including allogeneic hemopoietic cell transplantation.

O6.03
Post-transplant cyclophosphamide (PT-CY) regimen following unmanipulated Haploidentical bone marrow transplantation post myeloablative conditioning

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Background and aims: Haploidentical bone marrow transplantation with post-transplant cyclophosphamide (PT-CY) has been increasingly used for patients lacking a suitable HLA-matched donor.

In this study we assessed outcomes of 282 consecutive haploidentical transplantations followed by a uniform GvHD prophylaxis, as previously published by our group: cyclosporine from day 0, mycophenolate from day +1, and post-transplant cyclophosphamide 50 mg/kg on days +3 and +5.

Patients: All patients received a myeloablative conditioning containing thiotepa, fludarabine, busulfan (three or two doses), or TBI-fludarabine (n = 55). The median age was 48 years (17–74); at transplant 145 (51%) patients were in remission of disease (CR1 and CR2), and 137 had active disease (49%); all were first grafts. The diagnosis included AML (n = 111), MDS (n = 31),

ALL (n = 96), MPN (n = 43), NHL (n = 19), CLL (n = 9) and MM (n = 13).

Results: The median follow up was 562 days (range, 6–2241 days). Median number of infused cells was 3·4 × 10E8/kg (range 1·1–7·7). There were 7 pre-engraftment deaths and 21 graft failures. Full-donor chimerism on day +30 was reached in 254 (90%) patients. The median day for neutrophil engraftment was day +18 (range 13–60). The cumulative incidence of grade II-IV and III-IV acute GVHD (aGVHD) was 17% (n = 49) and 5% (n = 15) respectively. 2-years cumulative incidence of moderate–severe chronic GVHD (cGVHD) was 13% (n = 39). Sixty-one (21%) patients experienced haemorragic cystitis.

3-year cumulative incidence of non relapse mortality (NRM), relapse and relapse related death were 17% (n = 47), 32% (n = 91) and 25% (n = 69) respectively. Main causes of death were infections (n = 34) and hemorrhage (n = 7).

4-year overall survival and disease free survival were 55·7% and 47% respectively.

Conclusions: A modified PT-Cy schedule as GVHD prophylaxis after MA conditioning regimen followed by haploidentical BMT results in a low risk of aGVHD and cGVHD and encouraging rates of TRM and DFS.

O6.04
Therapy of high risk myelodysplastic syndrome

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Unfavorable karyotype and bone marrow blast over 10% are the principal risk factors for progression to acute myeloid leukemia (AML).

Biologically high risk myelodysplastic syndrome (MDS) and AML of elderly are the same entity.

For this reason, biological studies focused on epigenetic and genetic factors may be applicable to both clinical conditions.

In the past there weren't any efficacious treatment options, except intensive chemotherapy or allogenic stem cell transplantation; nowadays different therapies and targeted therapies are available and rely upon the results of the biological studies mentioned above.

Evidences of methylation of CpG –islands lead to the development of hypomethylating therapies, which are the milestone corner of the actual treatment and will be in near future the backbone of more targeted therapy with novel agents.

The founders of hypomethylating therapy are azacytidine and decitabine and as single agents can give hematological improvements in nearly 50% of patients with complete remission (C R) rate of 20-30%.

The overall response rate may improve with combination therapies.

The combination of lenalidomide and azacytidine is able to obtain durable response in nearly 60% of high risk MDS patients.

The combination of iron chelation therapy and azacytidine is able to reduce either the genetic damages induced by superoxide and thus to obtain CR in nearly 60% of patients.

In those carrying the FLT3-ITD mutation, Azacytidine plus sorafenib can obtain 30% of CR. It's unknown the potential of combining midostaurin and hypomethylating agents and/or quizartinib.

Potential new approaches include the combination of targeted therapies (i.e. IDH1 inhibitors, idasanutlin) and hypomethylating agents.

Newer strategies may include:

1 the inhibition of BCL2 (Venetoclax), which is able to restore the inhibited pro-apoptotic pathway;
2 combination of immune check point inhibitors with azacytidine. Indeed Azacytidine is able to overexpress PD1 and PD1-L.

Only with future biological research, newer therapeutic strategies will be available.

Workshop 7: Phagocyte Biology

O7.01
Vasoactive intestinal peptide dampens fMLF-induced ROS production and inflammation by targeting a MAPKinases-p47phox phosphorylation pathway in human monocytes

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Reactive oxygen species (ROS) produced by the phagocyte NADPH oxide (NOX2) are needed for microbial clearance but when produced in excess they will exacerbate inflammatory response and injure surrounding tissues. NOX2 is a multicomponent enzyme composed of membrane associated cytochrome b588 and cytosolic components $p47^{phox}$, $p67^{phox}$, $p40^{phox}$ and rac1/2. We investigated if vasoactive intestinal peptide (VIP), an endogenous immune-modulatory peptide, could also affect ROS production by NOX2 in primary human phagocytes. We show that VIP alone was without effect but it dose-dependently inhibited ROS production induced by the bacterial peptide fMLF in human monocytes. The action of VIP was essentially mediated by high affinity G-protein coupled receptors VPAC1 as the specific agonist $[ALA^{11,22,28}]$VIP mimic VIP inhibitory effect while the antagonist PG97-269 blunted VIP action. Further, we showed that VIP inhibited fMLF-induced phosphorylation of ERK1/2, p38 pathways and the phosphorylation of $p47^{phox}$ on Ser345 residue. Also VIP was able to inhibit rat paw edema, induced by carrageenan, representing the first step of inflammatory response. These results show that VIP exerts anti-inflammatory effect by inhibiting the "MAPKinases-p47phox phosphorylation-NOX2 activation" axis. These data suggest that VIP could a natural anti-inflammatory agent of the mucosal system and its analogues could be novel anti-inflammatory molecules.

O7.02
Diverse stimuli engage different neutrophil extracellular trap pathways

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Neutrophils, the first line of host defence against pathogen attack, release neutrophil extracellular traps (NETs) to ensnare pathogens. As such neutrophils are central to innate immunity. However, NETs also have pathogenic functions in diverse diseases. To date many of the investigations into the mechanism of NET formation have relied on the use of the mitogen phorbol 12-myristate 13-acetate (PMA). We aimed to examine if the stimuli A23187 (a calcium ionophore), nigericin (a potassium ionophore), *Candida albicans* (a fungus) and group B streptococcus (GBS, a gram positive bacteria) utilise a similar pathway as PMA to induce NET formation. To carry out this study healthy neutrophils were examined for NET production in response the chosen stimuli in the presence of inhibitors of proteins known to be central to PMA induced NET formation (ex: PKC, neutrophil elastase, myeloperoxidase and reactive oxygen species, ROS). Neutrophils from patients with mutations in the pathways involved in NET formation, such as chronic granulomatous disease patients (CGD, patients with a mutation in proteins of the NADPH oxidase complex resulting in a lack of ROS generation by granulocytes) or MPO-deficient patients were also use to verify the pathways required for NET formation formed by the ligands of interest.

Our findings reveal that PMA and the physiological ligands *Candida albicans* and GBS make use of a related pathway for NET induction whereas the ionophores require very few of these signalling molecules for NET induction. This demonstrates that NET induction does not occur through one signalling pathway but is a widely varied method of host cell defence against attack.

O7.03
Regulation of neutrophil responses by P-rex and norbin

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Phosphatidylinositol (3,4,5)-trisphosphate-dependent Rac exchanger1 (P-Rex1) is a guanine nucleotide exchange factor (GEF) for the RAC family of small GTP-binding proteins (GTPases). It catalyses the active conformation of RAC, thus regulating many different cell responses, including neutrophil adhesion, motility and ROS production. We recently identified a new regulator of P-Rex1, the GPCR adaptor protein Norbin. This study revealed that Norbin can directly stimulate the Rac-GEF activity of P-Rex1 and promote its plasma membrane localization (Pan D. et al., 2016, JBC). It showed furthermore that Norbin is expressed in neutrophils. In order to assess the functional importance of the P-Rex1/Norbin interaction in neutrophils, we generated genetically-modified mouse strains. Norbin-deficient neutrophils showed surprising functional defects, a subset of which were Prex1-dependent. Similarly, Norbin deficient-mice also showed intriguing levels of neutrophil recruitment to sites of inflammation. Altogether, our findings suggest unexpected roles of the GPCR adaptor and P-Rex1 regulator Norbin in neutrophil biology, as well as surprising underlying mechanisms.

O7.04
The role of PI3Kγ signaling within leukocytes in obesity-driven inflammation and insulin resistance

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We previously reported that the class-1B phosphatidylinositol-4,5-bisphosphate 3-kinase-γ (PI3Kγ) plays an important role in diet-induced obesity, metabolic inflammation, and insulin resistance, but the molecular mechanisms for this action remains unresolved. We now show that PI3Kγ activity in metabolic inflammation and metabolic homeostasis is partially indirect, through its role in the control of adiposity, and partly due to its direct action in leukocytes. Our data are in contrast with the emerging view that PI3Kγ is a major negative regulator of classical macrophage activation, and indicate that the role of PI3Kγ in macrophage activation depends on the specific inflammatory context.

We conclude that PI3Kγ activity in leukocytes is required for M1-polarizated gene expression in the obese adipose tissue and for efficient recruitment of adipose tissue neutrophils, which promotes early-onset of insulin resistance during obesity.

O7.05
Pro-tumor steering of cancer inflammation by p50 NF-κB enhances colorectal cancer progression

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Despite tumor-associated macrophages (TAMs) display a M2-skewed tumor-promoting phenotype in most cancers, in colorectal cancer (CRC) TAMs polarization and its impact remain controversial. We investigated the role of the M2-polarizing p50 NF-κB subunit in orchestrating the phenotype of TAMs, tumor microenvironment composition and CRC progression. We first demonstrate, by parallel studies in colitis-associated cancer (CAC) and in genetically driven Apc^{Min} mouse models, that the p50-dependent inhibition of M1-polarized gut inflammation supports CRC development. In accord, $p50^{-/-}$ mice displayed exacerbated colitis associated with fewer and smaller tumors, along with enhanced levels of M1/Th1 cytokines/chemokines, including IL-12 and CXCL10, whose administration restrained CAC development *in vivo*. The inflammatory profile supporting tumor resistance in colons from $p50^{-/-}$ tumor bearers correlated inversely with TAMs load and positively with both recruitment of NK, NKT, T cells and number of apoptotic tumor cells. Of note, while in mice myeloid-specific ablation of p50 promoted tumor resistance, in CRC patients high number of p50+ TAMs at the invasive margin was associated with decreased IL-12p35 and Tbx-21 expression and worse post-surgical outcome. Our findings point to p50 involvement in CRC development, through its engagement in the pro-tumor activation of macrophages, and identify a new candidate for prognostic and target therapeutic intervention.

O7.06
Immunogenomic analyses and response to checkpoint blockade

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Current major challenges in cancer immunotherapy include identification of patients likely to respond to therapy and development of strategies to treat non-responders. To address these problems and facilitate understanding of the tumor-immune cell interactions we inferred the cellular composition and functional orientation of immune infiltrates, and characterized tumor antigens in 19 solid cancers from The Cancer Genome Atlas. Decomposition of immune infiltrates revealed prognostic cellular profiles for distinct cancers, and showed that the tumor genotypes determine immunophenotypes and tumor escape mechanisms. Using machine-learning approach we then identified determinants of immunogenicity and proposed an immunophenoscore for the prediction of response to immunotherapy with checkpoint blockers.

Using a mouse model of colorectal cancer, we then characterized the extent of immunoediting that tumors undergo during progression or as a consequence of the targeting the PD-1/PD-L1 axis. The results show that targeting the PD-1/PD-L1 axis potentiates immunoediting and renders tumors more homogeneous, which could possibly explain the development of acquired resistance to checkpoint blockers.

O7.07
Inhibition of neutrophil necrosis controls *Mycobacterium tuberculosis* growth after removal by macrophages

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Increased numbers of infections with multi and extensively drug resistant *Mycobacterium tuberculosis* strains as well as the lack of efficient vaccines require novel approaches, such as adjunct host-directed therapies to deal with the global tuberculosis epidemic. Neutrophils represent the main infected cell population in lungs of patients with active tuberculosis. Here we show that *M. tuberculosis* induces necrosis of human neutrophils in an ESAT-6-dependent manner. Subsequent removal of infected, necrotic neutrophils promoted mycobacterial growth in human macrophages. Importantly, neutrophil necrosis was a prerequisite for subsequent *M. tuberculosis* growth in macrophages. After identification of reactive oxygen species (ROS) as drivers of necrosis, we were able to prevent necrosis by pharmacological inhibition of myeloperoxidase. Thereby, we restored the capability of efferocytic macrophages to control mycobacterial growth, highlighting ROS and ROS-producing enzymes as putative targets for host-directed therapy. Taken together, host cell necrosis represents the starting point for a vicious circle leading to subsequent uptake of infected necrotic cells by other phagocytes,

mycobacterial growth therein and, again, induction of host cell necrosis, a scenario that is very likely to take place in patients with active tuberculosis. Interruption of this vicious circle by inhibition of host cell necrosis and subsequent restoration of the anti-mycobacterial functions of macrophages represents an intriguing approach for host-directed therapy.

O7.08
IFNbeta is produced by resolution-phase macrophages and limits neutrophil responses during peritonitis onset and resolution

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Background: The engulfment of apoptotic leukocytes (efferocytosis) by macrophages during the resolution of inflammation is essential for tissue homeostasis and results in macrophage reprogramming/immune-silencing. However, a distinct subset of resolution phase macrophages loss their phagocytic potential, and hence were termed satiated macrophages.
Materials and methods: An unbiased RNA-Seq analysis was conducted to reveal distinct gene expression profile between phagocytic and satiated macrophages. Consequently, the levels of IFNβ, inflammatory cytokines and chemokines in peritoneal exudates during inflammation onset and its resolution were determined by ELISA or Luminex analysis at different times post peritonitis. Differences between WT and $IFNb^{-/-}$ mice in terms of PMN infiltration and apoptosis, as well as macrophage efferocytosis and reprogramming were determined by fluorescent microscopy and ELISA, respectively.
Results: Here, we show satiated macrophages express a distinct IFNb-related gene expression signature. Unexpectedly, we found peritoneal IFNb levels peaked during the onset as well as the resolution phase of peritonitis. Consequently, we used $IFNb^{-/-}$ mice to determine IFNb limited the onset of neutrophilic inflammation by reducing peritoneal neutrophil numbers and neutrophil-attracting chemokines and enhancing their apoptosis. Moreover, IFNb enhanced macrophage efferocytosis and reprogramming to an anti-inflammatory phenotype.
Conclusions: These findings indicate for the first time that IFNb is a key effector cytokine in resolving inflammation and could lead to novel resolution-promoting, IFNb-based therapies for inflammatory disorders.

O7.09
Neutrophil dysfunction in an inducible mouse model of glycogen storage disease type 1b

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Introduction: Neutrophils play a key role in host protection against microbial infections. Neutropenia and neutrophil dysfunction are common features of many diseases and are observed in patients affected by Glycogen Storage disease type 1b (GSD-1b). GSD1b is a rare genetic autosomal recessive disease caused by the defect of the glucose-6-phosphate transporter (G6PT).
Objectives: The objective of this study was to understand the pathophysiology of the disease focusing on neutrophils activity in an inducible KO mouse model.
Methods: G6ptlox/wmice were crossed with transgenic mice expressing a TM inducible Cre-mediated recombination system. To induce the excision of G6PT exons, five-week old mice were injected intraperitoneally with TM for five days. Histological examinations of tissues and functional analysis of bone marrow/ peritoneal exudate neutrophils were performed in TM-$G6PT^{-/-}$ mice and compared to controls.
Results: TM-$G6PT^{-/-}$ mice showed pathological abnormalities characteristic of the human disease, including hepatomegaly, nephromegaly and hyperlipidemia. Bone marrow and peritoneal neutrophils from G6PT-KO mice displayed impaired mobility, chemotaxis, as well as diminished phagocytic activities, compared to wild type mice. In addition our data demonstrated that G6PT-KO neutrophils exhibited an enhanced late apoptosis and necrosis.
Conclusions: We have developed an inducible murine model that mimics the pathophysiology of GSD1b in terms of tissues damage, neutrophils dysfunction, and susceptibility to bacterial infection. This model will be exploited to develop and test new therapeutic strategies

O7.10
NAMPT integrates metabolism and immunosuppressive activity of tumor-associated myeloid cells

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Disregulation of myeloid cell metabolism in cancer can dramatically alter their function and influence disease outcome. Despite little is known on the connection between the metabolism of MDSCs and cancer-related immunosuppression, metabolic fluxes have been recently indicated as novel therapeutic targets for the restoration of anticancer immunosurveillance.

Nicotinamide phosphoribosyl transferase (NAMPT) is the rate-limiting enzyme in nicotinamide adenine dinucleotide (NAD) biosynthesis, essential to maintain NAD pool in cells. NAD plays a crucial role in maintaining cellular energy by redox reactions and is actively consumed by NAD-dependent enzymes (*e.g.* sirtuins, PARPs and CD38/CD157). Therefore, as NAD biosynthetic enzyme, NAMPT is supposed to affect a variety of metabolic and stress responses. Indeed, NAMPT expression increases in various diseases, including chronic inflammatory conditions, metabolic alterations and cancer. Noteworthy, NAMPT inhibitors entered in clinical trials for solid and non-solid tumours thanks to their ability of dropping NAD and ATP levels and, in turn, of activating an anti-tumoural activity. Despite this evidence proposes NAMPT inhibitors to blunt the link between cancer metabolism and inflammation, the contribution of NAMPT in cancer-related inflammation remains elusive.

We reported for the first time that NAMPT is a key coordinator of myelopoiesis in cancer and orchestrator of cancer-related inflammation. NAMPT supports both differentiation and peripheral accumulation of myeloid-derived suppressor cell (MDSCs), by respectively enhancing the nitric oxide-mediated suppressive activity in response to interferon-g and inhibiting

their CXCR4-dependent retention into the bone marrow. Further, high NAMPT expression in TAM supports their M2 polarization. As a consequence, pharmacological inhibition or myeloid-specific ablation of NAMPT reactivated specific antitumor immunity, resulting in inhibition of tumour growth and metastasis formation. Our data identifies NAMPT as critical controller of immunometabolism in myeloid cells, which is exploited by tumours to reprogram innate and adaptive immune functions in a tumour promoting mode.

O7.11
Functional state of phagocytes from different locations in rat with C6 glioma

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Background: The development of malignant glioma is accompanied by the microglial activation, the influx of circulating phagocytes and the massive infiltration of systemic macrophage-like cells as a result of a disrupted blood-brain barrier. It results in dramatic alterations of the metabolism of this complex microglia. Circulating phagocytes as well as phagocytes from another distant sites sense metabolic stress in their microglial counterparts. Functional characteristics of these cells can reflect the pathological changes in the tumor microenvironment. However, the data concerning metabolic state of such phagocyte cells in glioma-bearing animals are controversial and sparse. The aim of the work was to investigate a metabolic profile of phagocytes from different locations in rats with C6 glioma.
Materials and methods: In the study, microglial cells, circulating phagocytes and peritoneal macrophages from Wistar adult male rats with C6 glioma were investigated. Functional state of phagocytes was determined based on reactive oxygen species (ROS) production, phagocytic activity, as well as CD14 and CD206 expression in these cells by flow cytometry.
Results: ROS production of microglial cells from tumor-bearing animals was in 1·5 times higher than that in intact rats. ROS production of circulating phagocytes was decreased slightly as compared to intact group. ROS production of peritoneal macrophages was in 1·5 times higher as compared to intact group. Phagocytosis of microglial cells from tumor-bearing animals was only moderately increased. Phagocytosis of circulating phagocytes was decreased slightly as compared to intact group. Phagocytosis of peritoneal macrophages was increased in 3 times than that in intact rats. All macrophages from tumor-bearing rats contained significant number of CD14+/ CD206+ (anti-inflammatory phenotype) cells.
Conclusions: The present study shows that functional state of phagocytes from different locations from C6 glioma-bearing animals has pronounced anti-inflammatory phenotype under the influence of glioma.

