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CONFERENCE/MEETING ABSTRACT PROCEEDING

Selected Congress Abstracts: 6th Annual European Congress on Clinical & Translational Sciences (EUSTM-2019), 18–20 October 2019, Vienna, Austria

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Keywords: EUSTM-2019; Translational Sciences

4: How to Operate on Inoperable Patients: Minimally Invasive Direct Coronary Artery Bypass

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Introduction

Conditions such as liver failure and severe COPD are a considerable challenge for cardiac surgery. Hybrid surgery combining minimally invasive direct coronary artery bypass (MIDCAB) and percutaneous coronary intervention (PCI) is a less invasive technique than conventional coronary artery bypass grafting (CABG) for coronary revascularisation and could promote the safe inclusion of these patients.

Case description

The first patient was a 70-year old man who was referred for coronary revascularization as part of a pre-transplant workup. He had decompensated NASH cirrhosis with a Child Pugh of C9, MELD of 25 and a HCC of 1,8 cm in liver segment 2 detected on MRI by the time of admission. Preoperative coronarography revealed triple vessel disease and echocardiography suggested mild valvular disease. The second patient was a 82-year old man in whom coincidentally arrhythmia had been detected without complaints of palpitations or angor. Two months before admission he had been diagnosed with COPD GOLD II/D with fibrosis on CT after complaints of decreased exercise tolerance due to dyspnea NYHA class III. His FEV1 was 1.59l (64% predicted) and MFEF was 0.55l/s (31% predicted). ECG showed paroxysmal atrial fibrillation, flutter, non-sustained ventricular tachycardia with low voltage QRS, and a non-ST elevated septal and inferior infarct. Coronarography revealed triple vessel disease and a critical 80% type B1 stenosis at the AV branch of the right coronary artery (RCA).

Results and conclusion

Both patients were scheduled for MIDCAB of diagonal branch and left anterior descendens, and percutaneous coronary intervention (PCI) of the proximal circumflex or RCA, respectively, one month later. The first patient could be extubated after 12 hours and recovered easily in ICU despite minimal confusion, mild increase in bilirubin with icterus, deterioration of known renal insufficiency and oedema. After 4 days, the patient was stabilized and could be transferred from ICU to the Hepatology Unit where he stayed until his liver transplantation six and a half months later. Seven months after transplantation, the patient is doing relatively well with a well-functioning liver graft and stable chronic renal insufficiency. The second patient recovered without complications in ICU and was transferred to the Cardiac Surgery Unit after one day. Here, he developed hypotension and desaturation (82% O_2) which improved with oxygen and aerosol therapy. The patient was discharged on post-operative day 9 and sent to a nearby hospital for further revalidation. Here, limited wound dehiscention at the thoracotomy site was noticed, for which debridement and VAC therapy were initiated. One week later, this was changed to treatment with wicks as wound cultures had already returned to negative.

Take-home message

Although perioperative management remains challenging, hybrid surgery with MIDCAB and PCI allows for revascularization in patients with otherwise inoperable coronary artery disease due to comorbidities.

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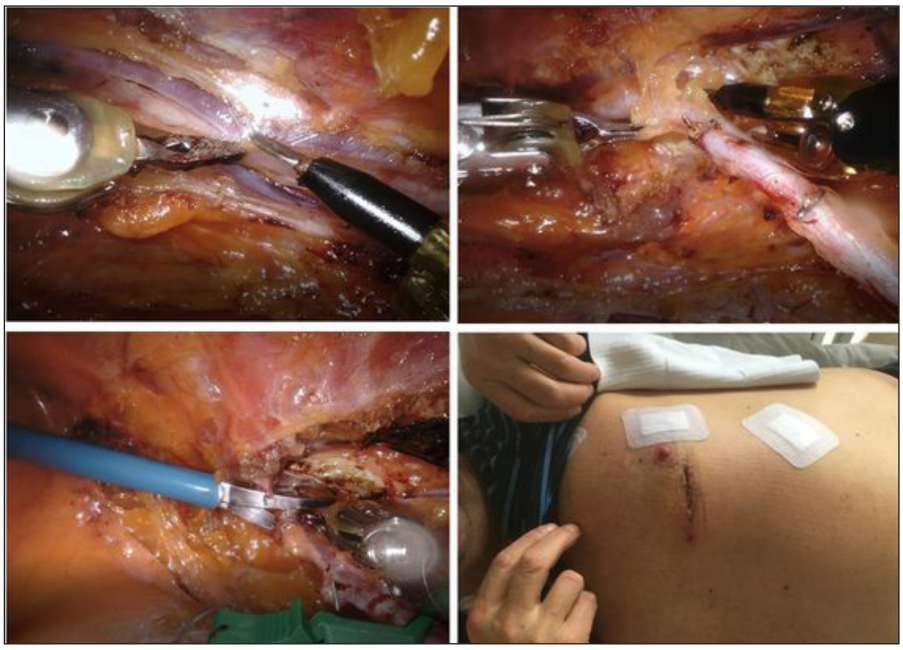

Figure 1.