O7.12
Excessive neutrophil recruitment and persistence during acute inflammation in ulcerative colitis

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Background: The early inflammatory response in ulcerative colitis (UC) has been shown to be protracted. Using an *in vivo* bacterial challenge model, we characterized the cellular and molecular determinants, and consequences, of the acute inflammatory response in patients with UC.
Methods: Acute inflammation was provoked in 26 UC patients off treatment or on 5-aminosalicylates, and 17 healthy controls (HC), by intradermal injection with killed *E. coli* or *S. pneumoniae*. Local vascular reactions were quantified by laser Doppler. Early and resolving inflammatory exudates were sampled by raising suction blisters over inoculation sites after 4 h or 48 h. Cells were characterised by flow cytometry; cytokines by multiplex array; and lipid mediators by mass spectrometry. *In vivo* findings were confirmed by *in vitro* stimulation of cultured peripheral blood-derived macrophages, supplemented by determination of levels of lipid biosynthetic enzymes by Western blot.
Results: UC patients off treatment demonstrated enhanced local blood flow within 24 h of bacterial exposure, with impaired resolution, to both Gram-negative and Gram-positive bacteria ($P = 0{\cdot}01$). Neutrophil accumulation ($P = 0{\cdot}04$) and prostaglandin E_2 production ($P = 0{\cdot}02$) was increased within 4 h of inoculation. This was not a cytokine-driven phenomenon. At 48 h, UC patients had persistent viable neutrophils ($P = 0{\cdot}001$) and T lymphocytes ($P = 0{\cdot}03$)at inflammatory sites, mimicking pathological appearances seen in acute flares of this disease. The exaggerated onset was normalised in patients taking 5-aminosalicylates, although these individuals had greater numbers of macrophages present at 48 h ($P = 0{\cdot}03$). Cytokine and prostaglandin findings were replicated in cultured macrophages *in vitro*, which also exhibited higher COX-1 up-regulation in UC, supporting the primary impact of these abnormalities in the generation of the excessive acute inflammatory response.
Conclusions: Neutrophil accumulation to acute inflammatory sites is exaggerated in UC and slow to resolve, associated with perturbed production of inflammatory lipid mediators. 5-aminosalicylates normalise this process, likely through harnessing novel pro-resolution mechanisms.

O7.13
Macrophages are an essential iron source for cutaneous stromal cells proliferation

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The role of erythrophagocytic macrophages in iron recycling for erythropoiesis is well established. However, macrophages may

be also involved in iron redistribution at a local level, thus impacting neighbouring cells. We previously showed that iron release from human macrophages supports in vitro cell proliferation

We used mice with iron retention in macrophages due to targeted inactivation of the iron exporter ferroportin (Fpn1 $^{fl/fl}$Lys$^{Cre/-}$) to investigate the role of macrophage iron in two conditions like hair follicle cycling and wound healing that share many similarities, including fast cell growth.

As compared to Fpn1 $^{fl/fl}$Lys$^{-/-}$ littermates, Fpn1 $^{fl/fl}$Lys$^{Cre/-}$ exhibited transient alopecia and showed lesions compatible with a delayed entry of the hair follicle into the growth (anagen) phase, which was accompanied by decreased expression of the proliferation marker Ki67 in hair bulb epithelial cells. Alopecia was present in mice with normal systemic iron homeostasis, thus suggesting that local iron release from macrophages is required to sustain the growth of hair follicle cells.

The closure of excisional skin wounds was delayed in Fpn1 $^{fl/fl}$Lys$^{Cre/-}$ mice, which displayed increased iron accumulation in wound macrophages together with sustained inflammatory response and granulocyte infiltration associated with defective granulation tissue formation and diminished fibroplasia. FACS analysis did not show any difference in leukocytes (CD 45^{+}) recruitment and macrophage polarization between Fpn1 $^{fl/fl}$Lys$^{Cre/-}$ mice and control littermates. Similarly, cytokines and growth factors, evaluated in FACS-sorted macrophages and wound tissue, did not differ significantly. Confocal analysis of CD31 and Lyve-1 showed a defect of both blood and lymphatic vessels formation in Fpn1 $^{fl/fl}$Lys$^{Cre/-}$ mice, accompanied by decreased expression of collagen-1, PDFGR and αSMA. These results indicate that disruption of iron export from macrophages does not affect the inflammatory processes of wound healing, but impairs stromal cells proliferation, leading to delayed skin repair. Altogether, these data unravel a new iron-related trophic function of macrophages.

O7.14
Assessment of neutrophil NADPH oxidase activity and phagosomal pH and area using the myeloid Hoxb8 cell line with and without HVCN1

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Background: This study uses a cellular model of murine neutrophils derived from myeloid progenitors maintained in an immortalised state by a genetically-inserted estrogen-responsive form of the Hoxb8 transcription factor. The HVCN1 proton channel normally compensates the charge across the neutrophil phagosome induced by the NADPH oxidase. We investigated NADPH oxidase activity and phagosomal pH and area in WT and HVCN1$^{-/-}$ neutrophils differentiated from this cell line compared to their bone marrow-derived counterparts.
Materials and methods: Hoxb8 progenitor cells were grown and maintained in cell culture with estrogen; they differentiated into neutrophils after removal of estrogen and addition of granulocyte-colony stimulating factor (G-CSF). Ex-vivo neutrophils were isolated from mouse bone marrow. Cell morphology was observed using Wright-Giemsa staining. Flow cytometry measured staining of cell surface differentiation markers. NADPH oxidase activity was measured with the Amplex UltraRed assay as hydrogen peroxide (H_2O_2) produced for 50 minutes after phorbol myristate acetate (PMA) stimulation. Phagosomal pH and area were measured using SNARF-1 with confocal microscopy.
Results: Increased nuclear segmentation accompanying the down-regulation of c-kit and upregulation of CD11b and Ly6G was observed when cultured Hoxb8-progenitors were differentiated, indicative of the maturation into neutrophils. There were no associated differences between WT and HVCN1$^{-/-}$ models. As previously described, HVCN1$^{-/-}$ neutrophils had very alkaline and swollen phagosomes compared to WT neutrophils – these changes were replicated in HVCN1$^{-/-}$ Hoxb8-neutrophils. The total H_2O_2 production in WT Hoxb8-neutrophils was normal, ~60% (SEM±4·2%) of normal in HVCN1$^{-/-}$ neutrophils, and ~70% (SEM±2·4%) of normal in HVCN1$^{-/-}$ Hoxb8-neutrophils.
Conclusions: Up until now, genetically-modified mice were required to study the effects of the loss of the HVCN1 channel on cell function. The Hoxb8 cell line provides a cheap and convenient alternative experimental model and this study serves as an example that can be applied to mutations or ablation of other genes in myeloid cells.

O7.15
5-aminosalicylate drugs generate anti-inflammatory hydroxy fatty acids that act upon macrophages to promote inflammation resolution in ulcerative colitis

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Background: 5-aminosalicylate (5-ASA) drugs constitute a major therapeutic option for ulcerative colitis (UC), but their mechanism remains incompletely understood. We used an *in vivo* model of acute inflammation in humans to determine their functional impact on the early inflammatory response, and *in vitro* stimulation of macrophages to elucidate the underlying molecular pathways.
Methods: Acute inflammation was provoked by intradermal injection with killed *E. coli* (EC) in 26 UC patients and 23 healthy controls (HC), with individuals in both cohorts either on no immunomodulatory treatment or on 5-ASAs. Inflammatory exudates were sampled at 4 h and 48 h by suction blister. Cells were characterised by flow cytometry; cytokines by multiplex array; and lipid mediators by mass spectrometry. The impact of lipid mediators on macrophage pro-inflammatory cytokine secretion was determined following *in vitro* stimulation with EC.
Results: Excessive inflammation clinically observed in UC was normalised in patients taking 5-ASAs. Enhanced resolution was associated with increased concentrations of the hydroxy fatty acids 9-oxo-octadecadienoic acid (OxoODE) and 13-OxoODE. To characterise the effect of these mediators, cultured macrophages were co-incubated with EC and 9- or 13-OxoODE. Both caused dose-dependent suppression of TNF-α (P = 0·0001 and P = 0·01, respectively), at concentrations reflective of those *in vivo*. 9-OxoODE was more potent, with an IC_{50} of 100 nM.

Effects were completely reversed by GW9662, a PPAR-γ antagonist ($P = 0{\cdot}006$). The *in vivo* profile of lipid mediators in HC treated with 5-ASAs differed from UC, associated with subtle variations in resolving inflammatory cellular and cytokine constituents, which has important implications for understanding hydroxy fatty acid generation in patients.

Conclusion: We have uncovered an important novel pathway through which 5-ASAs normalise the acute inflammatory response in UC. Generation of hydroxy fatty acids harnesses a previously unexplored pro-resolution pathway, exerting effects through the PPAR-γ receptor in macrophages. This provides potential new drug targets in this disease.

O7.16
Antibody-*dependent* destruction of B lymphoma cells by neutrophils

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Neutrophils kill antibody-opsonized cancer cells by a unique cytotoxic process of 'trogoptosis' that we have recently identified. Trogoptosis is essentially a lytic mechanism of cytotoxicity that involves physical destruction of the target plasma membrane, and this occurs when neutrophils rip fragments from the tumor cells by trogocytosis (*trogo* in Greek means 'gnaw'). Both trogocytosis and the actual killing process occur as a consequence of neutrophil Fc-receptor signaling. However, whereas neutrophils are quite efficient in the trogoptosis of solid cancer cells, their capacity to kill hematopoietic cancer cells, including B lymphoma cells in the presence of the anti-CD20 monoclonal antibody Rituximab (Rmab), appears surprisingly limited. Clearly, this may also restrict the clinical efficacy of Rmab and in order to be able to improve this, it is important to understand the basis of this limitation.

Our initial studies to explore this suggest that the inability of neutrophils to kill anti-CD20 opsonized B lymphoma cells is due to intrinsic feature(s) of the CD20 target antigen. In particular, we demonstrate efficient trogocytosis in the apparent absence of killing, suggesting that the latter is not caused by a general absence of neutrophil Fc-receptor signaling. Furthermore, we show that antibodies against other target antigens on the B lymphoma cells, such as e.g. HLA-DR, are able to cause efficient neutrophil-mediated killing, thereby implying that these tumor cells do not have an inherent resistance to neutrophil-mediated killing, but rather one that is specifically related to the nature of the CD20 target antigen.

O7.17
ACKR2 in hematopoietic precursors as a checkpoint of neutrophil release and antimetastatic activity

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Atypical chemokine receptors (ACKRs) are regulators of leukocyte traffic, inflammation and immunity. Here we report that ACKR2 is expressed in hematopoietic precursors and downregulated during myeloid differentiation. Genetic inactivation of ACKR2 resulted in increased levels of inflammatory chemokine receptors and release from the bone marrow of neutrophils with increased antimetastatic activity. In a model of NeuT-driven primary mammary carcinogenesis ACKR2 deficiency was associated with increased primary tumor growth and protection against metastasis, the latter neutrophil-mediated. Thus, ACKR2 is a key regulator (checkpoint) of myeloid differentiation and function and its targeting unleashes the antimetastatic activity of neutrophils.

O7.18
Neutrophils are protective in cancerogenesis by altering tumor microenvironment and controlling intestinal microbiota

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The view of neutrophil as a cell involved only in the early phases of inflammation has been challenged in the last years and neutrophils are now considered key players in the orchestration of the immune response. Several studies relied on antibody-based neutrophil depletion to determine their contribution to tumor development, but rigorous in vivo genetic evidence assessing the neutrophil role in cancerogenesis is missing.

We investigated this issue using key preclinical models of chemically-induced cancer (3-MCA induced sarcoma and AOM/DSS induced colitis-associated cancer, CAC) and taking advantage of a genetic model of neutrophil deficiency (i.e. *csf3r*$^{-/-}$ mice).

G-CSF receptor deficiency was associated with increased susceptibility to sarcoma and CAC, and tumor microenvironment displayed protumoral features (e.g. increased frequency of M2 macrophages, reduced IFNg concentration). In addition, neutrophil density within tumor significantly correlated with reduced proliferation rate of tumor cells in wild type mice. Importantly, adoptive transfer of naïve neutrophils reduced tumor growth in *csf3r*$^{-/-}$ mice. Finally, the increased susceptibility to CRC in *csf3r*$^{-/-}$ mice was dependent on intestinal microflora, and was abolished in cohousing experiments.

Collectively, our data support that genetic deficiency of neutrophils affects the anti-tumor response and is associated with increased susceptibility to chemically-induced cancerogenesis.

Until recently neutrophil function was mostly related to acute inflammation and defense against pathogens. We (and others) have challenged this dogma and demonstrated that neutrophils represent an essential component in the control of tumor onset and development.

O7.19

Bcl-2 overexpression in melanoma cells promotes recruitment and differentiation of macrophages towards a M2-like phenotype

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Introduction: Melanoma, one of the most aggressive skin cancers, is tightly related to chronic inflammation, which regulates its progression through expression of proinflammatory mediators. Being a relevant constituent of the tumor microenvironment, tumor-associated macrophages (TAMs) may regulate melanoma progression, through distinct M1 vs M2 polarized inflammatory programs. Our previous studies have demonstrated that bcl-2 overexpression in human melanoma cells increases tumor growth-associated properties. In order to define the mechanism through which bcl-2 affects tumor aggressiveness and to provide new insight into tumor progression, the aim of this study was to test if bcl-2 overexpression in melanoma cells could affect macrophage polarization.

Materials and methods: A panel of human melanoma cell lines and their derivatives overexpressing bcl-2 were used. Human monocyte-derived macrophages (M-DM) were differentiated from peripheral blood monocytes, in the presence of macrophage colony-stimulating factor, and then exposed to conditioned medium (CM) from control or bcl-2 overexpressing cells. ELISA, qRT-PCR and Western blot analyses were used to evaluate macrophage-derived cytokines and polarization markers, as well as melanoma-specific proteins.

Results: CM from bcl-2 overexpressing cells induced on macrophages a strong increase of M2 markers (CD206, IL-10, CCL1) while decreasing M1 markers (COX-2, IL-12), as compared to CM from control cells. To identify tumor-derived factors with M2-polarizing activity, CM from melanoma cells underwent to cytokine membrane array analysis. CM from bcl-2 overexpressing melanoma displayed increased levels of interleukin-1β (IL-1β), interleukin-8 (IL-8) and interleukin-17 (IL-17), cytokines involved in cancer-related inflammation and tumor progression. In addition, ELISA and Western blot analysis demonstrated an upregulation of COX-2 in bcl-2 overexpressing melanoma cells together with an increased production of prostaglandin E2, a known M2-polarizing factor. In support of these results, a massive *in vivo* recruitment of macrophages in bcl-2 overexpressing xenografts was evidenced.

Conclusions: Melanoma cells overexpressing bcl-2 protein secrete factors able to promote the recruitment of M2 polarized macrophages.

O7.20

ARHGAP25 is a predominant regulator of leukocyte transendothelial migration in mice

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Introduction: Rho/Rac family of small G-proteins are involved in fine tuning and spatio-temporal regulation of leukocyte trafficking as well as their effector functions. Previously, we identified and characterized a novel GTPase activating protein, ARHGAP25, which is expressed primarily in hematopoietic cells and regulates Rac. We demonstrated that ARHGAP25 is key regulator of neutrophilic effector functions such as phagocytosis, superoxide production and it is involved in control of actin depolymerization as well.

Aims: Aim of the present study was to reveal the role of ARHGAP25 in leucocyte recruitment from the vascular compartment to the site of inflammation.

Methods: *Arhgap25*$^{-/-}$ mouse strain was obtained from Knockout Mouse Project (KOMP) Repository. *In vivo* migration upon TNFα stimulus was investigated using intravital microscopy of cremaster muscle. *Ex vivo* and *in vitro* migration assays were carried out by flow chamber assay and transwell assay, respectively. Leukocyte extravasation was investigated by histological staining of cremaster muscle or peritoneal tissues. Cell surface expression of adhesion molecules and amount of filamentary actin was measured by flow cytometry. Amount of active Rac was measured by Pull-down assay.

Results: Absence of ARHGAP25 caused reduced leukocyte rolling velocity and prolonged crawling, as well as increased leukocyte extravasation upon TNFα stimulus *in vivo*. We confirmed these results using *ex vivo* and *in vitro* experiments, respectively. Using bone marrow chimeric mice, we revealed that alteration of leukocyte transmigration observed in absence of ARHGAP25 is due to primary changes in hematopoietic compartment but not in endothelial or other nonhematopoietic cell compartment. Investigating the potential mechanism of altered leukocyte function, we revealed that ARHGAP25 affects actin polymerization and depolymerization through its GTPase activating effect on Rac.

Summary: Taken together, we identified ARHGAP25 as predominant regulator of neutrophil recruitment and extravasation due to its effect on Rac-dependent cytoskeletal changes.

O7.21

Activation of neutrophils by immunoglobulin A (IGA) exacerbates pathogenesis of inflammatory bowel disease (IBD)

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Background and aim: Mucosal lesions in IBD, and particularly ulcerative colitis, are characterised by massive neutrophil infiltration and aggregation. Earlier we showed that binding of IgA, the prominent antibody in the gut mucosa, to its receptor FcalphaRI on neutrophils initiates chemotaxis of these cells. As such, we hypothesize that abnormal activation of neutrophils by mucosal IgA might explain enhanced neutrophil infiltration in IBD, which results in undesirable tissue damage.

Methods: Fresh blood and snapfrozen colon biopsies of IBD patients were stained for different immunological markers and analysed with FACS or immunofluorescence, respectively. Neutrophils were activated by IgA and co-cultured with CaCo2 epithelial cells. A humanised mouse model, expressing both human IgA and FcalphaRI, was used to study the role of IgA/FcalphaRI interactions in pathogenesis in a DSS-induced colitis model.
Results: Phagocytosis of IgA opsonised particles led to activation of neutrophils, leading to extensive reactive oxygen production and release of Neutrophil Extracellular Traps (NETs), which consisted of aggregated DNA strands covered by reactive enzymes like myeloperoxidase (MPO) and neutrophil Elastase (NE). Culturing 'NETosing' neutrophils with epithelial cells led to enhanced apoptosis and cell death of epithelial cells. In human IBD biopsies enhanced neutrophil infiltration correlated with presence of NETs positive for MPO and NE. FcalphaRI on neutrophils furthermore, resulted in enhanced DSS induced colitis in IgA/FcalphaRI mice, compared to mice that only expressed human IgA.
Conclusions: Aberrant activation of neutrophils in the gut mucosa via IgA/FcalphaRI interactions may lead to extensive NETs release, resulting in epithelial cell death and tissue damage. Inhibiting migration and activation of neutrophils by restraining IgA/FcalphaRI interactions can be a valuable new approach to dampen the disease burden.

O7.22
Start and stop signals for neutrophil swarming and phagocytosis

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Neutrophil swarms emerge frequently around areas of tissue damage or infections, bacterial and fungal. The complexity of interactions between the moving neutrophils and their targets makes the study of neutrophil swarming very difficult. Moreover, in the animal models developed, additional complexity due to uncontrolled microenvironment in live tissues, further complicate the situation. To circumvent the limitations of current experimental systems, we designed a platform technology for synchronizing large arrays of neutrophil swarms and investigated the chemical signals that mediate the cooperation between human neutrophils. We captured the molecules released during swarming in small volumes of fluid and identified a constellation of lipid and protein mediators that represent both go and stop signals. In addition, we found several enzymes and inhibitors of enzymes. Together, the mediators and enzymes coordinate the effective interactions between the neutrophils in swarms and with other immune and non-immune cells. The complementary of these signals reveals an unexpected complexity for human neutrophil swarming and provides novel leads in the orchestration of the innate immune responses and can enhance the ability to intervene during infections and uncontrolled inflammation.

O7.23
Granulocyte differentiation from X-linked chronic granulomatous disease induced pluripotent stem cells

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Gene therapy represents one of the most relevant tools for the treatment of a wide spectrum of inherited disorders. Several techniques involving viral and non viral vectors, homologous recombination mediated by targeting nucleases such as Cas9 in stem cells or in patient-derived induced pluripotent stem cells (iPSC) have been investigated, providing promising results. However, specific chromosomal abnormalities as large deletions and structural abnormalities such as duplications or complex genomic rearrangements cannot be corrected with currently available techniques. For this reason we have previously proposed precise chromosome transplantation to genetically correct these abnormalities (Paulis et al., Oncotarget 2015, 6: 35218). Here we focus on Chronic Granulomatous Disease (CGD), a primary immunodeficiency caused by defects in the *CYBB* gene, located on the X chromosome, since about 5–10% of the patients bear large deletions or complex rearrangements involving contiguous genes. We generated iPSCs from a mouse model of X-linked CGD and interrupted the HPRT gene by the CRISPR technology, making cells HAT sensitive. Next, we transferred a normal X chromosome by microcell-mediated chromosome transfer (MMCT). The selection of HAT resistant 40, XY or 40, XX cells identified clones in which an endogenous sex chromosome has been replaced by the exogenous normal X. We have then set up a protocol of differentiation to obtain functional phagocytes *in vitro* from wild type iPSC, and plan to apply this procedure to chromosome-corrected iPSCs, to confirm the rescue of the genetic defect. This work will provide the proof of principle of the "chromosome therapy" feasibility, which could be applied to the 10% of CGD cases due to gross deletions including the CYBB gene that cannot be cured by conventional gene therapy.