5: Activation of b-catenin by CAR agonist is accompanied by the Akt pathway activation through FoxM1-Nedd4-mediated repression of PTEN

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The liver is the organ that can fully regenerate even after a serious injury. The ability of the remaining part of liver to bear the consequences of resection depends on its ability to decrease the hepatocyte death, to resist metabolic stress, and to increase its regenerative function. Currently, liver failure is the most severe complication in liver resection surgery. The small-for-size syndrome is the key risk factor for developing liver failure. In this regard, researchers are therefore keen to discover how to optimise the liver's unique ability to regenerate. The key to achieving this goal is an understanding of the process of hepatocyte proliferation, as well as the identification and functional characteristics of the genes involved in this process. It has long been known that constitutive androstane receptor (CAR) activation causes a strong proliferating effect in the liver, which suggests that CAR is a therapeutic target for partial resection of this organ. At the same time, the clinical use of CAR agonists is debated, since activation of CAR can be one of important trigger for the hepatocarcinogenesis formation. Therefore, studies describing the CAR-mediated proliferative pathways can help us to conclude about the possibility of the clinical use of CAR agonists during liver resection or transplantation.

Our results showed that liver hyperplasia resulting from the activation of CAR is mediated by a decrease in the level of PTEN protein and the subsequent activation of the Akt-signaling pathway in the mouse liver, which ultimately leads to an increase in b-catenin level and its nucleus translocation. Moreover, the decrease of PTEN level produced by CAR agonist was accompanied by a increase in the level of FoxM1, which was correlated with increased expression of FoxM1 target gene *Nedd4-1*, an E3 ligase responsible for the promotion of PTEN ubiquitination and degradation. Thus, these findings revealed an important function of a CAR-FoxM1-Nedd4-1-PTEN-Akt signalling pathway in hepatocyte proliferation regulation via, at least in part, b-catenin activation.

This study was supported by RSF 18-15-00021.

12: Physiological cardiorespiratory parameters in a model of anesthetized pigs with preserved spontaneous breathing: A prospective translational pilot trial

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Introduction

Cardiac resynchronization therapy (CRT) is a valuable treatment for patients with heart failure with reduced ejection fraction and intraventricular conduction disturbances resulting in wide QRS complexes. However, the rate of CRT-non-responders is high. Up to now, heart rate adaption as well as atrioventricular and interventricular conduction has a poor dynamic range. An early prognosis indicator of heart failure is loss of physiological modulation of heart rate by respiration known as respiratory sinus arrhythmia. Restoration of respiratory sinus arrhythmia with cardiac chamber synchrony could increase cardiac output (Nogaret A et al. 2013 PMID: 23026190; O'Callaghan EL et al. 2016 PMID: 26869940). The aim of

the present project was to evaluate the resting and exercise-induced respiratory and hemodynamic physiological variables under experimental conditions for promoting the development of new pacemaker sensors in a later phase of the European Union supported CResPace project (grant number 732170).

Methods

Physiological baseline data of 13 anesthetized, intubated and spontaneous breathing pigs (30–40 kg body weight) were collected. Anesthesia was induced according to the protocol approved by the local Ethical Committee. Single-lead ECG was measured in 11 pigs by BioAmp (ADInstruments, Oxford, UK). Invasive blood pressure was measured in 13 pigs by a sheath introduced in the femoral artery. Respiratory volume, respiratory rate, respirator gas flow and fraction of inspired oxygen (FiO_2) were measured in 13 pigs. Arterial blood gas analysis (pH, pCO_2, pO_2, lactate, HCO_3^-, base excess, arterial oxygen saturation) was obtained in 13 pigs. Descriptive statistics with means and standard deviations were calculated.