O7.24
Competition for a restricted number of niches as a major parameter of the macrophage ontogeny equation

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Tissue-resident macrophages are found in all organs of the body where they are adapted to perform numerous functions required for tissue homeostasis. Contrary to most immune cells, they develop prenatally from embryonic progenitors, have self-renewal mechanisms and exist, at least in most tissues, independently of adult hematopoietic stem cells (HSCs). These insights have undermined the concept of the mononuclear phagocyte system, where the circulating monocyte was seen as the central progenitor of all tissue macrophages. Importantly, despite their common origin, tissue-resident macrophages are highly specialised to the tissue of residence. However, macrophages that develop from adult HSCs after irradiation neither display the full gene signature of their embryonic counterparts. Here, using a new model of selective diphtheria toxin-mediated depletion of Clec4F-positive liver-resident Kupffer cells and transfer of precursors into an empty lung niche ($GMCSFR^{-/-}$ mice), we found that

short-lived circulating adult monocytes engrafted in both the liver and lung. These monocytes differentiated into bona fide self-renewing Kupffer cells or Alveolar macrophages respectively, which adopted the full transcriptional profile of their embryonic counterparts as well as their morphological and functional features. Highlighting the physiological relevance of our findings we found that, contrary to recent studies, bone marrow-derived monocytes engrafted in the liver and spleen during the period of normal organ growth in the first weeks of life. Thus in conclusion, we find niche availability and nurture by the tissue, rather than nature of the progenitor, to be the principal factors controlling macrophage life-span and organ-specific functions.

O7.25
The role of the Syk tyrosine kinase in the development of experimental epidermolysis bullosa acquisita

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Background: The inflammatory form of epidermolysis bullosa acquisita (EBA) is caused by autoantibodies against type VII collagen (CVII), a crucial component of the dermal-epidermal junction. We previously showed that genetic deficiency of the Src-family kinases Hck, Fgr and Lyn protected mice from skin inflammation. Here, we investigated if another important member of the Fcγ receptor signaling, the Syk tyrosine kinase plays a role in the experimental form of EBA.
Methods: We generated bone marrow chimeras lacking Syk from the hematopoietic compartment and wild type chimeras as controls. EBA was triggered by subcutaneous injection of anti-CVII or control IgG. Clinical scoring was based on the specific dermatological abnormalities and the size of the affected skin area. Circulating anti-CVII antibody levels and local LTB_4 or CXCL2 levels were determined by ELISA, while neutrophil accumulation in the ear was detected by flow cytometry. In vivo neutrophil migration was tested in mixed bone marrow chimeras. Bone marrow isolated neutrophils were stimulated by immobilized CVII–anti-CVII immune complexes.
Results: In contrast to wild type controls, bone marrow chimeras lacking Syk in their hematopoietic compartment were protected from skin inflammation triggered by anti-CVII antibodies despite normal peripheral anti-CVII levels. Syk deficiency resulted in the massive decrease of CXCL2 and LTB_4 levels at the site of inflammation and caused a defective neutrophil recruitment, while the in vivo migratory capacity of Syk knockout neutrophils were unaffected. Syk-deficient neutrophils failed to release superoxide, LTB_4 and CXCL2 when stimulated by CVII–anti-CVII immune complexes.
Conclusions: Our results show an essential role for the Syk tyrosine kinase in experimental epidermolysis bullosa acquisita and raise the possibility that the protein could be a suitable therapeutic target in the treatment of the human disease.

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O7.26
TNF-alpha drives the migration and crawling of neutrophils into afferent lymphatic vessels during antigen challenge in vivo

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Neutrophils are now viewed as key effectors of both innate and adaptive immunities in many physiological and pathological conditions. Whilst the trafficking of neutrophils through blood vessels has been extensively studied, the mechanism that control their entry into the lymphatic system is poorly understood. For this purpose, we have developed a murine model of cremasteric inflammation to visualise by intravital confocal microscopy the interactions of neutrophils with tissue-associated afferent lymphatic vessels *in vivo*. In the present study, we report that neutrophils migrate rapidly into the lymphatic vessels upon both antigen challenge and TNF-alpha-induced inflammation of murine cremaster muscles in a CCR7-dependent manner. Interestingly, neutrophil intravasation into lymphatics (but not extravasation through blood vessels) upon antigen challenge is inhibited (~70%) in mice genetically deficient in both receptors for TNF-alpha; a response associated with a reduced expression of CCR7 on the surface of tissue-infiltrated neutrophils. Furthermore, in WT mice pre-treated with an anti-TNF-alpha blocking antibody, ICAM-1 expression by lymphatic vessels was reduced; leading to an alteration of the directional crawling of neutrophils onto the lumen of lymphatic endothelial cells. Collectively, our results demonstrate the critical role of TNF-alpha in promoting neutrophil recruitment into the lymphatic vasculature *in vivo* during the inflammatory response of antigen challenge.

Workshop 8: Neurology

O8.01

The National Institutes of Health Stroke Scale is a strong predictor of mortality and disability in patients with primary intracerebral haemorrhage

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Introduction: Primary intracerebral hemorrhage (ICH) has a worse prognosis than ischemic stroke. The National Institutes of Health Stroke Scale (NIHSS), attained a very good prognostic value in ischemic stroke, but it is not clear whether this is true in ICH, too.
Aim: Objective of our study is to investigate the accuracy of NIHSS in predicting fatality rate and dependency degree in an unselected group of patients with spontaneous ICH.
Materials and methods: We included in the study all consecutive patients admitted for ICH to our Stroke Unit since 1st august 2011 to 31st January 2016. NIHSS was evaluated in all subjects within 24 hours from the onset. We systematically followed up all patients and we used modified Rankin Scale (mRS) to evaluate functional outcome at discharge and after three-months. Spearman's Rank Correlation Coefficient analysis was used for statistics. Sensitivity, Specificity, Predictive positive (PPV), Negative predictive (NPV) values, Global Accuracy (GA), and ROC curve were also computed, using the median score 7 as NIHSS cut-off and the score 4 as mRS cut-off.
Results: We included 156 subjects. Mean NIHSS (±SD) at admission was 10·82 (±8·27). Thirty-two patients (20·5%) died within 30 days and other 10 (29·9%) within 3 months. Mean mRS (score 6 for patients who died) at three months was 3·38 (±2·42). We found a highly statistically significant correlation coefficient between initial NIHSS and mRS after 30 days (0·74) and three months (0·66, $P < 0·01$). Sensitivity was 88·7 and 80%, Specificity 83·5 and 79·01%, PPV 81·8 and 77·9%; NPV 89·9 and 81·1%, GA 85·9 and 79·48%, respectively at 1 and 3 months. The ROC curve showed a fitted area as 0·914.
Conclusions: NIHSS correlates very well with the 1 and the 3-months mortality and functional outcome in patients with ICH, showing good levels of Sensitivity, Specificity, and Global Accuracy.

O8.02

Ischemic preconditioning in rats: effects of nitric oxide and activation and inhibition of K-ATP - channels

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Activation of K^+_{ATP}-channels is considered to be a main component of response in preconditioning models. The role of NO in the development of ischemic cell damage is equally important. The properties of NO action depend on the intensity of its production and the state of the surrounding tissue. NO hyperproduction in stroke causes damage to structural and regulatory components of cells. Moderate activation of NO during preconditioning may exert a neuroprotective effect, activating antioxidant enzymes, triggering anti-apoptotic mechanisms, and increasing cerebral blood flow. The present study is focused on the relationship between K^+_{ATP}-channels and NO, as well as on the investigation of mitochondrial mechanisms in cerebral ischemia.

We found that 24 hours after ischemic preconditioning in rats, there is a two-fold decrease in expression of mitochondrial K^+_{ATP}-channels in nervous tissue, a comparable increase in expression of cytochrome c oxidase, and a decrease in intensity of protein S-nitrosylation and nitration. Pharmacological preconditioning with the K^+_{ATP}-channels opener diazoxide led to a 25–41% reduction of free NO concentration, statistically significant 9 and 72 hours after ischemic stroke simulation. We attribute this result to the restructuring of tissue energy metabolism, namely to the provision of catalytic sites in mitochondria and increased elimination of NO, which prevents a decrease in cell sensitivity to oxygen during subsequent period of severe ischemia.

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O8.03

System of antioxidant protection in rat after spinal cord injury and production of nitric oxide

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The high frequency of spinal injury (SCI) combined with the complexity of the pathogenesis, and the lack at present adequate methods of treatment and rehabilitation of patients with consequences of spinal cord (SC) render this problem beyond the purely medical aspects. Great interest attracts the participation in the mechanisms of development of various pathological conditions of the body free radical compounds - nitric oxide (NO). It is known that major damaging factor during the development of processes of apoptosis is the peroxynitrite ($ONOO^-$), which is formed when NO interacted with superoxide ($O2^-$). Dismutation of superoxide by cytosolic enzyme Cu,Zn-COD (superoxide dismutase) is the primary and primary protection against free-radical oxidation processes. During modeling of SCI it is studied the content of copper, which is an indicator of activity of COD, and also the production of NO in the SC. There was applied the method of EPR spectroscopy with the examination of ratio of complexes (DETC)2-Cu and (DETC)2-Fe2+ -NO (NO/Cu). Ratio NO/Cu in the SC of intact rats is averaged 1:80, which is, apparently, helps prevent the formation of peroxynitrite. In the end, the ratio of the NO/Cu in early posttraumatic period amounted

to an average of 1:50, and in late - 1:3. Changing of this ratio shows the impairment of the antioxidant protection of the spinal cord in chronic posttraumatic period.

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O8.04
Sensory deprivation and delirium after stroke

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Background: Delirium is a common syndrome especially in elderly people and also in acute phase of stroke.

There are 3 types of delirium, in accordance to clinical presentation: hyper kinetic (with psychomotor agitation and hallucination), hypo kinetic (with lethargy and apathy) or the mixed form.

Among risk factors for post stroke delirium, sensorial disturbances are common, in particular visual and hearing deficits.

Only one clinical study [Oldenbeuving et al., 2011] evaluated the association between post stroke delirium and sensorial deprivation, reporting a weak association between delirium and previous visual disturbance (OR 1·5, but $P = 0{\cdot}75$).

Complex visual or auditory hallucinations may appear in many organic neurological disorders such as cortical or subcortical lesions and/or degenerative diseases.

Methods: We studied 100 patients with acute stroke admitted to Stroke Unit of San Martino Hospital in Genova; we evaluated delirium with DSM-V criteria and we screened delirium with 4AT scale and the association between delirium and sensorial disturbances.

Results: DSM-V criteria revealed 32% of cases of post stroke delirium in the acute phase. 4AT scale was used for delirium screening revealing a 52% of cases of delirium, the same observed by the consensus diagnosis of two senior neurologist (that was 50%).

We found a statistically significant association between delirium, hallucinations and visual disturbance; in particular between delirium and visual deficits ($P = 0{\cdot}004$ Chi Square Test) and between hallucinations and visual disturbance ($P < 0{\cdot}001$, Chi Square Test). There is no association between hearing loss, delirium and hallucinations.

Conclusions: We showed that the 4AT is useful to identify delirium in stroke patients.

The presence of visual sensory deprivations is a risk factor for post stroke delirium and hallucinations; therefore it is important to identify all risk factors in order to prevent it.

O8.05
Treating tension-type headaches in schoolchildren: the efficacy of aminophenylbutyric acid hydrochloride

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The research covered 56 schoolchildren aged 14–15 with tension-type headaches (TTH): 11 boys and 23 girls with episodic tension-type headache (ETTH), and 8 boys and 24 girls with chronic tension-type headache (CTTH). All children were prescribed GABA-receptors agonist - aminophenylbutyric acid hydrochloride (Anvifen®) 250 mg 3 times a day for 3 weeks. The reduction of TTH intensity was assessed by 3-grade scale: "no change", "significantly reduced", and "completely stopped". We assessed accompanying symptoms over the last 6 months according to the Likert scale. ETTH/CTTH: (1) difficulty falling asleep, restless sleep ($0{\cdot}91 \pm 0{\cdot}83/1{\cdot}54 \pm 1{\cdot}26$), (2) difficulty concentrating during the day ($0{\cdot}61 \pm 0{\cdot}92/2{\cdot}04 \pm 1{\cdot}21$), (3) morning sickness ($0{\cdot}32 \pm 0{\cdot}87/1{\cdot}81 \pm 1{\cdot}29$), (4) feeling ill in the morning with improvement in the second half of the day ($0{\cdot}17 \pm 0{\cdot}57/1{\cdot}95 \pm 0{\cdot}95$), (5) meteosensitivity ($0{\cdot}58 \pm 1{\cdot}01/1{\cdot}59 \pm 1{\cdot}22$), (6) decrease in physical capability ($0{\cdot}20 \pm 0{\cdot}64/1{\cdot}72 \pm 0{\cdot}82$).

After 3 weeks, the pain in ETTH subgroup completely stopped in 27 children (79%), and significantly reduced in 7 (21%). In CTTH subgroup pain completely stopped in 9 children (41%), and significantly reduced in 13 (59%). Associated symptoms of ETTH/CTTH: (1) $0{\cdot}26 \pm 0{\cdot}44$, $P = 0{\cdot}000093/0{\cdot}45 \pm 0{\cdot}80$, $P = 0{\cdot}000224$. (2) $0{\cdot}17 \pm 0{\cdot}38$, $P = 0{\cdot}003707/0{\cdot}54 \pm 0{\cdot}85$; $P = 0{\cdot}000007$, (3) $0{\cdot}08 \pm 0{\cdot}28$, $P = 0{\cdot}058101/0{\cdot}77 \pm 1{\cdot}06$, $P = 0{\cdot}001682$. (4) $0{\cdot}08 \pm 0{\cdot}28$, $P = 0{\cdot}083119/0{\cdot}27 \pm 0{\cdot}45$; $P = 0{\cdot}000000$, (5) $0{\cdot}32 \pm 0{\cdot}63$, $P = 0{\cdot}010304/1{\cdot}36 \pm 1{\cdot}09$, $P = 0{\cdot}021450$, (6) $0{\cdot}058 \pm 0\ 23$; $P = 0{\cdot}133706/0{\cdot}95 \pm 1{\cdot}04$, $P = 0{\cdot}005055$.

Aminophenylbutyric acid hydrochloride (GABA-receptors agonist) reduces the intensity of tension-type headache and has a positive effect on associated symptoms. It can be recommended for inclusion into the scheme of tension-type headache treatment in schoolchildren.

O8.06
Speech disorders in patients with vascular encephalopathy

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The authors present the results of a three-year research project on speech disorders in patients with vascular encephalopathy. The research was conducted with the use of neuroimaging and psychometric instruments designed in Kazan Federal University. An in-depth neurolinguistic testing, also developed in Kazan Federal University for patients with different types of encephalopathy, included balanced diagnostic phonetic, lexical and grammar subtests, as well as test tasks aimed at discourse analysis. Testing involved qualitative and quantitative (point-based) scores. For each subtest, experiments were held in order to identify linguistically valid diagnostic units. As a result, a database of diagnostic materials was set up. Since the dynamics of speech status must be monitored in the course of treatment, each patient every time was offered a neurolinguistic questionnaire with unique structure but featuring different linguistic material. It can be concluded that speech disorders in patients with vascular encephalopathy differ from disorders in patients with focal lesions. Revealed speech disorders mainly refer to lexical, syntactic, discourse and pragmatic levels. Phonetic and morphological

disorders are not clinically significant. The reduction of short-term and long-term verbal memory is high in these patients. There was observed a gradual loss of ability to understand the phraseological units and other units with a figurative sense and lexical homonyms. Patients with vascular encephalopathy have a significant deficit in derivation which results in a great number of occasional new units and word-formation models. Also, the syntax suffers gradual disintegration: some violation of compatibility can be observed, the number of clausal sentences increases, while that of compound sentences decreases, as well as the quantity of minor parts of speech in syntactic structures. The lack of ability to perceive oral texts and to retell them correctly appears at the first stage of vascular encephalopathy; delayed reproduction of any types of texts is significantly deficient.

O8.07
Striatal glutamatergic transmission: astrocytic A2A-D2 receptor-receptor interaction and homocysteine

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The interaction between adenosine A2A and dopamine D2 receptors at the striatal neuronal plasma membrane is a well-established phenomenon and has opened up new perspectives on the molecular mechanisms involved in the pathophysiology of neuropsychiatric disorders such as Parkinson's disease or schizophrenia. However, it has barely been investigated in astrocytes, although involvement of astrocytes in neuropsychiatric disease vulnerability is increasingly recognized. Here, we investigate the presence of A2A and D2 receptors on astrocyte processes prepared from adult rat striatum, and on A2A-D2 receptor integrative processes at plasma membrane of striatal astrocyte processes.

We report here that A2A and D2 receptors were co-expressed on the processes, and A2A-D2 receptor-receptor interaction controlled glutamate release from the processes. The synthetic peptide VLRRRRKRVN corresponding to the D2 receptor region involved in electrostatic interaction underlying A2A-D2 heterodimerization abolished the ability of the A2A receptor agonist to antagonize the D2 receptor-mediated effect. The complexity of the integrative action of A2A-D2 receptor-receptor interaction is suggested by the effect of intracytoplasmic homocysteine, which inhibited the D2-mediated effect on glutamate release (i.e., homocysteine allosteric action on D2), without interfering with the A2A-mediated antagonism of the D2 effect (i.e., maintained A2A-D2 receptor-receptor interaction).

In conclusion, our findings indicate the crucial integrative role of A2A-D2 molecular circuits at the plasma membrane level of striatal astrocyte processes. The fact that homocysteine reduced D2-mediated inhibition of glutamate release could provide new insights into striatal astrocyte-neuron intercellular communications. In fact, hyperhomocysteinemia has been repeatedly reported in Parkinson's patients, especially during L-dopa treatment, and hypothesized to play a role in tardive side effects of L-dopa. As striatal astrocytes are increasingly known to be involved in the pathophysiology of Parkinson's disease, these findings may shed light on the pathogenic mechanisms of the disease and contribute to the development of new drugs for its treatment.

O8.08
Dysregulation of regulatory CD56 (bright) NK cells/T cells interactions in multiple sclerosis

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Recent evidence has shown that CD56bright NK cells (NKregs), a subset of NK cells abundant in lymph nodes, may have an immunoregulatory function. In multiple sclerosis (MS), expansion of NKregs has been associated to response to different treatments and to remission in pregnancy; however what function they exert in physiologic conditions and whether it is impaired in MS is not known. Our aim was to dissect NKreg function in healthy subjects (HS) and compare it with that of patients with MS.

We assessed proliferation and death of CD4+ T cells in presence of pre-activated NKregs and CD56dim NK cells, and degranulation of NK cells in co-culture. Through selective blocking we studied the NK receptors involved in NK cell-T cell interactions and through real-time PCR the expression of lytic enzymes by NK cells.

We found that NKregs from HS acquire, upon inflammatory cues, the capability of suppressing autologous CD4+ T cell proliferation through direct cytotoxicity requiring engagement of natural cytotoxicity receptors (NCRs) and secretion of granzyme B. NKregs from MS patients did not differ in frequency but had a significantly lower suppressor function towards autologous T cells. This impairment was not related to deficient NCR/granzyme B pathways. Rather, blocking HLA class I on T cells restored normal suppressor function in NKregs from MS. On par with this observation, expression of HLA-E molecule was upregulated in CD4+ T cells from MS patients compared to HS.

Our study suggests that in HS NKregs inhibit CD4+ T cell proliferation by direct cytotoxicity through engagement of NCRs and release of granzyme B. In MS, the function of NKregs appears to be impaired by increased inhibitory signal from HLA-E expressed on high level on T cells. The defect in NKregs in MS might contribute to the excess of autoimmune response that is associated to disease development.

O8.09

Possible role of microRNAs in the modulation of neuroinflammation by mesenchymal stem cells

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The degeneration of lower and upper neurons in amyotrophic lateral sclerosis (ALS) is associated with a neuroinflammatory reaction in which microglia play a prominent role.

Treatment of SOD1-G93A mice (mSOD1) with mesenchymal stem cells (MSC) improves clinical outcome and pathological scores, and modulates the neuroinflammation typical of this ALS model.

We postulate that MSC ameliorate clinical murine ALS in part via modulation of microglial phenotype through specific RNAs shuttled by exosomes present in their secretome.

Our microarray experiments revealed nine microRNAs (miRNAs) that were significantly upregulated in MSC cultured under inflammatory stimulus with IFNg.

We sought to ascertain whether or not the miRNAs that are upregulated in MSC could modulate the activated microglia phenotype. Accordingly, we transfected lipopolysaccharide-activated murine microglial N9 line cells with miRNA mimics and analyzed the expression of genes associated with proinflammatory/neurotoxic (M1-like) and anti-inflammatory (M2-like) phenotypes. Our results suggest that miR-466q and miR-467f are able to downregulate proinflammatory genes in activated microglia, while miR-466m5p, miR-5126 and miR-3082-5p significantly upregulated the M2-like phenotype. We observed that all nine miRNAs are present within the exosomes released by IFNg-primed MSC, with the immunomodulatory miRNAs miR-466q and miR-467f being significantly overexpressed. Exposure of activated N9 microglia to MSC-derived exosomes, which enter the cells, resulted in modulation of their activation state. We used the MirWalk database to predict possible target genes for miR-466q and miR-467f and KEGG Pathway and PANTHER databases to predict the pathways involved, including MAPK pathways, which we are presently validating. Experiments are ongoing to monitor the efficient release of the miRNAs and their predicted modulatory effect on microglia isolated from mSOD1 at relevant time points of the ALS-like disease. These data are prerequisite to obtain an in-vivo proof of concept that administration of exosomes to mSOD1 can recapitulate the beneficial effect of the MSC themselves.

O8.10

Dysregulation of Repressor Element 1-Silencing Transcription factor in a mouse model of multiple sclerosis

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The transcriptional repressor REST regulates neurogenesis and neuronal identity through cell-specific gene repression, allowing expression of its targets in mature neurons where REST is quiescent. REST dysregulation has been implicated in several neurodegenerative disorders, but whether it is deleterious or neuroprotective is as yet unclear. We have addressed REST expression in chronic experimental autoimmune encephalomyelitis (EAE), the mouse model of multiple sclerosis, characterized by inflammation, demyelination and axonal loss. Preliminary results at mRNA and protein levels indicated that REST expression increases significantly in EAE mouse spinal cord 24 hours after disease onset, with concomitant downregulation of its target gene, sodium channel Nav1·2, confirming REST transcriptional repression and suggesting neuronal dysregulation at this early stage. Time course analysis confirmed an increased expression of REST in the spinal cord during acute EAE phase, which was accompanied by downregulation of its target genes analyzed (Nav 1·2, somatostatin, synapsin, and NMDAR). Analysis of brain areas revealed a small upregulation of REST in the striatum at disease peak and in the hippocampus at the chronic phase that, unexpectedly, was associated with upregulation of REST target genes. While full-length REST represses the expression of neurogenesis genes, its splicing variant, REST4, has been shown to induce their expression. We therefore monitored the expression of REST4 mRNA, which, interestingly, was increased at the early phase in the striatum, where we observed upregulation of REST target genes, but not in the spinal cord where these are downregulated. These data suggest that REST dysregulation also occurs in EAE, affecting the response of neural cells to pathological stimuli. Whether or not an unbalance in the expression of full-length REST and REST4 underlies the anomalous expression of REST in EAE at specific stages and in particular CNS areas remains to be established.

O8.11

Creatine transporter deficiency: effect Of Di-Acyl-Creatine in Brain slices in vitro

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Hereditary creatine transporter deficiency is a severe incurable neurological disease where creatine is missing from the brain. Creatine crosses the blood-brain barrier (BBB) slowly and in a limited manner, and this is a serious obstacle to its therapeutic use. A possible solution could be to modify the creatine molecule in such a way to create a molecular structure that can cross the BBB while still conserving a biological activity similar to creatine. For this reason, we devised and synthetized di-acyl-creatine (DAC). Preliminary data show that DAC 1 μM is able to revert in vitro the deleterious effects of blocking the creatine transporter. To test the effects of DAC in brain slices, we used electrophysiological techniques. Specifically, we tested our slices by recording their compound action potential (Population Spike, PS). The latter is a waveform that normally occurs in the CA1 cell body layer after electrical stimulation of the Schaffer collateral. Its presence confirms the ability by the slice to maintain and express a normal synaptic transmission. Normal slice always show this waveform, with only occasional exceptions. To have a specific model of the harm that block of the creatine transporter causes in brain tissue, we incubated mouse hippocampal slices with the creatine transporter blocker guanidine-propionic acid (GPA) and testing their PS. We found that block of the creatine transporter with GPA has a harmful effect on brain slices

viability, indicating that block of the creatine transporter has by itself a harmful effect on nervous tissue functioning. We next investigated whether or not this harmful effect could be reverted. 1 mM GPA allows synaptic transmission in only 61 ± 31% of our slices, a statistically significant difference. Lacking its transporter, creatine supplementation was not able to revert this effect. By contrast, DAC 2 μM restored it in a statistically significant way (slices viability 83 ± 27%)

O8.12
Autologous haematopoietic stem cell transplantation in severe forms of multiple sclerosis

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Intense immunosuppression followed by autologous haematopoietic stem cell transplantation (AHSCT) has been evaluated as a possible new therapeutic tool in severe autoimmune disorders, especially as it has been shown to be efficacious in animal models of immune mediated diseases. In severe forms of multiple sclerosis (MS), AHSCT has been carried out in phase I and II studies and up to now more than 900 cases have been treated worldwide with this procedure. The target of this treatment is the eradication of self reactive abnormal immune system by intense immunosuppression, followed by the infusion of autologous haematopoietic stem cells (HSC) aimed to restore the hemato-lymphopoietic system. This procedure, besides its certain immunosuppressive properties, could also result in a resetting of the immune system that might become tolerant to self antigens for a long period of time. In the last years the best results have been obtained in MS still in the RR phase of the disease, with an aggressive clinical course.

Workshop 9: Internal Medicine with Fadoi

O9.01
Chronic care model: disease management for heart failure: The PONTE project

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The need to reduce the healthcare costs combined with the progressive increase of chronic diseases such as heart failure (HF) requires the testing of care model through the implementation of care pathways to ensure the continuity of care and the integration between territory and hospital. It will be necessary to promote the outpatient and home-based intervention with the aim of reducing the number of hospitalization and readmission improving the quality of patient's life. The P.O.N.T.E project (A Pilot Study for implementation of regiOnal guidelines for integrated maNagement of heart failure between hospital and Territory in ligurian populaTion) is a multidisciplinary disease-management program in elderly patients with HF and major co-morbidity conduct by Department of Internal Medicine with collaboration of cardiologists of ASL 2 Savonese. The P.O.N.T.E project is a disease management program for HF patients based on nurse case manager (continuity of care) integrated with a clinical follow-up by internists (multidimensional) with cardiologist's and nephrologist's supervision (multi-disciplinary). The nursing staff has a role in the coordination of the ambulatory and in data collection, promotes healthy life style modifications as diet and physical activity among heart failure patients. In a year of project, we enrolled 120 patients of which 56% female patients and 44% male patients. Among the most frequently associated comorbidity hypertension (75%), chronic renal failure (47%) and COPD (45%). 21% of the patients has necessitated an additional diuretic treatment intravenous. The rate of hospitalization for heart failure patients enrolled was 8%. In a year of project in our ASL the rate of hospitalization for heart failure dropped by 15%. Our project has demonstrated the effectiveness of these programs multidisciplinary patient-centred and continuity of care hospital-territory.

O9.02
Acute complex care model: an organizational approach for the medical care of hospitalized acute complex patients

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Background: Chronic diseases are the major cause of death (59%) and disability worldwide, representing 46% of global disease burden. According to the Future Hospital Commission of the Royal College of Physicians, Medical Division (MD) will be responsible for all hospital medical services, from emergency to specialist wards. The Hospital Acute Care Hub will bring together the clinical areas of the MD that focus on the management of acute medical patients. The Chronic Care Model (CCM) places the patient at the center of the care system enhancing the community's social and health support, pathways and structures to keep chronic, frail, poly-pathological people at home or out of the hospital. The management of such patients in the hospital still needs to be solved. Hereby, we propose an innovative model for the management of the hospital's acute complex patients, which is the hospital counterpart of the CCM.
Acute Complex Care Model (Accm): The target population are acutely ill complex and poly-pathological patients (AICPPs), admitted to hospital and requiring high technology resources. The mission is to improve the management of medical admissions through pre-defined intra-hospital tracks and a global, multidisciplinary, patient-centered approach. The ACCM leader is an internal medicine specialist (IMS) who summarizes health problems, establishes priorities, and restores health balance in AICPPs.
Conclusions: The epidemiological transition leading to a progressive increase in "chronically unstable" and complex patients needing frequent hospital treatment, inevitably enhances the role of hospital IMS in the coordination and delivery of care. ACCM represents a practical response to this epochal change of roles.

O9.03
Intenists' experiences in peri operative medicine

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Fragility fractures are a major health care problem worldwide. Both hip and non-hip fractures are associated with high mortality in the years following the fracture. The mortality rates can reach 10% during admission into the hospital and 30% after the first 12 months. The estimated socio-economic costs represent 01% of the global health care costs worldwide reaching 1·4% in the more developed countries. The mean age of patients with hip fractures and the presence of comorbidity are the main reasons warranting co-management of these individuals. We developed since 2010 a multidisciplinary care-model: a co- management of the patients with an Hospitalist dedicated to the Orthopedic Department. The Hospitalist dealt with antibiotic prophylaxis, thrombo-prophylactic protocols, correct analgesic regimens, assessment and treatment of cognitive deterioration and nutritional conditions during the in-hospital period, the prevention of re-fractures, the early diagnosis of dysphagia. The efficiency and benefits of a multidisciplinary approach is well documented in literature. We have six years of observational study: the outcome measures examined were in -hospital mortality, the length of hospital stay, the medical complications, the number of cardiology and neurological consultations. We also monitored the consumption of anti-microbic, anti-thrombotic, neurolectic and antipsychotic, analgesic /opiates, nutritional supplements, oral anti-diabetic/insulin to evaluate the adherence to guidelines (treatment of pain, therapy of diabetes in surgery, prevention and treatment of delirium, anti-microbic and antithrombotic prophylaxis). We reduced the total expense for

antimicrobial therapies, the length of stay in hospital, the incidence of delirium, the in–hospital mortality, the consumption of parenteral nutrition, the consultations of Cardiologist and Neurologist, and we introduced NOAC and protocols for thrombo-prophylaxis, we observed an increasing consumption of opiates and of calcium and VIT D, we reduced the employ of oral anti diabetic therapies and increased the use of insulin schedules.

O9.04
Hospital based progressive care

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The Progressive Patient Care is a model which aims at group patients according to their complexity in order to place patients in the most appropriate care setting.

The original model consists of three care levels: intensive care, intermediate care, low care. The I level (intensive care) need high level of medical and nursing care and appropriate technology. The II level (intermediate care) collects medical and surgical inpatient admission. The III level (low care) need medical monitoring, nursing and physiotherapic treatments.

The organization for intensive care levels is an opportunity for Italian healthcare facilities to reach continuity of care. This model emphasizes care processes looking to patients' needs rather than a division according to criteria of specialties.

O9.05
A relapsing hepatitis

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A 55-year old woman presented to the Emergency Department because of jaundice and asthenia, started 3 days before. She was affected by mild hypertension (treated with olmesartan/amlodipine 20/5 mg). She reported previous psoriatic arthritis (4 years before), nephrolithiasis and cholecystectomy. One year before she had been hospitalized at the Infectious Disease Department because of cholestatic hepatitis; no macroscopic hepatic or biliary alterations were found, nor evidence of major or minor hepatotropic viruses infection or autoimmune disease. In the same occasion, a lung CT scan demonstrated a single pulmonary nodule and multiple bilateral micronodules associated with mediastinal lymphoadenopathies. There were no evidences of pneumonia, and bronchoalveolar lavage didn't demonstrate neoplastic disease. A transbronchial lymphonodal fine needle aspiration biopsy evidenced granulomatous reaction without necrosis or mycobacterial or fungal infection. A 18-fluorodeoxiglucose positron emission tomography demonstrated high metabolic activity of lung lesions. Hepatitis self-resolved within two weeks and the patient refused lung biopsy. Lung CT follow-up began, and the micronodules spontaneously disappeared within 9 months.

When admitted to our Department, a relapse of cholestatic hepatitis was confirmed (ALT: 747 U/l, bilirubin: 8·7 mg/dl, gGT: 93 U/l), with a subtle progression through hepatic failure (INR: 1·5, bilirubin: 16·6 mg/dl). Again, imaging, virological and immunological exams were negative, including iron and copper metabolism. A liver biopsy was obtained, with demonstration of interface hepatitis, plasma cells and eosinophils infiltration, bridging and centrolobular necrosis, mild fibrosis but no granuloma. Oral prednisone 1 mg/kg with azathioprine 50 mg bis in die were started, with rapid improvement on liver tests. Given the high likelihood of autoimmune hepatitis, in the absence of classical autoimmune markers, we went deep the analysis and asked for IgG4 immunohistochemistry on the biopsy specimen, which resulted positive. Also serum IgG4 levels were above upper normal limit. A diagnosis of IgG4-related lung/liver disease was made.

O9.06
Dietary fat in asthma and respiratory diseases, a multi-case control study

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Introduction: Diet is an important factor associated to respiratory diseases. In particular, the role of fatty acids has been investigated, but results are conflicting. In the frame of the Genes Environment Interaction in Respiratory Diseases (GEIRD), a population-based multi-case control study, we aimed to assess the association between different types of dietary fats (total, animal and vegetable fats, monounsaturated (MUFAs), saturated (SFAs) and polyunsaturated fatty acids (PUFAs), and oleic acid) and respiratory diseases and to assess the association with fat containing foods (olive oil and butter).

Methods: We collected information about clinical status and nutrient intake of 962 subjects: 145 with Current Asthma (CA), 77 with Past Asthma (PA), 77 with CB, 301 with Allergic Rhinitis (AR), 164 with Non Allergic Rhinitis (NAR) and 344 Controls.

Food intake was collected through the Food Frequency Questionnaire EPIC (European Investigation into Cancer and Nutrition), validated for the Italian population.

The associations between fats and respiratory diseases were estimated by means of multinomial or logistic models. The different types of fat and foods were analyzed both as continuous variables and as quartiles and they were energy-adjusted.

Results: We found a negative association between the intake of vegetable fats, MUFAs, oleic acid and the risk of CA. The association is statistically significant both in the highest quartile and as inter-quartile trend. In a similar way, olive oil is associated to a reduction of the risk of CA (RRR 0·78 95%CI 0·64–0·96). Also, there is a positive association between AR and a moderate intake of animal fats.

Conclusions: These data show for the first time a protective role of olive oil in asthma. Furthermore, the results suggest the existence of a relationship between nutrition and respiratory diseases. This association seems to be dependent not only on the diet but also on the disease itself.

O9.07
Troponin elevation does not always mean cardiac ischemia

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Background: High serumtroponin (Tn) levels might not be only detected in acute myocardial infarction, but also in some

metabolic conditions. Here, the case of a 47 year-old woman suffering from Type 1 Diabetes (T1D) and presenting with ketoacidosis and acute troponin elevation was reported.

Materials and methods: Data were collected during hospitalisation after the informed consent signature by the patient.

Results: The patient was admitted to the Emergency Room for mental confusion, nausea, vomiting and dyspnea from about 5 days. No typical chest pain was referred. Insulin therapy compliance was sub-optimal. At the admission, blood glucose was 513 mg/dl, potassium 4·4 mEq/l; sodium 137 mEq/l, creatinine 0·8 mg/dl and Tn I 1·13 mcg/l increased to 9 mcg/l (peak); the arterial blood gas analysis showed: pH 7·3, pO_2 52·3 mmHg; pCO_2 27·0 mmHg; HCO_3- 15·6 mmol/l; Base Excess −9·6; lactates 2·8 mmol/l, anion gap 20·2, C-reactive protein 15·9 mg/l, ketonuria 40 mg/dl. Electrocardiogram showed a sinus rhythm with normal QT interval. Echocardiography showed normal kinesis of the right and left ventricles; the thoracic CT scan was negative for pulmonary embolism, but showed bilateral consolidations. The patient was daily infused with 0·9% sodium chloride; insulin and antimicrobial treatments were started and well tolerated until pneumonia resolution and metabolic balance. Main causes of myopericarditis were excluded by negative autoimmune assays (ANA, ENA, ANCA), serology for most common myocarditis-associated pathogens, and cardiac magnetic resonance imaging.

Conclusion: We presented the case of a T1D patient with ketoacidosis, in which a relevant increase in troponin levels was not apparently related to an ischemic heart disease. We would recommend to consider metabolic disorders as a potential condition associated with troponin elevation in diabetic patients. Also the cardiovascular prognostic value of positive troponin in diabetic patients with ketoacidosis represents an interesting topic for future research and clinical practice.

O9.08
Intermittent fasting in heart failure: a pilot study

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Background: The effects of Calorie Restriction (CR) on cardiovascular system have been deeply studied, with an overall beneficial impact on systemic inflammation, traditional risk factors, vascular oxidative stress and endothelial function. Also, studies conducted on laboratory rodents and primates have shown that CR ameliorates the age-associated cardiovascular impairment of left ventricular diastolic function and arterial elasticity.

However, sustained CR is hard to follow for the majority of people and it is associated with side effects (i.e. reduced bone mineral density).

The term "Intermittent Fasting" (IF) encompasses different paradigms involving alternating between periods of unrestricted feeding and periods of dietary restriction. Data from preclinical experiments in animals suggest that IF leads to improved cardiovascular risk profile and it can be easier to follow.

Heart Failure (HF) is increasingly recognized as a multisystem disease with important metabolic comorbidities. Inflammation, endothelial dysfunction and oxidative stress are key pathophysiologic elements.

Aim: 40 overweight/slightly obese HF outpatients (NYHA I-II) will be randomly assigned to IF or control group for six months. IF scheme is a '5:2-diet' (ad libitum food for 5 days during the week and fasting -i.e. a maximum of 500 Kcal intake- for two nonconsecutive days).

Primary objective of this study is to determine whether IF reduces the level of chronic inflammation (decrease in high sensitive C-reactive protein level).

The secondary objective is to determine whether IF mimics the metabolic and cardiovascular effects of CR, evaluating different outcomes:

- body weight, waist circumference and body composition modifications measured by DEXA
- lipid profile, glucose tolerance
- oxidative stress markers
- blood pressure
- arterial stiffness measured by Pulse Wave Velocity and Augmentation Index
- arterial endothelial function measured with the flow dependent vasodilation technique
- heart stiffness measured by transthoracic echocardiogram

Serum samples will be collected for future analysis (hormones, adipokines).

Samples collection regarding oral microbiota will be held too.

Workshop 10: Tissue Calcification

O10.01
Pathophysiology of obstructive acute renal failure

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Obstructive acute renal failure is a heterogeneous entity as the pathophysiology of intratubular obstruction is quite different from upper tract obstruction. In the former case, tubules are dilated due to a high hydrostatic pressure whereas pressures are normal in urinary upper tract. In the latter case, a high pressure above the ureteral obstacle is responsible for dilated renal cavities leading to extrinsic compression with no or only few dilated tubules though high hydrostatic pressure are recorded within tubules. Obstruction within tubules may be related to crystal formation, exfoliated cells, cellular debris and/or protein gels altogether with cell proliferation, and recruitment of inflammatory cells. Though fibrosis may develop, occurrence of atubular glomeruli in several nephrons due to an initial loss of tubular patency highlights the critical importance of maintaining a fluid flow within tubules in order to avoid uncontrolled tubular cell proliferation. The onset of tubular obstruction in few tubules especially in crystal nephropathy is underestimated especially in chronic kidney disease patients, thus suggesting that some macromolecular solubilizing factors may be potential relevant therapy to prevent (and/or reverse) chronic kidney disease progression or decrease acute renal failure sequelae.

O10.02
Prevalence of hyperparathyroidism and hypovitaminosis D in morbid obese patients and their correlation to early atherosclerosis markers

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Obesity is a risk factor for hypovitaminosis D with a potential consequent secondary hyperparathyroidism. Many pathophysiological mechanisms have been claimed to explain the link between obesity and hypovitaminosis D, such as sequestration of vitamin D in the exceeding fat tissue. On the other hand hyperparathyroidism has been claimed as a possible effector of vascular dysfunction. The aim of the study was to evaluate the link among iPTH, vitamin D and early vascular damage in morbid obesity.

We evaluated the levels of 25(OH)vitamin D and iPTH in a cohort of 150 morbid obese patients with a BMI > 35 kg/sqm and stable normal levels of calcemia and eGFR.

Ninety-seven percent of patients had levels of 25(OH) vitamin D < 30 ng/mL and 72% of them had levels of iPTH ≥65 pg/mL. Dividing our population according to the median level of BMI (44 kg/sqm), we found that patients with a BMI above median level had also significantly higher levels of iPTH ($P = 0{\cdot}021$) with no significant difference in 25(OH) vitamin D levels. Performing univariate correlations, we confirmed a significant positive correlation between iPTH, BMI (rho = 0·176, $P = 0{\cdot}030$) and waist circumference (rho = 0·189, $P = 0{\cdot}021$); we found also a significant positive correlation between iPTH and HOMA index (rho = 0·222, $P = 0{\cdot}007$), systolic blood pressure (rho = 0·202, $P = 0{\cdot}013$) and carotid IMT (rho = 0·179, $P = 0{\cdot}032$). At the stepwise regression analysis age, 25(OH) vitamin D and waist circumference were the independent predictors of iPTH.

Our data confirm the high prevalence of hypovitaminosis D and hyperparathyroidism in obese patients, although hypovitaminosis D could not be the only factor causing hyperparathyroidism in these subjects. Furthermore, levels of iPTH correlate with markers of metabolic and vascular damage, supporting the hypothesis that hyperparathyroidism could be an additional risk factor for cardiovascular disease in morbid obese.