Results

Results of ECG recordings are displayed in **Table 1**. Respiratory rate and volume were 39 ± 9/min and 4.1 ± 1.0l/min, respectively. Invasive systolic and diastolic blood pressure were 130 ± 11 mmHg and 110 ± 10 mmHg, respectively. Arterial blood gas analysis was conducted with a mean FiO_2 of 30 ± 0% and a mean gas flow (respirator) of 2.2 ± 0.2l/min. According to these setting, pO_2 levels of 144.7 ± 11.7 mmHg and an arterial oxygen saturation of 100 ± 0% were achieved. Further results of the arterial blood gas analysis were: pH 7.39 ± 0.03; pCO_2 55.1 ± 4.5 mmHg; lactate 1.2 ± 0.6 mmol/l; HCO_3^- 31.0 ± 1.9 mmHg, base excess 7.7 ± 2.5 mmol/l.

Conclusion

Physiological parameters of the cardiorespiratory system stayed constant within the setting of anesthesia with preserved spontaneous breathing. As a next step, invasive sensors for an improved CRT will be tested and reported.

Table 1: Results of single-lead ECG recordings (n = 13) in anesthetized, intubated, spontaneous breathing land race pigs. (SD – standard deviation, ms – milliseconds, mV – millivolts).

Parameter	Mean (SD)
RR Interval (ms)	687 (81)
Heart Rate (bpm)	88 (10)
PR Interval (ms)	105 (10)
P Duration (ms)	48 (7)
QRS Interval (ms)	57 (13)
QTc (ms)	443 (26)
P Amplitude (mV)	0.17 (0.06)
R Amplitude (mV)	0.99 (0.38)
T Amplitude (mV)	0.42 (0.16)

Data of sensor testing will be presented at EUSTM-2019 in October 2019.

This work was financially supported by the European Union's Horizon 2020 Future Emerging Technologies Programme (CResPace, grant number 732170).

Competing Interests

The authors have no competing interests to declare.

13: Evaluating biomarkers for laboratory medicine; a practical toolbox

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The translation of promising biomarkers to clinical application is a critical opportunity for laboratory medicine; to provide information that enables clinicians to make better decisions about the care of their patients. The realisation of this goal is itself critically dependent on the appropriate evaluation of novel biomarkers for use in the clinical setting.

Inherent to this concept is consideration of the *unmet clinical need* that a laboratory test is aiming to address. However, as testing guides downstream clinical interventions to improve patient outcomes, the link between testing and outcomes is often indirect. As such, a full mapping of the clinical care pathway to define the purpose and role of the laboratory test and importantly, the clinical management decisions that the test will inform, thus enables the unmet clinical need to be addressed and furthermore complemented by the anticipated impact on patient outcomes.

The corollary from this approach is the early specification of analytical and clinical performance criteria to subsequently guide evaluation studies in a cyclical manner, keeping the clinical care pathway and outcomes as the key drivers in the process. In doing so, biomarker development can be aligned to address existing gaps in clinical care and mitigate research waste and inappropriate utilisation of laboratory tests where clinical benefit is uncertain or at worst potentially harmful.

There is a major opportunity here for laboratory professionals to play a key role in the development and implementation of clinical care pathways for new and existing laboratory tests. Stakeholder involvement; working together to overcome the conventional silos across disciplines is paramount to drive the adoption of innovative tests with robust implementation planning, so that test results are available and acted upon in an appropriate and timely manner, with a strong link to clinical intervention and outcomes. To realise the value of laboratory medicine in this context, the EFLM Working Group for Test Evaluation (WG-TE) has developed a 'practical toolbox' providing a Test Evaluation Framework and Interactive Unmet Clinical Needs Checklist (hosted on the EFLM eLearning platform) to assist laboratories and other key stakeholders in clinical translational research, to undertake clinical needs assessment and clinical care pathway development.