O10.03
Crystal nephropathy in transplanted patients

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Background: Tubulo-interstitial microcalcifications in renal transplant are described with wide difference of incidence (4–78%) according to time and goal of biopsies. Biopsies of transplanted kidneys are commonly stained for tissue examination and search for possible crystal deposits which are then identified by polarizing microscopy and staining by von Kossa's method revealing mainly calcium deposits. However, such identification is not accurate enough to help in optimizing the clinical management of the patients.

Materials and methods: Here we have analysed kidney transplants presumably containing crystal deposits with the Spotlight 400 Fourier transform infrared (FTIR) imaging System in the mid infrared spectral range to obtain infrared maps of tissue slices at high spatial resolution, down to 10 microns. First, microcalcifications observed in 118 allograft biopsies were studied by FTIR; second, we compared 55 patients with to 100 patients without calcifications. In all cases, biopsies were performed within two years following transplantation.

Results & discussion: FTIR showed that a major proportion (92%) of calcium deposits were made of calcium phosphate (CaP) crystals, mainly carbapatite and/or amorphous carbonated calcium phosphate (ACCP) as pure or mixed forms; including 71·4% of ACCP which appeared early in post-transplantation. Quantitative score (0 to 4) of CaP deposits revealed a significant relation between high score values (3 or 4) and a high level of calcemia at the time of kidney graft and further the decrease in renal function. Among other deposits,

we found pure or mixed calcium oxalate monohydrate crystals in 16% of the samples. These crystals have been reported to evolve more slowly, inducing delayed renal failure post-transplantation. The patients with calcifications show persistent hyperparathyroidism and tubular cell vacuolization as circumstance of crystal deposition.

Conclusion: These preliminary data suggest the clinical interest of an accurate characterization and quantification of crystals deposits with FTIR microspectroscopy.

O10.04
Stone composition is a witness of some specific environmental exposure: illustrations through epidemiological studies in Burkina Faso and in France

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Background: Urolithiasis is increasing in most countries, suggesting that the same risk factors, mainly of dietary origin, are involved in stone formation. However, the comparison of stone composition in various populations may reveal specific environmental risk factors. Thus, data collected from a country cannot be systematically applied to another one. Two examples may be provided from France and Burkino-Faso.

Materials and methods: A series of 100 consecutive stones removed by urologists in the CHU of Ouagadougou was analyzed by infrared spectroscopy according to the same protocol used for thousands stones analyzed over the past fifteen years in France. The data collected from the two series have been compared.

Results: The main component of calculi from Burkina Faso was calcium oxalate (70·9% of cases), followed by opaline silica (17·4%) and uric acid (7%). In France, the main component was calcium oxalate (69·5%), followed by calcium phosphate (15·5%) and uric acid (10·1%). However, we found a major difference between the two series of calculi when considered the stone nucleus. In Burkina Faso, 46·5% of stones were developed from an opaline silica core and only 5·8% were initiated from calcium phosphate. By contrast, in France, 52·4% of all stones were initiated from a calcium phosphate core (mainly Randall's plaque carbapatite).

Discussion: The high occurrence of opaline silica nuclei in Burkina Faso is explained by geophagy, a common traditional practice. By contrast, in France, a high consumption of vitamin D + calcium was suggested to be an important factor involved in the high occurrence of Randall's plaques found in stone nuclei and in the kidneys of French population, especially in predisposed stone former patients.

Conclusion: Stone analysis is an important key to better understand the origin of environmental factors involved in stone formation.

O10.05
Crystalluria due to drugs: an update

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It is well known that several drugs may precipitate as crystals in the urine. Drug crystalluria can be isolated, or with haematuria (either microscopic or gross), leukocyturia, or even acute kidney injury, due to the intratubular precipitation of crystals.

Several factors may favour the precipitation of drug crystals namely, drug overdose, hypoalbuminemia, intravascular volume depletion, urinary pH, or the presence of chronic renal dysfunction.

Two types of drug crystals can be found in the urine: 1. Those which are made up of the drug itself, which can be suspected on the basis of their unusual morphology and birefringence features (e.g., shocks of wheat, needles, sheaves, hexagons, star-like structures, etc.), and can be identified with certainty only by infrared spectroscopy investigation. 2. Those which come as calcium oxalate, due to the drug metabolic transformation into oxalic acid; these crystals can be identified only by the knowledge of the clinical history.

Well known drugs which can cause crystalluria are: sulfadiazine, amoxicillin, ciprofloxacin, indinavir, acyclovir, triamterene, piridoxylate, primidone, naftidorofuryl oxalare, felbamate, vitamin C and orlistat.

More recently, other drugs have been added to the list: sulphamethoxazole, zonisamide, sulfasalazine, atazanavir and darunavir.

O10.06
A clinical case with an unexpected sulfamethoxazole crystalluria

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Crystalluria due to N-acetylsulfamethoxazole hydrochloride (SMX), which is the main component of the antibacterial agent co-trimoxazole, is rare. We describe a peculiar case of SMX crystalluria.

On July 15th, 2014 the examination of the urine sediment of a 50-year-old kidney transplant recipient showed the presence of hexagonal crystals. These appeared as plates of variable size, some of which were heaped one upon another, while others appeared as individual thin plates with a smooth surface. By polarized light, the largest and heaped crystals were strongly birefringent and polychromatic (? uric acid crystals), while the individual thin plates were either no birefringent or displayed a pale to shining white birefringence (? cystine crystals). Because of these contrasting features, infrared spectroscopy analysis was performed. This demonstrated that the crystals were made up of SMX, which the patient was actually receiving - 1 tablet/day – to prevent *Pneumocystis jirovecii* infection.

This case demonstrates the importance of the use of polarized light and of infrared spectroscopy in the identification of crystals when they come with unusual morphological and/or birefringence features.

O10.07
"Daisies": an unusual type of calcite urinary crystals

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Calcium carbonate (calcite) crystals are among the rarest crystals in humans, as demonstrated by the fact that we did not find them in a retrospective study, in which 807 urine samples containing crystals samples were identified. Accordingly, printed

Atlases on urinary sediment or monographs on urinalysis, published in English language, either do not mention these crystals or show of them only few (one to three) images, the commonest reported morphology of such crystals being that of "dumbbell" particles and, less frequently, "spheres or spheroids" or "amorphous granules".

We describe 1. A cohort of 10 new cases (8 women, 1 man, age 3 to 82 years; 1 dog) with an unusual type of calcite crystal in their urine, which were brought to our attention from 8 different institutions from 5 European countries, after the publication of our first case with this type of crystals in 2004 2. The result of the search of such crystals in the urine of two horses, in which calcite crystals are common due to their diet based on herbs and 3. The result of a prospective search of such crystal on the urine of 2280 pregnant women, which was stimulated by the fact the 3 out of the 11 cases identified so far were pregnant.

O10.08

Structural and biomechanical properties of fixed bovine pericardium heart valve prostheses are not affected by ionizing radiation: an in-vitro study

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Objective: Precocious structural degeneration of bioprostheses in irradiated survivors of chest malignancies has been reported. Aim of this in-vitro study was to evaluate any structural and/or biomechanical radiation-induced changes on a fixed bovine pericardium used to manufacture prosthetic heart valve leaflets.

Methods: Fixed-pericardial bovine patches underwent irradiation with a γ-rays source IBL 437C, according to experimental radiobiological protocols (T1: single-2-Gray (Gy) dose in 15·3 sec; T2: single-10-Gy dose in 76·7 sec; T3: single-30-Gy dose in 230 sec; T4: fractioned radiation 3-Gy dose in 23 sec /session, ×10 sessions; T0: controls). Specimen assessment included mechanical traction test (strain-stress and load to breakage point), shrinkage test (contraction temperature) and histological examination (hematoxylin/eosin for tissue features, Azan for collagen fibers, Verhoeff/VanGieson for elastin fibers).

Results: Mechanical traction tests did not show remarkable variations in traction load values (MPa:T0/T1 = 9·3 ± 1·3/11·8 ± 1·6; T0/T2 = 14 ± 4·6/14·3 ± 2·2; T0/T3 = 12·7 ± 0·2/12·7 ± 1·7; T0/T4 = 10·1 ± 1·8/10·3 ± 4·3; P = NS). Contraction temperature (°C) did not show any significant difference among the specimen, with only a trend towards a mild increase in the mean shrinkage temperature in all radiated samples vs T0 (T0/T1 = 85·07 ± 0·43/85·22 ± 0·27; T0/T2 = 85·05 ± 0·17/85·28 ± 0·48; T0/T3 = 84·94 ± 0·27/85·61 ± 0·23; T0/T4 = 85·01 ± 0·33/85·34 ± 0·5; P = NS). There were no remarkable histological differences, with irradiated samples maintaining the same architecture of elastic fibers and collagen bundles, as well as the same cellular and vascular content of controls.

Conclusions: In-vitro, ionizing radiations do not seem to induce any biomechanical and/or structural changes on fixed bovine pericardium. In-vivo studies are required to evaluate the potential role of endogenous factors in inducing radiation mediated prostheses tissue degeneration.

O10.09

Acute kidney injury: the importance of crystalluria identified at urine sediment examination

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Many different conditions can affect the renal parenchyma and cause acute kidney injury (AKI). Among these, there also is "acute crystalline nephropathy", which is due to the intratubular precipitation of crystals, with consequent tubular obstruction and interstitial inflammation.

Today, acute crystalline nephropathies include: acute uric acid nephropathy (precipitation of uric acid crystals); ethylene glycol intoxication (precipitation of monohydrated calcium oxalate crystals); inherited metabolic disorders, such as adenine phosphoribosyltransferase deficiency (precipitation of 2,8-dihydroxyadenine crystals), primary oxaluria (precipitation of monohydrated calcium oxalate crystals); monoclonal gammopathies (precipitation of monoclonal light chain crystals), and drugs (precipitation of crystals made of the drug itself or of calcium oxalate).

The examination of the urinary sediment, performed with the correct methodology (= standardized sample preparation + knowledge of urine pH) and the proper equipment (bright field or phase contrast microscopy + polarized light devices) by expert and motivated persons, is of paramount importance in the early diagnosis and follow up of crystal-induced AKI. Clue to diagnosis is the correct identification of crystals (either free or within casts) and of other possible markers of acute tubular damage (renal tubular epithelial cells and epithelial casts).

O10.10

Crystal nephropathy, nephrocalcinosis and human monogenic disease

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Nephrocalcinosis and other crystal-induced forms of nephritis are a potential mechanism behind irreversible and progressive renal damage leading to ESRD. They are associated with chronic interstitial nephritis, cellular infiltration, tubular atrophy and glomerular sclerosis. Many of these conditions have a mendelian inheritance. The most frequent and earliest clinical manifestation is stone formation; renal failure is also very frequent. However, renal failure is not invariably preceded by symptoms or episodes of stone formation in patients. Sometimes, these patients may have a silent progression to ESRD. In up to 60% of adults with primary hyperoxaluria type 1, the diagnosis is established when they already have chronic renal failure, and disappointingly this may occur in a transplanted kidney. In patients with acute or chronic renal failure due to intratubular crystal precipitation or crystalline interstitial nephropathy, the diagnosis of 2,8 dihydroxyadeninuria is unsuspected until renal biopsy is performed in the absence of any previous stone episodes. Unfortunately, in this condition too, the diagnosis could be done only when disease has recurred in a transplanted kidney.

O10.11
25 (OH)D-24-hydroxylase mutations, nephrocalcinosis and brushite stones

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Mutations of the CYP24A1 gene, encoding for the enzyme 25 (OH)D-24-hydroxylase, can cause hypercalcemia, hypercalciuria, nephrolithiasis and nephrocalcinosis in children and adults. Since the first report of ten pediatric patients presenting with idiopathic infantile hypercalcemia and biallelic mutations of the CYP24A1, an increasing number of both pediatric and adult cases have been reported. Most reports are consistent with an autosomal recessive inheritance, whereas others have suggested a dominant inheritance with incomplete penetrance. In fact, the presence of a single mutation has been linked to an intermediate clinical and biochemical phenotype between that of biallelic mutations and wild-type status; the specific mutation, together with environmental factors such as exposure to sun and vitamin D supplementation, might play a role in the development of the clinical phenotype. Some reports suggest that patients with mutations of the CYP24A1 might be more prone to forming brushite stones, so clinicians should be aware of such association.

Workshop 11: Flow Cytometry Advancements in Clinical Investigation

O11.01
Standardization and reproducibility in flow cytometry

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Flow cytometry enables sensitive detection of even very rare cell types and the reliability of cell identification and accuracy of enumeration depends on the specificity of the marker antibody used to stain the cells.

REAfinity™ Antibodies are a new generation of engineered flow antibodies for human, mouse, and rat antigens that can eliminate variation and ensure your data is reproducible from lot to lot, differently from traditional monoclonal hybridoma antibody production technology that is marked by inherent variability from one lot to the next.

The optimal clone for REAfinity antibodies production is established for each specificity by identifying the best antibodies in terms of antigen recognition and binding affinity. Then it is recombinantly engineered to introduce the human IgG1 Fc region and a specific mutation in the human IgG1 Fc region. Therefore, no FcR blocking step is required resulting in a significant savings on FcR blocking reagents.

O11.02
Immunoregulatory T circuits, not only CD4+ Treg lymphocytes

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Regulatory T cells (Treg) are specialized populations of T lymphocytes (Treg) that play a key role in maintaining tolerance and immune homeostasis. Regulatory T cells are deeply involved in numerous chronic disorders such as autoimmune diseases, chronic infections and cancer. In all these clinical settings alteration of frequency and /or function of Treg cells were observed and these abnormalities may contribute to disease progression and may impact on patient condition.

Since the discovery of the first Treg population as T CD4+ FoxP3+ CD25high+ CD127low, it is now evident that there is a system of Treg populations in the CD4+ subset, that can operate through numerous and diverse mechanisms in different microenvironments. Furthermore, It has been defined and demonstrated that regulatory / suppressor populations are included and generated from T CD8+ compartment, which are also involved in the maintenance of homeostasis and in many chronic inflammatory situations.

The multi-color flow cytometry thanks to the combination of different markers and use of appropriate strategies gating provides an opportunity to measure CD4+ and CD8+ Treg populations and to define the state of maturation and functional aspects.

Evidences provided by our lab have demonstrated the impact of both CD4+ and CD8+ Treg populations in many clinical settings, from autoimmunity, to tumor immune escape. During the report, it will be treated the biology of regulatory populations in maintaining homeostasis and their comprehensive analysis in diametrically opposed chronic inflammation situations, as autoimmune diseases and cancer.

O11.03
MAIT cell are activated by yeast in multiple sclerosis patients

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The composition of the intestinal microbiota plays a critical role in mammalian metabolism and, likely, in human health. With modern changes in diet, reduced exposure to environmental microorganisms and reduced incidence of infectious disease, our finely tuned immune systems which emerged both to tolerate more than a trillion commensal microorganisms and to respond rapidly and aggressively to invading pathogens appears to be out of sync with modern human lifestyles.

Very recent studies have suggested that the microbiota may have a role in immune-mediated CNS diseases such as multiple sclerosis (MS). Multiple sclerosis is an autoimmune disease that affects the CNS. We have previously shown that MS patients present a specific subset of CD8+ T cells expressing high levels of CD161, called mucosal-associated invariant T (MAIT) cells. These cells, mostly present in the gut, display a semi-invariant T cell receptor, Vα7·2 and recognize bacterial products. Their expansion in the peripheral blood of MS patients could be due to stimulation by gut flora strains with prominent pro-inflammatory inducing abilities. Thus, it is possible to hypothesize that in MS patients such an imbalance favours the generation and amplification of proinflammatory effector cells whose effects reach way beyond the intestinal mucosa. Our immunological in vitro studies consistently show a higher reactivity to yeast extracts in cells isolated form MS patients, also in the context of genetically identical monozygotic twins discordant for disease. Specifically, cells of the innate arm of the immune system (mDC and monocytes) produce higher amounts of proinflammatory cytokines in the MS twin compared to the healthy one. CD8+ MAIT cells are more activated and readily proliferate and produce proinflammatory cytokines and chemokines, particularly in response to yeast extracts obtained from MS patients. In addition, we show for the first time that MAIT cells respond to Saccharomyces Cerevisiae, which is more represented in the faeces obtained from MS patients.

O11.04
Malonyl-CoA/CPT1 axis and muscle pathophysiology

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Adult skeletal muscle plays crucial roles in physical activity, heat production and energy expenditure, and has a remarkably plasticity in response to environmental changes. Muscle plasticity is illustrated by endurance exercise training that induces myofiber type switching from fast/glycolytic to slow/oxidative and increases capillary, thus allowing higher oxygen capacity. Conversely, sedentary lifestyle, aging, obesity and type 2 diabetes (T2D) have been reported to be associated with decreased muscle oxidative capacity. Muscle performance relies partly on the capacity to match fatty acid (FA) and glucose oxidation to both the organism energy supply and demand. Mitochondrial FA ß-oxidation (mFAO) is usually the main muscle energy source during rest or endurance exercise. Moreover, reduced mFAO and ectopic lipid accumulation in the skeletal muscle have been proposed as a link to explain insulin resistance, a hallmark feature of obesity and T2D. mFAO is tightly controlled by carnitine palmitoyltransferase 1 (CPT1) that converts long-chain acyl-CoA to acylcarnitine, which then enters the mitochondria and undergoes ß-oxidation. Given its inhibition by malonyl-CoA, an intermediate of lipogenesis, CPT1 is considered the key regulatory step of mFAO. In contrast to the liver CPT1 isoform (CPT1A), the muscle isoform (CPT1B) is highly sensitive to malonyl-CoA inhibition, suggesting a tight control, whose physiological relevance remains poorly understood. During the past years, we addressed this question by expressing *in vitro* (muscular cells) and *in vivo* (*in vivo* electrotransfer of mouse tibialis anterior, muscle-specific transgenesis) a mutated constitutively active CPT1 that is active but insensitive to malonyl-CoA inhibition. The objective of this presentation is to describe the pathophysiological consequences of modulating the muscle malonyl-CoA/CPT1 partnership in relation to muscle mFAO capacity, palmitate-induced lipotoxicity, aging and obesity-induced insulin resistance.

O11.05
Searching for rare immune cells

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The study of rare cells can provide indeed valuable information on the status of the patient in several clinical settings. For example, using peripheral blood, it can be of interest to count the number of circulating tumour cells, tumour stem cells, endothelial cells and their precursors, hematopoietic progenitor cells and their subpopulations, antigen specific T-cells, invariant natural killer T cells and fetal cells in maternal circulation, among others. Not only rare cells can be useful to understand disease mechanisms, but also to find novel targets. Flow cytometry is clearly the best technique for this sort of quantitative and qualitative analysis, because of its capability to identify as many as 30 (if not more) parameters at the single cell level. The main issues of the pre-analytical phase, including amount of blood to use, the use of pre-enriched populations, the number of markers to use and of cells to acquire will be presented, along with the importance to exclude doublets and using a DUMP channel. Careful attention, optimal methodologies in all phases, including collection of biological samples, adequate controls and adequate use of software and hardware are crucial in this field, and several "next generation" instruments have been developed that allow a fast analysis with very high speed and sensitivity.

O11.06
28-color, 30 parameter flow cytometry to dissect the complex heterogeneity of tumor infiltrating T cells in human lung adenocarcinoma

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The heterogeneity of tumor-infiltrating lymphocytes and their relative pro- or anti-tumor potential can only be addressed by more powerful single cell approaches. By using antibodies conjugated to new synthetic dyes excited by 5 lasers mounted on the FACS Symphony A5 (BD Biosciences), we developed 28-color FACS to profile millions of single T cells from human lung adenocarcinomas. BUV, BV and BB dyes were brighter than many standard dyes regularly used in polychromatic FACS, thereby allowing extreme flexibility in panel development and better sensitivity in detecting dimly expressed proteins. Spreading Error (SE), resulting from errors in the measurement of fluorescence, but not spillover compensation was the main determinant of the success of 28-color panels: indeed, dye combinations with >400% compensation but limited SE could be easily included. We used computational barcoding coupled to single cell PCA and t-distributed stochastic neighbouring embedding (t-SNE) to reduce dimensionality and identify correlated or anti-correlated markers and thus putative functional subsets in the blood, the tumor and in the non-tumoral portion of the lung from the same patient ($n = 16$). We thus revealed that PD-1^{bright} exhausted CD8+, enriched at the tumor site, are mainly confined to the CD69+ tissue-resident memory compartment and are T-bet + HLA-DR+, while the same are nearly absent from the early-differentiated CCR7+ compartment. The latter, in turn, appeared to be recruited from the circulation. Pure naïve CD8+, defined by 5 markers, were virtually absent in tumors, hence suggesting that neoantigen priming might not occur in situ. Terminally-differentiated CD57+T-bet^{low}Eomes^{high} T cells did not include PD-1^{bright} cells, confirming that exhaustion and senescence are divergent states. High-content profiling is currently being performed in dozens of samples and integrated with clinical and metabolic parameters.