20: Strategies for cancer immunotherapy with genetically engineered T cells

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Conventional cancer therapies are limited by their toxicity and lack of specificity. Thus, immunotherapy of cancer with genetically engineered T cells has been developed in recent years. Chimeric Antigen Receptor (CAR) and T Cell Receptor (TCR) are the two major types of frequently used immune receptors to engineer T cells. CAR-T cell therapy has demonstrated promise in treating patients with several hematological malignancies, including acute B-cell lymphoblastic leukemia and B-cell lymphomas. However, safety is an important consideration in CAR-T cell therapy given the potential for serious adverse events, including the high risk of cytokine release syndrome (CRS) and patient death. TCR-T appeared not only to have less serious adverse events, but also effective in treating some solid tumors. Compared with CAR-T that can only recognize surface antigens, TCR-T can not only recognize surface antigens, but also intracellular antigens. Especially, recent studies of check point blockade therapies revealed that patients and tumor types with higher tumor mutation burden or higher frequency of tumor infiltrating lymphocytes (TILs) responded better to such therapies, suggesting that tumors harboring more mutations are more likely to generate tumor-specific neo-antigens (neoAgs), which would be recognized by neoAg-specific TILs. However, current vaccination studies show that it is much harder to obtain clinical improvement than to obtain an immune response. This suggests that vaccination strategy requires further improvements to elicit sufficiently strong immune responses. For example, too few CTLs may be induced, or the induced CTLs may be unable to infiltrate tumors, infiltrating CTLs may not become activated after encounter with tumor antigen *in vivo*, or may be suppressed within the tumor microenvironment. Thus, we propose a combination therapy strategy to overcome these challenges, and in particular an opportunity to use checkpoint inhibitors to activate neoAg-specific TILs, enhance co-stimulations, generate long-term memory T cells, and create an inflammatory environment at the tumor site to promote further CTL recruitment. Such strategies would convert 'cold' tumors lacking TILs into 'hot' tumors inside which the antitumor activity of these tumor-specific TILs could be released and even further enhanced by the checkpoint blockade therapies. An alternative approach that should also be tested in parallel is to isolate neoAg-specific CTLs from responding patients, especially the CTLs specific for the neoAgs harboring driver mutations, then expand them *in vitro* in large numbers, and adoptively transfer back into patients to generate a sufficiently large number of properly activated T cells. In a second step, we propose to isolate the TCRs from these neoAg-specific CTLs, and construct a TCR library that can be used to generate cell therapies for patients where vaccination is not effective. We believe this approach is easily scalable across different tumor types, and may provide a general strategy for the eradication of multiple cancers.

21: Hereditary Haemochromatosis: time for routine testing?

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This talk will review recent findings on the iron overload condition, hereditary haemochromatosis (HH) Type 1. HH is the most common genetic condition in western countries, with approximately 1 in 150 people of northern European ancestry being homozygote for the most severe genotype, HFE C282Y. The mutations also occur in southern European populations, with lower prevalence in other ancestry groups. Fortunately, HH is easy, safe and cheap to treat if found early, with regular blood donation being effective at maintaining low iron levels.

Family based studies suggested moderate penetrance, but a major US community based study in 2002 suggested that less than 1 percent of C282Y homozygotes actually developed clinical disease. As a result of this low penetrance and the high costs of testing at the time, routine testing or screening has not been recommended.

My group retested penetrance of the haemochromatosis mutations in the UK Biobank genetic study, including 2890 C282Y homozygotes aged 40 to 70 at baseline, followed-up for over 7 years. This provides data on nearly ten times more C282Y homozygotes than previous similar studies.

We found that associated clinical disease was far more common than previously reported. Arthritis, liver disease and diabetes were relatively common, and people with the mutations also experienced chronic pain and tiredness. At a mean age of 63 in an earlier follow-up, haemochromatosis was diagnosed in 21.7% of men and 9.8% of women with *HFE* C282Y mutations. More disease developed at older ages, and there was also a nominally significant increase in mortality in the *HFE* C282Y mutations group overall, including 14 deaths from liver cancer. This excess disease is not rare in clinical settings: in men, 1.6% of all the hip replacements and 5.8% of all liver cancers occurred in those with the C282Y homozygous genotype.

Many patients appeared to have been misdiagnosed and many were only diagnosed only after substantial delays, likely after irreparable disease had occurred.

Our results are consistent with an analysis showing that nearly 10% of male C282Y homozygotes eventually developed severe liver disease, and a US hospitals based study that reported that by the end of life, 50% of male and 25% of female C282Y homozygotes were diagnosed with haemochromatosis.

This talk will provide updated estimates of outcomes, with data on longer follow-up times. The data on the cost-effectiveness of screening will also be reviewed. Overall the evidence suggests that haemochromatosis fits criteria for routine testing or screening, and may be an excellent focus for expanding precision medicine.

22: Lymphocytes' CD-Profile, HLA-DR, CD25 and Perforin Expression in responders and non-responders during Standard Antiviral Hepatitis C Therapy

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Aim

To compare changes of peripheral blood lymphocytes profile in patients with chronic viral hepatitis C, activation markers HLA-DR и CD25 expression, perforin expression on CD8+, CD56+, CD16+ cells depending on the response to standard antiviral therapy using peg-interferon and ribavirin.