O11.07
Flow cytometry evaluation of extracellular vesicles in health and disease

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Flow Cytometry represents a reference technique for Extracellular Vesicles (EV) characterization and quantification.

Yet, due to their small size, pre analytical purification steps are required to enrich sample purity and facilitate EV identification on the basis of scatter parameters.

Up to date a strong debate is currently ongoing on how pre analytical manipulation may alter the outcome of final analysis,

and the need of protocols limiting manipulation steps is gaining importance over time.

In this study we present a patent-pending manipulation-free procedure to identify and enumerate MV from different origins (platelet, leukocyte or endothelium) in untouched peripheral blood by flow cytometry. This protocol takes advantage of different probes allowing the identification of sub-cellular compartment, and guaranteeing particle membrane integrity.

Characterized particles display membrane antigen patterns consistent with parental cells and respond to MV definition criteria in terms of size (0·1–1 μ)

Our preliminary data suggest that absence of manipulation in EV detection protocol strongly enhance sensitivity and reproducibility of the assay, thus offering researcher and clinicians a new valuable and easy to use tool to exploit the potential of EV as biomarkers in different pathological settings.

Workshop 12: Vaccination and Autoimmune Disease: From Theory to Practice

O12.01
Co-localization of hantaviral nucleocapsid and Rab proteins during co-expression in vitro

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Hantaviruses (*Bunyaviridae)* are classified as category A pathogens due to the high mortality rate and potential for major health impact. Hantaviruses infect endothelial cells and utilize cellular transporting system for intracellular trafficking and replication. Viral particles enter the cell via endocytosis and utilize the intracellular membrane trafficking system for uncoating, replication and assembly. However, little is known about the role of endosome in Hantavirus trafficking and replication.

Using Gateway cloning technology, lentiviral system expressing hantavirus nucleocapsid protein was generated and used to investigate the role of early and late endosomes in hantavirus trafficking. To study co-localization and role of endosomes in hantavirus transport, A549 cells were infected with recombinant LV-HTV-S, LV-SNV-S, LV-PHV-S and transfected with plasmids encoding various Rabs (Rab5, Rab7, Rab11).

Co-localization of Hantavirus N protein with early and late endosome associated proteins (Rab5 and Rab11) was analyzed using confocal microscopy. We found that N protein co-localized with Rab 5 and Rab 11 at the early stages of infection. Additionally, expression of dominant negative Rabs inhibited N protein expression without changes of its transcriptional level. These data indicate the importance of endosomes in trafficking and accumulation of hantavirus N protein in infected cell.

O12.02
Current recommendation to reduce the burden of bacterial and viral infections in patients affected by systemic lupus erythematosus

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Objective: Infections are among the most important causes of morbidity and mortality in Systemic Lupus Erythematosus (SLE) patients. In fact, these patients have an increased frequency of severe bacterial and viral infections possibly due to inherited genetic and immunologic defects and to immunosuppressive therapies. In addition, infectious agents can switch on .lupus disease expression and activity. In this narrative review, we aimed to summarize and report the preventive strategies currently recommended to reduce the burden of bacterial and viral infections in SLE.

Methods: A narrative review was conducted upon studies published in the international literature, recommendations and guidelines regarding the prevention of infections in SLE patients.

Results: Among the various strategies which can be applied to try and reduce the risk of infection in SLE patients, vaccination can be considered the most reliable option. Inactivated vaccines are safe to administer to SLE patients on immunosuppression and the induction of autoantibodies is usually of short term and of little clinical significance. Live vaccines are contraindicated in patients on immunosuppressive medication, although they may be considered in mildly immunosuppressed patients on a case-by-case basis. In order to obtain acceptable level of response and minimize adverse events, vaccines should be administered while disease activity is quiescent and, if possible, before starting immunosuppressive treatment, especially when high-dose corticosteroids or biologics are scheduled.

Conclusions: The current available evidences and recommendations suggest that most vaccines are effective and safe in SLE patients, although in certain cases immunogenicity may be sub-optimal and vaccination can trigger the induction of autoantibodies, even though for a short term. Although these issues are currently unresolved, the risk benefit balance is in favour for vaccination to reduce the risk of infection in SLE patients.

O12.03
Human Papillomavirus in patients with Systemic Lupus Erythematosus: a systematic review. Insights and implications for vaccination policies and cancer screening programs

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Objectives: Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder, whose etiopathogenesis is still poorly understood, being a complex, multi-factorial and multi-systemic disease. It imposes a considerable epidemiological, clinical and economic burden. A possible association between SLE and increased frequency of human papillomavirus (HPV) infection, squamous intraepithelial lesions, and cervical cancer – the tenth most frequent cancer and the third most common cancer worldwide in terms of mortality, after breast and colorectal cancer – as well as other HPV-related cancers is not well defined. The aim of this study is to comprehensively review and evaluate the extant scientific evidences on the relationship between SLE, HPV infection, pre-cancerous cervical lesions, cervical cancer, and other HPV-related cancers.

Methods: According to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines, a systematic review was conducted
Results: HPV infections are more prevalent in SLE patients when compared to the healthy counterpart, leading to a considerable number of cancers. Three studies have shown that HPV vaccines can be safely administered, and are capable to induce an effective immunogenic response in this group of patients.
Conclusions: Given the increased incidence of cervical abnormalities due to HPV in SLE patients, HPV vaccination should be encouraged, especially among those at increased risk for persistent infection and for cervical dysplasia. Moreover, further research in the field is warranted.

O12.04
Melatonin significantly reduces viral load by action on oxidative stress and autophagy, not by melatonin receptors

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Background: Herpes simplex virus type 1 (hsv-1) is a neurotropic virus that, during infection of humans, may cause many diseases include primary and recurrent epithelial lesions as well as disseminated disease and encephalitis. Like many other virus, HSV-1 increases oxidative stress into the host in order to facilitate the infection. Considering this fact, melatonin, as effective antioxidant, may reduce oxidative stress and enhances autophagy as protection against infection.
Materials and methods: HSV-1 infected and uninfected Vero cells were cultured in absense and presence of Mel 1 mM. Expression of genes encoding melatonin receptors [membranal (MT1) and nuclear (ROR) were tested by a nested retrotranscriptase (RT)-PCR. Viral load in supernatants were quantified by real time-PCR. Antioxidant enzymes: Superoxide dismutase (SOD) and catalase (CAT) activities were measured. Autophagy markers were studied by immunoblotting.
Results: ROR and MT1 ARNs were not detected in Vero cells, what implies that these cells do not express these membranal and nuclear melatonin receptors and, therefore, its effect has to be direct into the cell. These data can be related to antioxidant activities. SOD and CAT reduced their activities in infected primary cultures under melatonin treatment. Melatonin can emulate SOD enzyme, doing more efficient the balance between SOD and CAT and reducing oxidative stress. Also melatonin enhanced autophagy. Ratio LC3-II/LC3-I and p62 as aggregate marker were significantly increased under melatonin treatment. Both mechanisms hamper increase of viral load and Mel 1 mM was able to reduce viral load at 48 h of post-infection
Conclusion: This study suggests a possible therapeutic implication of melatonin in counteracting HSV-1 infection, not by melatonin receptors, but also autophagy and oxidative stress modifications.
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O12.05
Vaccines and autoimmunity: real association or false myth?

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Concerns about vaccine safety are one of the most important determinants of vaccine hesitancy. Alleged adverse events following immunisation represent a real obstacle to reaching high immunization coverage. Rumours about false correlation between vaccination and serious diseases or conditions have caused in the past large panicking reactions among the public and consequent dramatic drop in vaccine uptake. A recurring myth related to vaccination is that vaccines can trigger autoimmune reactions. Multiple sclerosis and rheumatoid arthritis have been allegedly related to anti-hepatitis B vaccine; more recently an entirely new syndrome (ASIA – Autoimmune syndrome induced by adjuvants) has been postulated by a small group of rheumatologists that suggest a link between some vaccines or adjuvants and a large series of autoimmune diseases or disorders. None of the hypothetic links listed above between autoimmune diseases and vaccines have been proven by either clinical or epidemiological studies. On the contrary, some influenza vaccines have been proven to be linked with an increased risk of some diseases having an important immune component like Guillain-Barre syndrome or, more recently, narcolepsy. Health care professionals should better know the real risk of abnormal immune reactions following vaccination in order to better communicate the risk and avoid false concerns among the public.

O12.06
Adjuvanted influenza vaccines

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Vaccination is universally considered as the most effective tool for the prevention of seasonal influenza, which represents a significant burden, both from the healthcare and the socio-economic viewpoint. Conventional non-adjuvanted vaccines have shown suboptimal immunogenicity in the elderly, in patients with serious chronic diseases or the immunocompromised and in young children. The protection offered by non-adjuvanted vaccines may be further reduced by periodic antigenic drifts.

Between the several strategies proposed to address the need for more immunogenic vaccines than the conventional ones, the most successful strategy is represented by the use of adjuvants. Several adjuvant substances have been tested in both preclinical and clinical trials. Among these, MF59 represents the most used molecule in seasonal influenza vaccines.

The MF59-adjuvanted seasonal vaccines have been shown to enhance immunogenicity and to confer cross-reactivity against heterologous influenza viral strains in the elderly, in adults with serious underlying medical conditions and in healthy infants and young children. Furthermore, its effectiveness has been demonstrated in older adults, reducing hospitalizations for pneumonia, cardiovascular and cerebrovascular diseases. The MF59-adjuvanted pandemic A/H1N1v vaccines demonstrated similar results in terms of immunogenicity.

In addition to MF59, other adjuvant substances, such as AS03 and AF03, have been tested in candidate pre-pandemic influenza vaccines. Pandemic vaccine against influenza A/H1N1v adjuvanted with AS03 demonstrated good immunogenicity and cross-reactivity against heterologous viral challenge.

All licensed adjuvanted influenza vaccines are safe, with an acceptable tolerability profile, thus representing a feasible option to optimize the control of seasonal and pandemic influenza.

O12.07
Vaccines and patients with autoimmune disease: the experience from the teaching hospital of Genoa

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University of Genoa, Italy

Infections are among the most important causes of morbidity and mortality in autoimmune diseases patients, particularly those with connective tissue disease as Systemic Scleroderma (SSc) and Systemic Lupus Erythematosus (SLE). In fact, these patients have an increased frequency of severe bacterial and viral infections possibly due to inherited genetic and immunologic defects and to immunosuppressive therapies. Among the various strategies which can be applied to try and reduce the risk of infection in these patients, vaccination can be considered the most reliable option.

At the IRCCS AOU San Martino – IST, the adult-acute care teaching hospital in the Liguria region, North-West Italy, a SLE Clinic and a Scleroderma Unit are active respectively since 2011 and 2014 for the care of patients. To these patients, vaccines recommended by international and national guidelines are routinely proposed.

In the period September 2016 – January 2017, 20 patients received different vaccines according to the above mentioned recommendations. Vaccines were administered at the vaccine outpatient clinic of the Hygiene Unit of the same hospital.

Twenty patients received the seasonal influenza vaccine. Six of them received also immunization against pneumococcal bacteria. We did not observe severe adverse events following immunization; among these, we reported local adverse events in 3 patients (15%) and only fever as systemic event in 5 (25%). Adverse events were mild and self-limited in few days. No flares of disease and no hospitalization for influenza were recorded.

Our experience confirms the efficacy and safety of the vaccination in patients with connective tissue disorders and highlights the recommendation of immunization for both viral and bacterial infections in order to reduce the risk of infective complications.

O12.08
Immunization of the immunocompromised patient

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University of Ferrara, Italy

In immunocompromised individuals the incidence and severity of vaccine-preventable diseases is particularly high; besides, these patients are at increased risk, as in constant contact with health care environments. Therefore, immunization of immunocompromised individuals is an important prevention tool. However, the safety, immunogenicity, efficacy/effectiveness data for vaccinations in these patients are still limited and vaccination coverage rates in immunocompromised subjects are generally low.

In addition, the types of immunodeficiencies are many and with a variable degree of immunological impairment, so it is difficult to have precise and always applicable guidelines.

In general, live vaccines are contraindicated in immunocompromised patients, and limited evidence suggests that inactivated vaccines have the same safety profile in both immunocompetent and immunocompromised patients.

In theory, therefore, inactivated vaccines can be administered, but the amount and duration of the immune response in immunocompromised patients can be reduced or absent.

Depending on the type of immunodeficiency, in fact, various mechanisms of the immune system can be compromised and consequently, according to the pathology, there will be a different response to different vaccines also.

Each immunocompromised patient must therefore be evaluated individually, carefully assessing the potential benefit related to the prevention of the disease, the safety, tolerability and efficacy/immunogenicity of immunization, and indications or contraindication to each vaccine.

In general, immunization of patients affected by primary or secondary immunodeficiency allows to improve the quality of life and prognosis, to decrease infectious complications and could be a lifesaving intervention.

Even if available data highlight the need for further studies on the level of effectiveness of different vaccines in relation to different immunodeficiencies, we absolutely need to increase awareness of the importance of vaccinations in immunocompromised patients.

Workshop 13: Gerontology

O13.01
Pathophysiology of delirium

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Delirium is a clinical condition characterized by abnormal arousal and disturbed attention, associated to altered cognition and behavioural disturbances. It is a multifactorial disease

that cross aging, mental and physical health.

This acute brain failure is a complex interplay between individual' vulnerability and predisposing and precipitating factors.

So far, the complete phenomenology of delirium is partially understood and several pathophysiologic pathways are considered to be causally involved in the genesis of delirium.

Therefore, the pathophysiological changes associated with delirium may add to the pre-existing cerebral disease, including dementia, and accelerate the progression rate of neurodegenerative processes.

In particular, brain neuroinflammation with blood-brain-barrier breakdown, with cytokine activation and microglial activation is considered a key relevant mediator of delirium. In the context of aging and neurodegeneration, adaptive changes to acute insults result in microglia priming coupled with dysfunction of brain to immune pathways. These changes are responsible for the sickness maladaptive behaviour syndrome, frequently associated to delirium clinical manifestations.

Moreover, cerebral hypo perfusion, oxidative stress, mitochondrial dysfunction and hypothalamic-pituitary adrenal axis hyper responsiveness may contribute to the onset and long-term sequelae associated to delirium.

In addition, the impaired balance of different neurotransmitters such as acetylcholine, dopamine, noradrenaline, glutamate and gamma-amino hydroxybutyric acid (GABA) cloud underlie different symptoms associated to delirium.

This presentation is aimed at providing an up to date on the pathophysiology of delirium with potential new fields of investigations and related clinical implications.

O13.02
Updates in delirium

S.E.J.A. De Rooij
Head University Medical Geriatric Medicine, University of Groningen

Thinking about the future and about how to improve the care for our vulnerable patients with delirium we need to answer a few questions first: should we elaborate on better strategies for treatment? Should we improve knowledge and skills or focus more on preventive strategies or predominantly on strategies to decrease the severity or shorten the duration of delirium episodes and on strategies to improve the outcome of delirious persons?

The last decade many articles have been written about prevention and both epidemiological studies as studies on biomarkers showed the adverse effects of delirium suggesting increased brain injury resulting in dementia and premature mortality. New future treatment should focus on all these themes: improve knowledge, skills, prevention, severity & duration and improve attention after delirium to ameliorate the worse outcomes of delirium.

O13.03
Dementia and delirium

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Delirium is a cause of altered level of consciousness and confusion, with old age being a major risk factor. This condition affects as many as 25–60% of hospitalized old age subjects. Individuals affected by dementia are highly susceptible to delirium and recent evidence show that the frequency of delirium in patients hospitalized with dementia was as high as 89%. Unfortunately, it can easily go unrecognized even by healthcare professionals because many symptoms are shared by both conditions. Most importantly the pathological processes involved in delirium may cause neuronal damage which in turn triggers or accelerates persistent cognitive changes. In addition, the overall prognosis of patients with dementia is worse if they also experience an episode of delirium than if they do not. Thus, the identification of the presence of the neurobehavioral abnormality and the differentiation of delirium from dementia or some other neuropsychiatric condition are necessary for a quick therapeutic intervention.

O13.04
Clinical presentations of delirium

G. Bellelli
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Delirium is a clinical problem in routine practice, replaced by a myriad of labels (e.g., acute organic brain syndrome, acute confusional state, toxic encephalopathy and intensive care psychosis). These synonyms have contributed to increase potential misunderstandings and low quality of care in this field. Now, the fifth version of the DSM criteria (DSM-5) supports the use of "delirium" as the umbrella term subsuming these multiple synonyms. Key diagnostic features of delirium include an acute onset and fluctuating course of symptoms, inattention, impaired consciousness, and disturbance of cognition. Occasionally, disturbance in sleep–wake cycle, perceptual disturbances (hallucinations or illusions), delusions, psychomotor disturbance (hypoactivity or hyperactivity), inappropriate behavior, and emotional lability are present. Delirium may present in hyperactive, hypoactive, or mixed forms. Although these two forms are distinctive clinically, patients can wax and wane between them during the course of a day or the course of the disorder (mixed type). The mainly hypoactive form is more common in elderly patients with a worse prognosis.

Delirium is essentially a clinical diagnosis. Recognition of delirium necessitates brief cognitive screening, clinical observation and accurate informant's interview. As a result, delirium may result undetected in up to 80% of cases. There are more than twenty-four diagnostic tools for delirium, each of them

with pro and cons. The 4 AT is a new tool, recently validated in the screening for delirium of older patients: it's brief (generally <2 min), no special training is required, it's simple to administer, and does not require physical responses, allows for assessment of 'untestable' patients. Using this tool in a point-prevalence study including 1867 older patients (aged 65 years or more) across 108 acute and 12 rehabilitation wards in Italian hospital, we found a prevalence of delirium of 22·9%, similar to previous studies. This instrument is reliable even with physicians untrained in the diagnosis of delirium.

Workshop 14: New Therapeutic Approaches to Epilepsy

O14.01
From Hippocrates to 'next-generation sequencing' era: the impact of genetics on
P. Striano
University of Genova, Italy

In last few years there has been rapid increase in the knowledge of epilepsy genetics. Nowadays, it is estimated that genetic epilepsies include over than 30% of all epilepsy syndromes.

Several genetic tests are now available for diagnostic purposes in clinical practice. In particular, next-generation sequencing has proven to be effective in revealing gene mutations causing epilepsies in up to a third of the patients. This has lead also to functional studies that have given insight into disease pathophysiology and consequently to the identification of potential therapeutic targets opening the way of precision medicine for epilepsy patients.

This talk is focused on the most recent advances in genetics of epilepsies. I will also overview the modern genomic technologies and illustrate the diagnostic pathways in patients with genetic epilepsies. Finally, the potential implications for a personalized treatment (precision medicine) are also discussed.

O14.02
Causes and consequences of gray matter heterotopia
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Malformations of Cortical Development are the most frequent causes of childhood epilepsies carrying a lifelong perspective of disability and reduced quality of life. Gray matter heterotopia corresponds to either macroscopic cortical malformations (e.g. subcortical band heterotopia) or more subtle malformations (e.g. periventricular heterotopia). Their precise incidence is not known but they have been diagnosed with increased frequency since the use of brain imaging techniques. In many cases, molecular genetics have led to an improved understanding of intracellular pathogenic signaling pathways. However, these important advances have so far failed to lead to better antiepileptic therapies. This is more likely due to our lack of understanding of the mechanisms that underlie hyperexcitability in these disorders. Recent and preliminary data suggest a common mechanism underlying hyperexcitability in models of neurodevelopmental disorders, namely a profound cortical excitation-inhibition imbalance.

In my presentation I will examine first the normal development of neocortex analyzing the different steps and mechanisms involved in the genesis of gray matter heterotopia. I will discuss then the mechanisms involved in the of epileptogenesis based on the analysis of an original rat model of double cortex associated with mutations in the doublecortin (DCX) gene with the neuropathological hallmark of aberrantly migrated neurons. I will scrutinize the neuronal basis of seizure generation in this model and set the conceptual basis for new experimental treatment strategies.

O14.03
Promoterapy: increasing endogenous gene promoter efficiency as a strategy to rescue intractable epilepsy
G. Lignani
UCL, United Kingdom

Epilepsy is one of the most important health burdens within the clinical neurosciences, and finding tools that open new mechanistic and therapeutic insights is a high priority. CRISPR is a powerful gene editing approach and it is now starting to be used to cure several pathologies. A variant of CRISPR, the CRISPR-On, allows to directly regulating the expression of endogenous genes by directly targeting their promoters (PromoTerapy), which allows expression of the full panoply of splice variants and untranslated regulatory sequences. In order to determine whether this strategy can be effective in non-genetic focal epilepsies, we applied CRISPR-On technology to increase KNCA1 (encoding for Kv1·1) expression in excitatory pyramidal neurons in a mouse model of focal epilepsy. The overexpression of Kv1·1 leads to a decreased neuronal excitability restoring physiological network activity. We have combined the functional analysis of neurons *in vitro* with the *in vivo* characterization of its translational potential through telemetry video-EEG. This approach is considered the proof of principle that PromoTerapy can be used to treat intractable focal epilepsies through the direct regulation of endogenous genes.