Methods

Phenotype and expression of molecules were studied using the flow cytometry method at treatment start, at 4th and 20th weeks of antiviral therapy. 38 patients with hepatitis C genotype 1b, viral rate 10^6 copies/ml, F0, IL28b CC/CT. The first group (A) was composed of 18 patients, who did not have virologist response at weeks 4 and 12 (based on which the treatment was stopped), 20 patients (B) with rapid virologist response, in which the treatment was terminated at week 48, and then the SVR noted.

Results

In the A group at week 4, decrease of B-lymphocytes ($P < 0.01$) and NK (CD56+CD16- was noted. In the B group – decrease of T-lymphocytes ($P < 0.001$), B-lymphocytes ($P < 0.01$) and T-helpers ($P < 0.001$) (diag.1, 2). No increase of killers (CD8+, CD56+, CD16+) in this group was noted neither at week 4 nor 20 (diag.3). But perforin expression increased significantly – 8 times on CD3+CD8+, 4 times on CD3-CD56+CD16- to week 20 of treatment. At the same time, in CD56-CD16+ cells no significant difference in perforin expression was noted in all treatment periods. A group before the treatment HLA-DR expression was higher than in the B group, but at week 4 the number of CD3+HLA-DR+ matched in both groups.

Conclusion

Decrease of main lymphocyte populations (T, B, T-helpers) when reaching a RVR reflects interferon therapy phenomena: cells re-distribution between peripheral blood and tissue compartment, activation apoptosis of lymphocytes, antiproliferative effect of interferon. An absence of such decrease in non-responders reflects the low level of interferon effect because of interferon receptors tolerance or blockade of the signal. In treatment response develop-

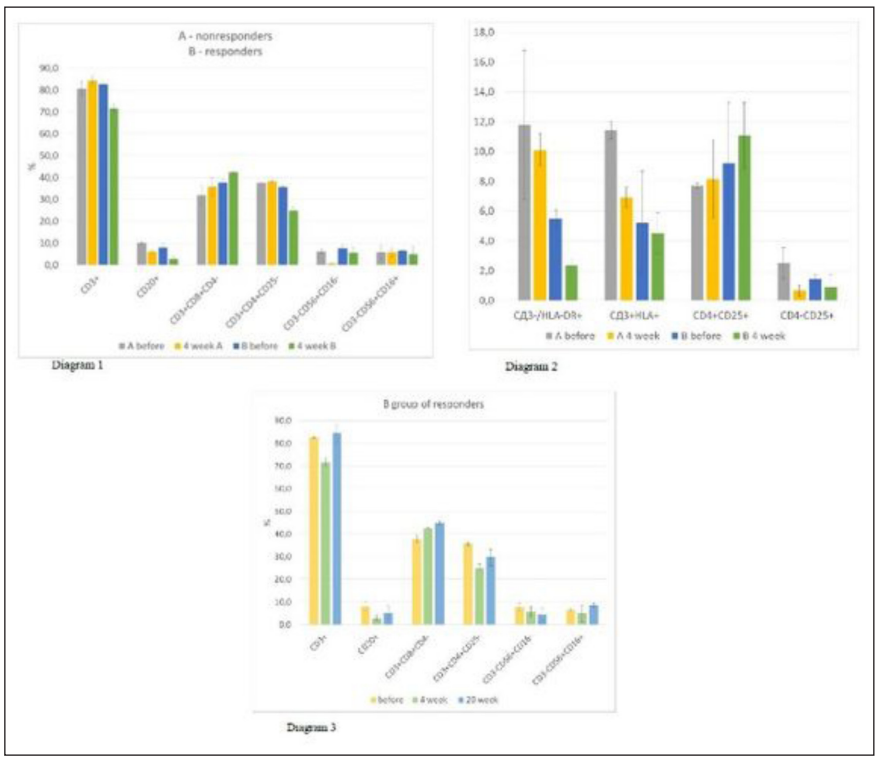

Figure 1.

ment, the most important has the functional activity of T-cytologic cells and CD56+CD16-natural killers, but not of CD56-CD16+ cells.

23: Type 2 Diabetes Mellitus in Patients with Chronic Viral Hepatitis C And B

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Background
The hypothesis that HCV can cause diabetes mellitus first made 25 years ago. Since then, several dozen research papers on studies of the relationship between HCV and type 2 diabetes have been published. Several studies conducted in various parts of the world found that between 13% and 33% of patients with chronic HCV-infection has diabetes, more often 2 types.

Although insulin resistance (IR) can develop independently of hepatitis C, a significant amount of clinical and experimental data suggests that HCV plays a role in its pathogenesis. This aspect is important because IR can not only accelerate the development of cirrhosis and HCC in the outcome of chronic viral hepatitis C, but it can also reduce the response to antiviral therapy.

Aim
To study the frequency of type 2 diabetes mellitus and the characteristics of its course in patients with chronic viral hepatitis C and B.

Methods and Materials
The data of 213 patients with chronic viral hepatitis B and C were analyzed. Among them, male patients were 50% (107), female – 50% (106). The average age of patients with hepatitis B was 35.9 ± 7.5 years, in patients with hepatitis C average age was 36.3 ± 8.9 years.

Among 213 examined, HCV was detected in 77% (164) of patients, HBV was detected in 33% (49) of patients, only 9% (19) were patients with co-infection: hepatitis B + C – 7% (15 patients), hepatitis B + D – 2% (4 patients). The 1ab genotype determined in 79 (48%) patients with HCV, 2a genotype in 23 (14%) patients, 3a genotype in 52 (32%) patients and 10 (6%) patients whose genotype is not typed.

Results
The study found that the occurrence frequency of type 2 diabetes mellitus (DM) differed in patients depending on the etiology of viral hepatitis: at HCV – 13.4% of patients had DM, and 6.1% of cases in patients with HBV. So, in the group of patients with hepatitis C type 2 diabetes mellitus occurred 2 times more often than in groups with hepatitis B with a certainty of $p < 0.01$. Among patients with hepatitis C type 2 diabetes is more common in individuals with the 3 genotypes. It is 2.5 times more often than in genotype 1 with a confidence level of $p < 0.001$. The correlation coefficient between the 3 genotype of HCV and the presence of type 2 diabetes was 0.77.

24: Sustained Virological Response in Patients with Chronic Viral Hepatitis C And Type 2 Diabetes Mellitus Currency

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Background
Some recent studies showed a significant decrease (normalization) in the level of glycated haemoglobin (HbA1c) in patients with HCV while the sustained virologic response to treatment had achieved.

Aim
To study the frequency of type 2 diabetes mellitus and the characteristics of its course in patients with chronic viral hepatitis C during antiviral therapy.

Methods and Materials
The 164 patients with chronic viral hepatitis C were enrolled in the study. Among them, male patients were 47%, female – 53%. The average age of patients was 36.3 ± 8.9 years. The 1ab genotype determined in 79 (48%) patients with HCV, 2a genotype in 23 (14%) patients, 3a genotype in 52 (32%) patients and 10 (6%) patients whose genotype is not typed.

Patients received standard antiviral therapy (AVT) (Peg-interferon + ribavirin). The fasting blood glucose levels checked out daily in the first 8–12 weeks of treatment. The level of glycemia was determined laboratory at least once every 2 weeks. Glycated haemoglobin was detected every 3 months during treatment and following 6 months after ending of AVT.

Results
During the treatment, it was revealed that when the early virologic response was reached, 17 of 22 (77.3%) patients with CVH C had a normal level of glycemia within 2–3 weeks already without using of hypoglycemic medicaments. Due to the achievement of complete glycemia control, the sugar-reducing preparations in patients were cancelled. Patients continued to follow the diet. The correlation coefficient between the achievement of the immediate virologic response and normalization of the glycemia level was 0.75.

When analyzing the long-term treatment outcomes it was observed that in all patients who had glycemic control at the early *virologic response* (EVR) within 6 months after the ending of treatment, the level of glycated haemoglobin remained below 7.0% – an average of 5.60 ± 0.552%. Thus, the control of glycemia levels persisted for 6 months after the ending of anti-viral therapy (AVT) on the background of achieving a sustained virologic response.

Conclusion

Achieving of glycemia level normalization while reducing and total cancelling of antidiabetic preparations in 77.3% of patients with SVR is a weighty argument that the hepatitis C virus, especially genotype 3, plays a significant role in the pathogenesis of type 2 diabetes. Successful eradication of the virus may lead to the reduction of insulin resistance. However, this effect was not obtained in all patients. Either the remaining 22.7% of diabetes has its own causes, that is independent upon the presence of HCV, or the eradication of the C virus has not eliminated all pathogenetic mechanisms in the development of diabetes.