O14.04
Modeling of epileptogenesis in genetically altered mice and reprogrammed neurons
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Proline-rich transmembrane protein 2 (PRRT2) has been identified as the single causative gene for a group of paroxysmal syndromes of infancy, including benign familial infantile seizures, paroxysmal kinesigenic dyskinesia/choreoathetosis and migraine. A large number of PRRT2 nonsense, frameshift, and missense mutations have been associated with diseases with a variable phenotypic spectrum, ranging from mild forms that improve with age to severe phenotypes. PRRT2 mRNA is almost exclusively expressed in neurons in the cortex, hippocampus, basal ganglia, and cerebellum, which are all regions putatively involved in the pathogenesis of the PRRT2-linked diseases. To model the disease and dissect out the physiological role of

PRRT2 we studied the phenotype of PRRT2 knockout (KO) mice and established iPSC lines from fibroblasts of patients carrying the PRRT2 c.649dupC mutation that were differentiated into functional cortical neurons. Analysis of synaptic function in primary KO neurons showed a slowdown of the kinetics of exocytosis, weakened spontaneous and evoked synaptic transmission and increased facilitation at excitatory synapses. These effects were accompanied by an increased intrinsic excitability of excitatory neurons and by a heightened the spontaneous and evoked electrical activity in cortical networks. Interestingly, electrophysiological analysis of hiPSC-derived neurons from patients homozygous for PRRT2 mutation showed a high level of Na^+ currents associated with an increase of intrinsic neuronal excitability that was rescued by reintroduction of PRRT2 gene. The data obtained with the mouse and human model of PRRT2 KO indicate the existence of network instability as the possible basis of the paroxysmal phenotypes associated with PRRT2 mutations. IPSC

O14.05
The universe of epileptic mToropathies

R. Guerrini
University of Florence, Italy

Several syndromes have been associated with megalencephaly and include neuromatosis type 1 due to NF1 microdeletions that also involve the RNF135 gene and Sotos syndrome (with NSD1 mutations), Weaver syndrome (with EZH2 mutations), in addition to Bannayan-Riley-Ruvalcaba syndrome, Cowden syndrome, and severe megalencephaly with autism (all 3 with PTEN mutations). Megalencephaly with polymicrogyria occurs in megalencephaly-capillary malformation syndrome (with mutations of PIK3CA) and megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome (with mutations of PIK3R2 or AKT3). Hemimegalencephaly most often occurs without syndromic features, and has recently been associated with mosaic mutations of PIK3CA, AKT3, and MTOR. Hemimegalencephaly has also been associated with the linear nevus sebaceous syndrome and, rarely, with CLOVES syndrome (congenital lipomatous overgrowth with vascular, epidermal, and skeletal anomalies), tuberous sclerosis, hemihypertrophy, and hypomelanosis of Ito. The highly focal and variable nature of FCD type 2b, and the pathological resemblance to tubers in tuberous sclerosis, led to the hypothesis that somatic mosaic mutations of genes that encode proteins in the PI3K-AKT-mTOR pathway, which includes the tuberous sclerosis associated genes TSC1 and TSC2, were implicated in FCD. This hypothesis has been in part confirmed by studies documenting pathogenic germline and mosaic mutations in the mTOR gene or in other genes belonging to the PI3K-AKT-mTOR pathway in the dysplasic tissue of FCD type 2a and 2b. In addition, somatic duplications in the 1q chromosomal region encompassing the AKT3 gene have been associated with megalencephaly, hemimegalencephaly, and FCD type 1b. When performing genetic testing for disorders possibly caused by mosaic mutations, the screening of multiple tissues is advisable. Indeed, although testing DNA extracted from blood is the gold standard for identifying de novo constitutive mutations, the analysis of different tissues may help in identifying mutations that are only present in a subset of somatic cells

Workshop 15: Cancer Clinics

O15.01

Clinical practice ultradeep massively parallel sequencing for predictive genes in metastatic colorectal cancer sheds light on clinicopathological associations of the RAS/RAF mutational module and reveals high frequency of concomitant RAS/RAF mutations

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Background: Over the last few years, ultradeep massively parallel sequencing (uMPS) has become reliable and cost-effective, and its use in clinical practice is becoming a reality. A relevant role for uMPS is the prediction of response to anti-EGFR agents in metastatic colorectal cancer (mCRC), where multiple exons from KRAS, NRAS, and BRAF must be sequenced simultaneously.
Aim: We optimized a 14-amplicon uMPS panel to assess, in a monocentric, prospective, consecutive cohort of patients affected by mCRC the presence and clinicopathological associations of mutations in the KRAS, NRAS, BRAF, and PIK3CA genes from formalin-fixed, paraffin-embedded specimens collected for diagnostic and research purposes at the time of diagnosis in our referral basin.
Results: Over two years, we analyzed 219 mCRC specimens with the aforementioned method. We observed an unexpected, statistically significant association of RAS mutations with gender, young age and tumor site (right colon). We further demonstrated, by transversal validation using digital polymerase chain reaction (dPCR), that concomitant mutations in the KRAS/BRAF/NRAS genes, are not infrequent in mCRC, and that on the other hand, as anticipated by large, whole-genome studies, RAS and PIK3CA tend to be concurrently mutated. Finally, we corroborated associations such as the higher prevalence of BRAF mutations in right mCRC tumors, in concomitance with microsatellite instability.
Conclusions: To our knowledge, this is the first monocentric, consecutive, prospectively accrued clinical mCRC cancer cohort tested on a routine basis by uMPS for the RAS genes, BRAF, and PIK3CA. Our study has highlighted in clinical practice findings such as the concomitance of mutations in the RAS/RAF module, the co-occurrence of RAS and PIK3CA mutations, as well as possible associations of gender with specific mutations. These results need clinical follow-up in the context of treatment to assess their translational relevance.

O15.02

Abiraterone Acetate (AA) in pre- and post-docetaxel (DX) metastatic castration resistant prostate cancer (mCRPC): a monoinstitutional experience focused on cardiovascular (CV) events

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Background: AA is a therapeutic option for mCRPC patients (pts). Treatment is effective in pre e post DX setting and it is commonly well tolerated. However it requires the concurrent administration of corticosteroids and can induce relevant adverse events (AEs) including: fluid retention (FR), hypertension (HP), cardiac disorders (CD) and hypokalemia (HK).
Methods: We retrospectively analyzed mCRPC pts treated with AA and low-dose prednisone to evaluate the incidence of AEs as a function of baseline pts demography. The putative impact of AEs on clinical outcome (PFS and OS) was also investigated.
Results: 105 pts were analyzed (30 DX naïve). Demography: median age: 74; ECOG PS 1-2: 19%; Pain: 22%; median PSA: 37·8 ng/mL; Gleason S > 7: 52·4%; previous CD: 40%; baseline HP: 63%; median LVEF: 55%; BMI > 25: 47·6%. Median OS: 24·6 months and median PFS 14·9 months; pre or post DX: OS: 24·8 months and 19·9 months, PFS: 20·9 months and 13·8 months respectively. At multivariate analysis PSA (≥10 ng/mL) ($P = 0{\cdot}007$) Gleason S > 7 ($P = 0{\cdot}008$), PS 1-2 ($P = 0{\cdot}002$), prior hormone therapy (OT) duration <43·2 months ($P = 0{\cdot}01$) and BMI > 25 ($P = 0{\cdot}03$) predict for PFS (PCWG2) while pain ($P = 0{\cdot}01$) PS 1-2 ($P = 0{\cdot}004$) OT duration <43·2 months ($P = 0{\cdot}05$) and BMI ($P = 0{\cdot}04$) predict for OS. Incidence of CV AEs in the whole series: HP 17·2%; FR 4·8%; CD 8·6% and HK in 16·1%. Of tested variables, age (≥75) is the only one that predict incidence of CD ($P = 0{\cdot}001$) and FR ($P = 0{\cdot}03$). HK doesn't influence PFS and is associated with a longer OS ($P = 0{\cdot}01$). This association is confirmed also after multivariate analysis ($P = 0{\cdot}04$).
Conclusions: AA is confirmed to be effective and safe, though >75 pts requires rigorous CV monitoring, being at higher risk for CV AEs. Intriguingly, treatment related HK appear to be associated with a better OS.

O15.03

Immunophenotyping of Endometrial Carcinoma: beyond a dualistic characterization

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Endometrial cancer is traditionally divided in TypeI and TypeII, but some cases seem to escape from any rigid classification.

151 consecutive endometrial carcinomas surgically treated at the IRCCS San Martino-IST of Genoa in the period 2013-2016 were collected and immunohistochemical evaluation for estrogen receptor (ER), progesterone receptor (PR), Ki67, p53, Bcl-2, Cyclin D1, PTEN, E-cadherin, Beta-catenin and mismatch repair proteins (MLH1, PMS2, MSH2, MSH6) related to microsatellite instability (MI) was performed.

Patients were divided into Low Grade Endometrioid Carcinoma (LGEC $n = 109$; 72·19%), High Grade Endometrioid Carcinoma (HGEC $n = 11$; 7·28%) and Other High Grade Endometrial Carcinoma (OHEC; $n = 30$; 19·87%).

LGEC, compared to HGEC, showed significantly lower age, low Ki67 but higher PR and E-cadherin expression, less frequently advanced stage, infiltrative invasion, desmoplasia, tumor necrosis and infiltration of lymphatic vessels.

LGEC, compared to OHEC, showed lower age, low Ki67 and p53 expression, higher ER and PR expression and less frequently advanced stage, infiltrative pattern of invasion, desmoplasia, lymphatic infiltration, tumor necrosis and infiltration of lymphatic vessels.

HGEC, compared to OHEC, showed lower p53 and higher PR expression.

MI was observed in 31 cases: MLH1/PMS2- in 25 cases (16·52%), MSH2/MSH6- in 6 cases (3·97%). There were no significant differences between microsatellite stability, MLH1 /PMS2- and MSH2/MSH6- cases, except for lower age in MSH2/MSH6-, almost exclusively composed of LGEC ($n = 5$). MI was rare in OHEC ($n = 2$) and limited to mixed carcinomas.

The dichotomous classification seems effective for paradigmatic cases. HGEC showed more aggressive characteristics than LGEC but were distinct from OHEC for less evidence of adverse prognostic factors such as low PR expression and high expression of p53. The cases MSH2/MSH6- showed a younger age and greater propensity for well-differentiated endometrioid forms. MI seems to be uncommon in OHEC and limited to mixed carcinomas, indicating a possible deviation of the pathogenic pathway.

O15.04

The potential predictive role of peripheral circulating T lymphocyte sub-populations during treatment with nivolumab for advanced non-small cell lung cancer

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Background: In spite of the emerging role of immune checkpoint inhibitors in non-small cell lung cancer (NSCLC), consistent predictive factors are still lacking. Since these agents enhance anti-tumoral immune response, distinctive patterns among circulating immune cells might be associated with responsiveness. Our aim is to determine whether variations in immune sub-populations might predict response to nivolumab in NSCLC.

Methods: Patients receiving nivolumab for advanced NSCLC underwent blood sample collection before each administration for 4 consecutive cycles, followed by response assessment using the response evaluation criteria in solid tumors (RECIST) v. 1·1 and the immune-related response criteria (irRC). Peripheral blood mononuclear cells were analyzed for the frequency of the major adaptive cell subsets, including B cells, natural killer cells, and T-cells, which were further divided into sub-populations; additionally, the proportion of PD-1+ lymphocytes was assessed The relative frequencies and the ratios between the sub-populations at each sample collection were compared with radiological response.

Results: Fifty-four patients were considered eligible. Patients achieving partial response (PR) at the first RECIST assessment had a significant up-regulation of Tregs ($P = 0·021$), as well as a decreased CD8+/Treg ratio at baseline ($P = 0·033$) and at cycle 2 ($P = 0·029$). Conversely, patients experiencing progressive disease (PD) at the first RECIST assessment had a significantly up-regulated CD8+/Treg ratio at cycle 2 ($P = 0·029$). Patients experiencing PD at the first irRC had a higher proportion of PD-1+ T cells compared to the other patients ($P = 0·009$) at cycle 2. Finally, patients achieving PR at the first RECIST and irRC assessments had an increased proportion of exhausted T cells ($P = 0·012$ and $P = 0·06$, respectively).

Conclusion: The proportions of T cell sub-populations appear to be correlated with responsiveness to nivolumab. While the mechanisms at the basis of these findings have to be defined, further studies are highly advised.

O15.05

Retrospective assessment of cardiac troponin-I levels in patients receiving nivolumab for non-small cell lung cancer

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Background: Peculiar immune-related adverse events (irAEs) might onset during treatment with immune check-point inhibitors. While cardiac irAEs are seldom reported, animal data suggest that the myocardium might be sensitive to PD1/PD-L1 blockade. The aim of this study is to determine whether Cardiac Troponin-I (CTnI) might be used as a biomarker of cardiologic irAEs during treatment with nivolumab in advanced NSCLC.

Methods: Serum samples were collected from patients receiving nivolumab for advanced NSCLC at baseline and at each cycle up to 5 cycles, and then every 2 cycles. Cardiac Troponin-I was retrospectively quantified and defined as undetectable (<0·015 μg/l) or detectable (>0·015 μg/l); a value of 0·045 μg/l was considered significantly increased. Cardiologic anamnesis of the patients with detectable CTnI was collected from clinical documentation; additionally, patients alive at the time of the analysis underwent cardiologic evaluation.

Results: Fifty-nine patients were evaluable, and 26 out of 351 collected samples had detectable CTnI levels. Thirteen patients (22%) had detectable CTnI levels in at least one sample; among these, 6 (10%) patients had significant alterations in at least one sample, and in 3 cases (5%) this alteration was reported in multiple samples. No specific time-related pattern was identifiable for CTnI alterations. Five patients with detectable CTnI, including 2 with significant alterations (0·292 μg/l and 0·285 μg/l), had neither evident cardiovascular disease, nor cancer-related para-cardiac infiltration. Two patients had pericardial effusion, while two other had concurrent irAEs (hyperthyroidism and hepatitis).

Conclusion: Troponin-I was altered in a considerable number of patients receiving nivolumab, in some cases with no evident concurrent cardiovascular disease or manifest indirect noxae. Although a rationale for immunotherapy-related myocardial inflammation is acknowledged, further investigations on the cardiovascular effects of PD1/PDL1 inhibitors are required to draw meaningful conclusions, such as studies involving prospective cardiovascular assessments of patients receiving these agents.

Workshop 16: Diabetes with Italian Society of Diabetes

O16.01

Non-alcoholic fatty liver disease strongly predicts incident diabetes in patients with coronary artery disease

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Aim: Both coronary artery disease (CAD) and non-alcoholic fatty liver disease (NAFLD) are associated with type 2 diabetes. Whether NAFLD predicts future diabetes in CAD patients who do not have diabetes yet is unknown.

Methods: We therefore prospectively recorded diabetes incidence in a large cohort of 1018 consecutive non-diabetic patients with angiographically proven CAD; for the diagnosis of NAFLD we used the validated fatty liver index (FLI); diabetes was diagnosed according to ADA criteria.

Results: At baseline, 44·3% of our patients had impaired fasting glucose (IFG) and 55·2% had an HbA1c of 5·7–6·4% and thus were at risk of diabetes according to ADA categories. The prevalence of NAFLD was significantly higher in patients with IFG than in those with normal fasting glucose (46·8 vs. 34·0%; $P < 0·001$) but not between patients with an HbA1c of 5·7–6·4% and those with an HbA1c < 5·7% (40·8% vs. 38·5%; $P = 0·478$). Prospectively, 11·2% of our patients newly developed diabetes during a follow-up period of 6·3 ± 3·7 years; both IFG (OR 3·24 [2·03–3·32]; $P = 0·001$) and an Hba1c of 5·7–6·4% (OR 2·90 [1·50–5·61]; $P = 0·002$) significantly predicted incident diabetes. Importantly, diabetes incidence was significantly higher in patients with NAFLD than in those who did not have NAFLD (18·4 vs. 8·5%; $P < 0·001$), and NAFLD strongly predicted incident diabetes both univariately (OR 2·41 [1·56–3·73]; $P < 0·001$) and after multivariate adjustment including both baseline fasting glucose and HbA1c (OR 1·76 [1·11–2·79]; $P = 0·017$).

Conclusion: We conclude that NAFLD in patients with CAD strongly predicts incident diabetes independently from the baseline glycemic state.

O16.02

Pharmacological Sirt6 inhibition improves glucose tolerance in a type 2 diabetes mouse model

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Sirtuin 6 (Sirt6) is a sirtuin family member involved in a wide range of physiological and disease processes, including glucose homeostasis. Based on the roles played by Sirt6 in different organs, including its ability to repress the expression of glucose transporters and glycolytic enzymes, inhibiting Sirt6 was proposed as an approach for treating type 2 diabetes mellitus (T2DM). However, so far, the lack of small molecule Sirt6 inhibitors has hampered the conduct of in vivo studies to assess the viability of this strategy. Here we took advantage of a recently-identified Sirt6 inhibitor, compound 1, to study the effect of pharmacological Sirt6 inhibition in a mouse model of T2DM, i.e. in high fat diet (HFD)-fed animals. The administration of the Sirt6 inhibitor for ten days was well tolerated and improved oral glucose tolerance, it increased the expression of the glucose transporters GLUT1 and GLUT4 in the muscle and enhanced the activity of the glycolytic pathway. Sirt6 inhibition also resulted in reduced insulin, triglycerides and cholesterol levels. This study represents the first in vivo study of a Sirt6 inhibitor and provides the proof-of-concept that inhibiting Sirt6 may be a viable strategy to improve glycemic control in T2DM.

O16.03

Increased expression of toll like receptors 2 and 4 in normoglucose tolerant subjects WITH 1-hour post-load glucose ≥155

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Toll Like Receptors (TLRs) 2 and 4 are inflammatory mediators involved in the pathogenesis of type 2 diabetes (T2D) and cardiovascular diseases. Several evidences show that normoglucose tolerant subjects with 1-hour post load glucose (1hPLPG) ≥ 155 mg/dl (NGT ≥ 155) during an oral glucose

tolerance test (OGTT) have a high risk to develop T2DM and subclinical organ damage. The aim of the study was to evaluate the expression of TLR2 and TLR4 in monocytes of subjects at risk for T2DM, stratified by (1hPLPG). Patients have been selected from the Catanzaro Metabolic Risk Factor Study (CATAMERIS). All patients underwent medical examination and OGTT. Selected patients have been divided into six groups: normotensive subjects with 1hPLPG < 155 mg/dl (NT < 155, group 1) and ≥155 mg/dl (group 2); hypertensive subjects with 1hPLPG < 155 mg/dl (group 3) and ≥155 mg/dl (group 4); obese normotensive subjects with 1hPLPG < 155 mg/dl (group 5) and ≥155 mg/dl (group 6). Monocytes were obtained from peripheral blood, isolated by Ficoll and stained anti-CD14 antibody conjugated to PE, anti-TLR2 FITC, anti-TLR4 APC. Fluorescence was detected by flow cytometer. Expression of TLRs was represented as ratio of mean fluorescence intensity (MFI) of sample versus the MFI of isotype control. The preliminary data show a 1·1, 2·4, 2·7, 3·3, 5·5 ($P < 0·0002$) fold increase of TLR2 levels from the second to the last group respectively, compared with healthy subjects (NT < 155); and a 2·7 ($P < 0·0028$), 2·1, 2·9, 4·2 ($P < 0·0002$), 5·2 ($P < 0·0038$) fold increase of TLR 4 levels from the second to the last group respectively, compared with controls. In conclusion, non-diabetic subjects with 1hPLPG ≥ 155 mg/dl show an upregulation of TLRs 2 and 4. Through the development and exacerbation of inflammation, increased TLRs activity may be associated with a higher risk of cardiometabolic diseases.

O16.04
Practical implication of glucose continuous monitoring (GCM) in patients with diabetes mellitus insulin-dependent

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Introduction: Glycate hemoglobin (HbA1c) is a gold standard surrogate of glucose control in patients with diabetes. CGM measurement up to 288 glucose values/24 h allows a more detailed analysis of glucose control. HbA1c and GCM are complementary.
Aim: We evaluated the usefulness of CGM associated HbA1c in establishing glycemic control of diabetes patients ($n = 108$) treated with insulin.
Patients: These patients have been divided into 3 different groups: HbA1c ≤7% (group 1), 7% ≤HbA1c ≤8% (group 2), HbA1c ≥ 8% (group 3). These group were divided into subgroups according to the percentage of time spent in hypoglycemia (<70 mg/dl), euglycemia (70–180 mg/dl) and hyperglycemia (>180 mg/dl). The three group of patients were also divided on the basis of percentage of time spent in euglycemia (≥75%; 50–75%; <50%).
Results: Mean age of patients was 53·5. Mean of HbA1c was 7·9 ± 1·4%. Mean of percentage of time into euglycemia was 61·8 ± 21·1 for group 1, 53·8 ± 20·4 for group 2, 58·9 ± 19·8 for group 3 ($P = 0·077$; $P = 0·299$; $P = 0·267$). Mean of percentage of time into hypoglycemia was 11·3 ± 14·9 for group 1, 11·1 ± 17·7 for group 2, 4·1 ± 6·8 for group 3 ($P = 0·303$; $P = 0·008$; $P = 0·203$). Mean of percentage of time into hyperglycemia was 26·9 ± 24·7 for group 1, 35·2 ± 21·9 for group 2, 37·0 ± 20·3 for group 3 ($P = 0·071$; $P = 0·017$; $P = 0·725$). The time of euglycemia was <50% for the 30·6% of all patients.
Conclusions: The time of hypoglycemia correlated with HbA1c. The 50% of patients presented a percentage of time into euglycemia <50% and the patients with HbA1c ≥ 8% presented a percentage of time into euglycemia 50–75%. Glycated hemoglobin target to be achieved may not be 7% but between 7% and 8% because there is no significant difference between the 2 groups with regard to the average time spent in euglycemia. Integrating HbA1c with GCM can indicate blood glucose results that have a better risk / benefit ratio in insulin-treated patients.

O16.05
Type diabetes (T2DM) remission rate in overweigh and mildly obese patients at long term after biliopancreatic diversion (BPD)

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Background: In the severely obese patients with T2DM the metabolic benefits observed after BPD are largely due to mechanisms independent of weight loss. This prompted to investigate the anti-diabetic effect of the operation in T2DM patients with mild obesity or simply overweight.
Materials and methods: A cohort of 91 T2DM patients with BMI ranging from 25 to 35 kg/m^2 were submitted to standard type of BPD and evaluated one, three and five years after the operation (follow-up rate 100%, 100% and 83%, respectively).
Results: The T2DM remission (Hb1Ac 6·0% without anti-diabetic therapy) was observed in 52% of the cases at 1 years, 40% of the cases at three years and 25% of the cases at five years, and the long term positive metabolic outcome was predicted by the baseline BMI while it was independent of gender, age, T2DM duration, use or insulin therapy, and preoperative fasting blood glucose (FBG) or HbA1c values. Both before the operation and throughout all the follow-up period, the insulin resistance parameters (body weight and HOMA values) were similar in the metabolically successful and unsuccessful subjects, while only in the former C-Peptide values normalized for FBG values, as marker of insulin secretion, increased progressively from 1·06 ± 0·64 to 1·44 ± 1·08 mcg/l* mL/dL-1*100 ($p < 0·002$).
Discussion: In the T2DM patients with overweight or mild obesity the post- BPD metabolic remission rate was disappointing as it was markedly lower that that observed in their counterparts with severe obesity, and the number of T2DM remitter patients after the operation was progressively decreases throughout the follow up period. The increase of insulin secretion plays a key role in the T2DM remission after the operation.

O16.06
Myostatin not only a miokine: a new possible actor in diabetic nephropathy

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Background: Myostatin (MSTN) is a TGF-β superfamily member and is primarily expressed in skeletal muscle, although weak expression in other tissues such as Heart Muscle, Adipose, Kidney, and Fibroblasts has been observed. MSTN shares with TGF B1 similarities in structure, signaling pathway, and function. MSTN stimulates fibrosis and inflammation in skeletal muscle. MSTN may play a role in the development of diabetes: MSTN mRNA was elevated in skeletal muscle from type 2 diabetes

and from non-obese but hyperinsulinemic relatives of type 2 diabetics as well as in skeletal muscle and plasma from subjects with obesity and insulin resistance. To date, nobody has demonstrated that human kidney expresses MSTN and its receptor and that it could be a trigger of renal damage in diabetic nephropathy (DN).

Objective: The aim is to evaluate MSTN and its receptor in normal human kidney (NK) and in renal biopsies of diabetic nephropathy patients (DN).

Materials and Methods: MSTN and Activin Receptor 2B (AR) were evaluated by immunohistochemistry. mRNA MSTN was analyzed in microdissected tubuli and glomeruli in 10 NK and 15 DN biopsies.

Results: NK glomeruli, tubuli and interstitium expressed MSTN. In DN, tubular MSTN mRNA and protein were 5-6 folds increased ($P < 0{\cdot}05$–$0{\cdot}001$). In the glomeruli, MSTN mRNA and protein were highly upregulated (~40 folds, $P < 0{\cdot}05$). MSTN was localized in podocytes and mesangial cells. Interstitium and infiltrates were MSTN positive. AR protein expression was expressed in glomeruli, tubuli and interstitial cells both in healthy and DN. In DN AR was downregulated in all compartments and was highly expressed in infiltrating cells.

Conclusions: MSTN is not only a myokine, but it is also expressed in kidney. We found MSTN and its receptor in normal human kidney and their expression were significantly modified in DN. These data suggest a new role of Mstn in DN.

O16.07

MafA as a marker of β-cells of the islets of pancreas in rats

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As markers of progenitor β-cell are known transcription factors, one of them is MafA. It is assumed that it activates the expression of the insulin gene in progenitor β-cells, that is a marker of the PC. According to others, MafA is required only for the regulation of insulin secretion in mature β-cells, as a marker of differentiated β-cells.

The aim of the study was to investigate the expression features of insulin and MafA and the relationship between their expression patterns in experimental diabetes.

The study was made on 33 white mongrel male rats that were injected with alloxan. Paraffin sections were stained immunohistochemically with antibodies against MafA and insulin.

It is established that in normal pancreas is observed the maximum number of insulin+ and MafA+ cells in the pancreatic islets of rats. After alloxan injection the number of cells of both populations in the early stages (1- 3 days) of the experimental diabetes was decreased. In the later stages (14–28 days) the number of MafA+ cells continued to decrease, while the number of insulin+ cells began to increase, but did not reach the standards. This increase is attributed to the fact that after the 14-day experiment began the regeneration of insulin-producing cells from the PC population. The continued decrease of MafA+ cells with the increase of insulin+ cells casts doubt MafA participation in the activation of the expression of the insulin gene in PC - in this case, we would see the increase of MafA+ cells before starts the increase of insulin+ cells. Thus, the results of our study do not support the hypothesis that MafA can be the marker of progenitor islet cells. Our results testify in favor of the second assumption, which relates MafA to a marker of mature β-cells.

O16.08

Levels of miR-126 are regulated in an opposite manner by hyperglycemia in a co-culture system of endothelial and retinal pigment epithelial cells

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Vascular Endothelial Growth Factor-A (VEGF-A) has pathologic roles in microvascular diabetic complication, including Diabetic Retinopathy and Diabetic Macular Edema. MicroRNAs have been implicated in the epigenetic regulation of key pathways in many diseases including type 2 Diabetes. Levels of miR-126, which regulates expression of VEGF-A, have been found down-regulated in diabetes. The aim of this study is to investigate whether hyperglycemia affects expression of miR-126 in a co-culture system of endothelial (HECV) and retinal pigment epithelial cells (ARPE-19).

HECV cells were co-cultured for 24 hours with ARPE-19 cells in DMEM normal glucose (5·5 mmol/l; CTR) or DMEM high glucose (25 mmol/l; HG) supplemented with 10% FBS. Total RNA was extracted from cells and levels of miR-126 were detected using RT-qPCR. mRNA expression of the miR-126 targets VEGF-A and HIF-1α was evaluated by RT-qPCR. Another set of cells was lysed to evaluate HIF-1α protein expression and subcellular localization.

Levels of miR-126 were decreased in HECV cells cultured with HG compare to CTR. Lowered levels of miR-126 in HG condition are associated with increased mRNA expression of VEGF-A and HIF-1α. Protein levels and nuclear localization of HIF-1α were up-regulated in HECV cells cultured under hyperglycemic condition. On the contrary, in ARPE-19 cells cultured with HG levels of miR-126a was up-regulated; mRNA levels of VEGF-A were significantly down-regulated; HIF-1α protein was significantly increased.

Here we demonstrate that hyperglycemia affects levels of miR-126 in endothelial and RPE cells, in an opposite manner. Up-regulation of miR-126 in RPE cells may lead to decreased levels of VEGF-A, whereas down-regulation of miR-126 in endothelial cells may impair endothelial function. These findings suggest that hyperglycemia may contribute to microvascular complication of diabetes by impairing levels of miR-126.

O16.09

Abscisic acid enhances glucose disposal and induces brown fat activity in adipocytes in vitro and in vivo

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Abscisic acid (ABA) is a plant hormone also present in animals, where it is involved in the regulation of innate immune cell function and of glucose disposal, through its receptor LANCL2.

ABA stimulates glucose uptake by myocytes and pre-adipocytes in vitro and oral ABA improves glycemic control in rats and in healthy subjects. Here we investigated the role of the ABA/LANCL2 system in the regulation of glucose uptake and metabolism in adipocytes. Silencing of LANCL2 abrogated both the ABA- and insulin-induced increase of glucose transporter-4 expression and of glucose uptake in differentiated 3T3-L1 murine adipocytes; conversely, overexpression of LANCL2 enhanced basal, ABA- and insulin-stimulated glucose uptake. As compared with insulin, ABA treatment of adipocytes induced lower triglyceride accumulation, CO_2 production and glucose-derived fatty acid synthesis. ABA per se did not induce pre-adipocyte differentiation in vitro, but stimulated adipocyte remodeling in terminally differentiated cells, with a reduction in cell size, increased mitochondrial content, enhanced O_2 consumption, increased transcription of adiponectin and of brown adipose tissue (BAT) genes. A single dose of oral ABA (1 μg/Kg body weight) increased BAT glucose uptake 2-fold in treated rats compared with untreated controls. One-month-long ABA treatment at the same daily dose significantly upregulated expression of BAT markers in the WAT and in WAT-derived preadipocytes from treated mice compared with untreated controls. These results indicate a hitherto unknown role of LANCL2 in adipocyte sensitivity to insulin-stimulated glucose uptake and suggest a role for ABA in the induction and maintenance of BAT activity.

Workshop 17: Rheumatology

O17.01
Optimization of low-dose long-term glucocorticoid therapy should follow the circadian rhythms in chronic inflammatory diseases: the case of rheumatoid arthritis

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Chronic inflammation in patients with autoimmune diseases seems correlated with an altered function of the HPA axis. Administration of exogenous glucocorticoids (GCs) is today recommended at low doses in almost all the chronic inflammatory autoimmune diseases, since may act like a "replacement therapy" in presence of decreased endogenous cortisol availability. As matter of fact, reduction of mean initial low-dose from 10·3 to 3·6 mg/day on long-term GC therapy in RA, has been reported in one recent analysis during the period 1980-2004. Indeed, the more specific items of the European League Against Rheumatism recommendations for the management of RA, and more recently also the American College of Rheumatology, relate to starting disease-modifying anti-rheumatic drug (DMARD) therapy in early disease using a conventional DMARD strategy in combination with low doses GCs. There is evidence that GC therapy, especially long-term low-dose treatment, may slow radiographic progression by at least 50% when given to patients with early RA, in agreement with the conventional definition of DMARD. As mentioned, low-dose GCs exert important genomic effects on cellular immunity and given the existence of cellular circadian rhythms, the prevention of the night up-regulation of immune cell activity (and related flare of cytokine synthesis) with their exogenous administration between 6:00 and 8:00 seems not be optimal since administered too late. Since join pain, stiffness and functional disability show maximum level in the early morning hours, it is now clear that preventing the nocturnal rise of pro-inflammatory cytokines by GCs, is more effective than treating established symptoms in the morning. Large-scale trials documented that modified-release prednisone has greater efficacy for long-term low-dose GC treatment in RA patients, showing a significant reduction in morning joint stiffness/fatigue in addition to all known therapeutic effects with conventional prednisone and a similar safety profile but without additional suppression of the HPA axis.

O17.02
Glucocorticoids in RA: efficacy and safety in 2017

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Glucocorticoids (GC) were for the first time used in patients in 1948, notably in a rheumatoid arthritis (RA) patient, with an impressive effect, the bedridden patient started to walk again. This "miracle" led to awarding the Nobel prize for Medicine to Kendall, Reichstein and Hench in 1950. Evaluating the disease modifying effect of GC in patients with RA a Cochrane review was published in 2007. In all but one trials in RA patients in which GC were compared to placebo, GC significantly retarded progression of erosions, assessed after one as well as after two years duration of therapy. Remarkably, in different studies it was shown that the described retardation of erosion progression persisted, even years after stopping the GC. Therefore, it may be suggested that GC have a greater beneficial effect on joint structure than can be explained by their anti-inflammatory effects only. Adverse events of low dose glucocorticoids are very well known, but need to be evaluated in the context of the treated inflammatory condition. From a large observational cohort study in the UK it has become evident that low to medium dosages of GC are associated with an increase in heart failure, but not with myocardial infarction, stroke, transient ischemic attack or cardiovascular mortality. A recent EULAR task force defined the conditions where long-term glucocorticoid treatment in patients with RA has an acceptably low level of harm to facilitate implementation of existing recommendations and concluded that the risks of long-term glucocorticoid therapy are defined by both drug- (dose, duration) and patient-specific characteristics. In conclusion, GC have a clear disease-modifying anti-rheumatic drug effect in early RA; a starting dosage ranging from 7·5 to 10 mg prednisone equivalent seems best regarding efficacy as reported in literature and the balance between efficacy and safety.

O17.03
Epigenetic meets rheumatic musculoskeletal diseases including systemic sclerosis

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Epigenetic modifications such as DNA methylation, histone modifications and small and long non-coding RNA expression can stably alter gene expression and have been found to be altered in immune as well as in stromal cells from patients with rheumatic and musculoskeletal diseases (RMD). These changes in the epigenome patients with RMD influence key inflammatory and matrix-degrading pathways and are suspected to play a major role in the pathogenesis of RMD. However, up to date it is not clear whether epigenetic changes are a cause or a consequence of chronic inflammation. Nevertheless, therapeutic targeting of epigenetic mechanisms might be a successful approach in the treatment of RMD and there have been substantial efforts to develop drugs targeting epigenetic mechanisms. In the field or rheumatoid arthritis and systemic sclerosis early clinical data are available.

O17.04
From the Raynaud's phenomenon to the microvascular damage in systemic sclerosis: the role of nailfold capillaroscopy in the diagnosis and management

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Several connective tissue diseases, in particular systemic sclerosis (SSc), have Raynaud's phenomenon (RP) as their first clinical manifestation. Primary RP represents a benign condition often observed in otherwise healthy subjects, especially women: it is due to an exaggerated response to the physiological cold-induced vasospasm, whereas the secondary form of RP is typically associated with connective tissue diseases, especially SSc. Nailfold videocapillaroscopy (NVC), particularly after the recent technological advances, is a safe and reliable method to observe the microvascular structure and its early changes, especially during the transition from primary to secondary RP. In case of SSc, by considering validated patterns and scoring systems, NVC is the main tool that rheumatologists can rely on, besides the presence of specific auto-antibodies, to perform a very early diagnosis of the disease. This implies the possibility of early treatment of SSc, with an eye of predicting and preventing its major clinical complications. Next to being paramount for the "(very) early" diagnosis of SSc eyes are also geared toward capillaroscopy with the aim to be able to use it as a biomarker, especially in the prediction of future occurrence of digital ulcers (DU) in SSc. Progression to secondary RP is unlikely for subjects affected by RP when average capillary diameter is under 30 μm. Subsequently, the execution of the qualitative/quantitative integrated analysis should be part of the NVC follow-up of RP subjects. Microcirculatory imaging by NVC, along with the laser Doppler analysis, seems useful in the prediction of complications and prognosis) and in monitoring therapeutic trials. Several studies have shown that in SSc patients, the absolute nailfold capillary number/mm as analyzed by NVC during long-term therapy with the endothelin receptor antagonist bosentan (BOSE) and the synthetic analog of prostacyclin PGI2 iloprost (ILO) improved statistically after even from 1 up to 4 years of treatment.

O17.05
How to assess skin involvement in systemic sclerosis

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Systemic sclerosis (SSc) is a connective tissue disorder early characterized by microvascular damage with progressive fibrosis and skin impairment, the last being a marker for disease classification and activity. Skin involvement may be recognized and studied by the modified Rodnan skin score (mRSS), the validated method to evaluate the severity of skin thickening in SSc, and to distinguish between patients with either limited (lcSSc) or diffuse (dcSSc) cutaneous involvement [1]. As per definition, the affected skin is confined to the extremities (hands, forearms, feet, lower legs and face) in lcSSc, whilst it is also present on upper arms, chest, abdomen and thighs in dcSSc patients. Modified RSS, the classical method to evaluate skin involvement in SSc, has some drawbacks, as it is unable to identify slight alterations in skin thickness and has high intra- and inter-observer variability. Conversely, several studies reported the utility of high frequency ultrasound (US) for early identification of skin involvement in SSc patients. US may identify the different skin layers and offers a wide range of values for dermal thickness (DT) measure, compared with semi-quantitative mRSS scale composed by only 4 integer values. However, mRSS and US do not measure exactly the same properties of the skin. Modified RSS measures skin thickness, texture and fixation, while US measures accurately the DT, even if it is difficult to differentiate between oedema and fibrosis. Interestingly a very recent crucial study strongly suggests that a subclinical dermal involvement may be detectable by US even in skin areas showing a normal mRSS in patients classified as having lcSSc. This should be taken into account during SSc subset classification in clinical studies/trials.

O17.06
Systemic Sclerosis management in 2017

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Systemic sclerosis (SSc, scleroderma) is a connective tissue disease which affects skin, blood vessels, heart, lungs, kidneys, gastrointestinal tract and musculoskeletal system. Involvement of internal organs results in significant morbidity and mortality of SSc patients. Clinical heterogeneity of SSc makes treatment of this disease very challenging and until now, no disease-modifying drug that treats all aspects of SSc, specifically the underlying autoimmune and vascular fibrosis, could be identified and established for everyday clinical practice. However, more advance could be achieved for treatment of the individual organ manifestation and comorbidities, and the recent update of the previous recommendations for SSc management were performed according to the EULAR standard operating procedures. The updated recommendations address treatments of Raynaud's phenomenon (RP), digital ulcers (DUs), pulmonary arterial hypertension (PAH), skin and lung disease, scleroderma renal crisis, and gastrointestinal involvement. Compared with the 2009 recommendations, the 2015 recommendations include phosphodiesterase-5 (PDE5) inhibitors in the treatment of SSc-related RP and DUs, riociguat and new aspects for endothelin receptor antagonists, prostacyclin analogues and PDE5 inhibitors for SSc-related PAH. The new recommendations added also the use of fluoxetine for SSc-related RP and haematopoietic stem cell transplantation for selected patients with rapidly progressing SSc. Taken together These updated and improved, consensus-derived recommendations will help rheumatologists to manage treatment for patients with SSc in an evidence-based way, and will also facilitate the directions for future clinical research in SSc.

Workshop 18: Hypertension, Uric Acid and Cardiovascular Risk

O18.01
Effect of apple polyphenols on vascular oxidative stress and endothelium function: a translational study

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Apple polyphenols are mostly acknowledged for their hypoglycaemic properties, but represent in general an apple active fraction with many pharmacological functions. We aimed to examine their effect on uricemia and endothelial function in a sample of overweight subjects. This was a two-phase study. The in vitro experiment aimed to evaluate the apple polyphenols' ability to lower uric acid in comparison with well-known xanthine oxidase antagonist allopurinol. The in vivo study consisted in a randomized, double-blind, parallel placebo-controlled clinical trial involving 62 overweight volunteers with suboptimal values of fasting plasma glucose (100 mg/dL ≤FPG ≤125 mg/dl). The treatment period was 8 weeks long and consisted in an indistinguishable pill of placebo or an active product, containing 300 mg of apple polyphenols extract. Apple polyphenols extract inhibited xanthine oxidase activity, with an IC50 = 130 ± 30 ng/mL; accordingly, the extract was able to reduce uric acid production with an IC50 = 154 ± 28 ng/mL. During the trial, after the first 4 weeks of treatment, FPG decreased in the active treated group (−6·1%, $P < 0·05$), while no significant changes were observed regarding the other hematochemistry parameter, the hemodynamic variables and the anthropometric characteristics ($P > 0·05$ always). After 4 more weeks of treatment, the active-treated patients had an improvement in FPG compared to the baseline (−10·3%, $P < 0·001$) and the placebo group ($P < 0·001$). Uric acid (−14·0%, $P < 0·05$ vs baseline; $P < 0·05$ vs placebo) and endothelial reactivity (0·24 ± 0·09, $P = 0·009$ vs baseline; $P < 0·05$ vs placebo) significantly improved too. In conclusion, in vivo, apple polyphenols extract has a positive effect on the vascular oxidative stress and the endothelium function and reduce FPG and uric acid by the inhibition of the xanthine oxidase, as our in vitro experiment attests.