25: Pre-clinical evaluation of EP4 receptor agonist for the treatment of chronic lymphocytic leukemia

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Prostaglandin EP4 receptor signalling was shown to prevent B cell receptor (BCR)-mediated proliferation, and represents a novel approach toward improving the therapy of B cell malignancies, known to be depend on BCR signals for survival. The aim of our preclinical study was to evaluate the EP4 receptor as a potential therapeutic target and EP4 receptor agonist PgE1-OH as a drug candidate for the treatment of chronic lymphocytic leukemia (CLL), the most common haematological malignancy diagnosed in adults.

Malignant B cells were isolated from whole blood obtained after informed consent from CLL patients. EP4 receptor agonist PgE1-OH induced apoptosis in all 151 CLL cells tested. The anti-cancer effects were mediated via EP4 receptor as evident from stronger cytotoxic effects of selective EP4 receptor agonist compared to endogenous ligand PgE2 and the fact that EP4 receptor antagonist prevented PgE1-OH induced apoptosis.

We evaluated the selectivity of PgE1-OH towards CLL cells using LCLs and peripheral blood mononuclear cells (PBMCs) obtained from healthy individuals. The average EC50 values for PgE1-OH after 24 h were 13.53 μM on CLL cells (N = 151), and 55.43 μM on LCLs (N = 24) and 46.36 μM on PBMC (N = 21), indicating that PgE1-OH was significantly more cytotoxic to malignant B cells compared to immune cells isolated from healthy individuals.

PgE1-OH exerted cytotoxic effects in all CLL cells with EC50 values ranging from 2 to 55 μM indicating inter-individual variability in response to PgE1-OH, which is in agreement with the fact that CLL is very heterogeneous disease. The analysis of the results revealed sex-dependent sensitivity of CLL cells to PgE1-OH, which was more cytotoxic to the cells of male compared to female donors. PgE1-OH was also more cytotoxic toward the cells of the carriers of the variant A allele of EP4 receptor expression-modulating polymorphism rs4495224. Moreover, male patients had higher expression levels of *Ptger4*, coding for EP4 receptor, compared to female patients as did the donors with the rs4495224 AA genotype compared to those with rs4495224 AC/CC genotype. A weak, but significant correlation between an increased expression of the *Ptger4* gene and lower EC50 values was also shown.

Furthermore, PgE1-OH was cytotoxic in CLL cells obtained from patients in all stages of disease and induced apoptosis in CLL cells isolated from patients with p53 deletion, known to be resistant to the standard cytotoxic therapy. Moreover, a very strong synergism was detected when cells were treated with PgE1-OH and idelalisib, well as in combination with ibrutinib and fludarabin, which might suggest novel therapeutic opportunities for the treatment of CLL. In conclusion, we identified EP4 receptor as promising therapeutic target and EP4 receptor agonist PgE1-OH as a promising drug candidate for the treatment of CLL.

28: Assessing The Impact of Switching to Tobacco Heating System (THS2.2) on Cardiovascular Events: Translating Basic Science into Clinical Benefit

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Background

Cigarette smoke (CS) is causally linked to the development of cardiovascular disease (CVD) through different pathophysiologic pathways, which include endothelial injury and dysfunction, oxidative stress, status, inflammation, and an abnormal lipid profile, all contributing to development of atherosclerosis. Tobacco harm reduction, by substituting cigarettes with less harmful products, is a complementary approach to current strategies for smokers who would otherwise continue to smoke. The Tobacco Heating System (THS) 2.2 is a novel tobacco product that heats tobacco instead of burning it, never allowing the temperature to exceed 350°C, thereby preventing the combustion process from taking place and producing substantially lower levels of toxicants compared with cigarette smoke.

Methods

Philip Morris International's (PMI) assessment program aims to demonstrate that switching to THS has the potential to reduce the risk of smoking-related diseases versus continued smoking. The program includes *in vitro*/*in vivo* toxicology testing methods that follow OECD guidelines and Good Laboratory Practice, a systems toxicology approach, and randomized, controlled clinical studies following the principles of Good Clinical Practice.

Results

The results of the THS translational assessment program demonstrated that cardiovascular toxicants are reduced by >92% in THS aerosol versus CS and that THS aerosol contains no solid carbon-based nanoparticles. The effects of THS aerosol on the adhesion of monocytic cells to

human coronary endothelial cells *in vitro* are significantly reduced. Switching to THS halted the progression of CS-induced atherosclerotic changes in ApoE$^{-/-}$ mice *in vivo*.

Endpoints linked to the development of smoking-related disease were analyzed following a six-month randomized, controlled clinical study with THS, which demonstrated a consistent improvement of endpoints in different pathophysiologic pathways leading to atherosclerosis.

Conclusions

The evidence available to date indicates that switching to THS has the potential to reduce the risk of smoking related diseases, such as CVD.

29: Endometrial Perforin positive lymphocytes in Women Undergoing In-Vitro Fertilization-Embryo Transfer

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Background

Cytotoxic cells, like CD8+ and natural killers CD16+, CD56+, have been associated with pregnancy loss. It is necessary to investigate levels of expression of perforin receptors on cytotoxic lymphocytes. This fraction can be used as an indicator of following successful implantation and pregnancy maintenance.

Aim

To evaluate the predictive value of expression of perforin receptors by cytotoxic lymphocytes in Women with a history of recurrent failed implantation and undergoing In-Vitro Fertilization (IVF)–Embryo Transfer.

Material and methods of investigation

The study included 25 endometrial samples from 15 women with a history of recurrent failed implantation and undergoing IVF-embryo transfer, and from 10 women with normal menstrual function and without reproductive losses. Uterine endometrium cells CD8+, CD16+, CD56+ PE marked and Perforin+ FITC marked after permeabilization were evaluated by flow cytometry.

Results

In patients with miscarriage in history, the level of perforin negative CD8+Perf– endometrial lymphocytes was reduced ($P < 0.05$). The average content of endometrial perforin negative natural killers with the phenotype CD16+Perf– of the main group did not differ from those of the control group, while the level of perforin negative CD56+Perf– cells was suppressed almost 3 times ($P < 0.05$) compared to the control.

Expression of perforin receptors on cytotoxic endometrial lymphocytes CD8+Perf+ and CD56+Perf+ did not differ from the control. At the same time, the average expression on CD16+Perf+ lymphocytes was increased 2-fold but did not differ significantly due to the large scatter of the indices. It should be noted that in a third of patients with miscarriage in the history of the level of CD16+Perf+ was increased almost 6 times.

30: Comparing the degradation of a lean MgZnCa alloy in old, osteoporotic and juvenile, growing rats

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Introduction

The advancing prevalence of post-menopausal osteoporosis is associated with increasing age of the population. Osteoporosis is characterized by weak bone mass and density consecutively increasing the risk of fractures. In 2010, 3.5 million incident fragility fractures were recorded in the European Union, which also increases the economic burden associated with high healthcare costs. Permanent titanium (Ti) and stainless steel

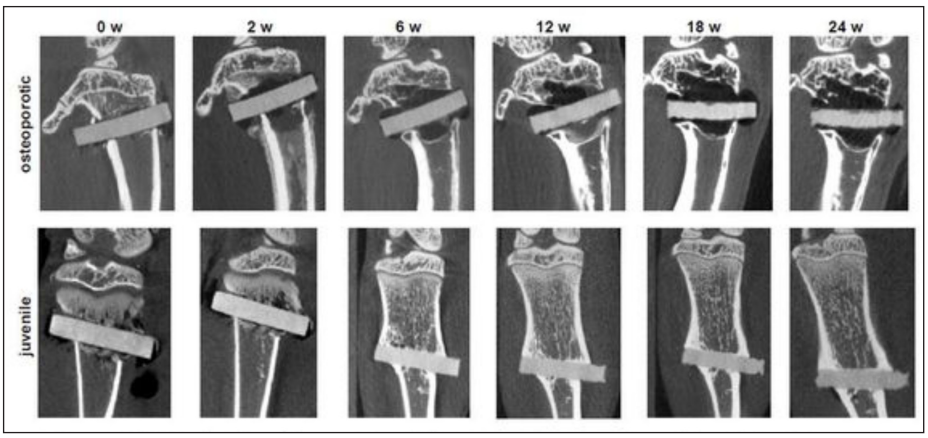

Figure 1: ZX00 implant degradation over 24 weeks in osteoporotic and juvenile rats observed via μCT scans.

implants are currently used to stabilize bone fractures. In elderly, permanent implants can induce stress-shielding leading to bone loss and increased risk of peri-implant fractures. Especially osteoporotic patients with weak trabecular and cortical bone have an increased risk for refractures due to the rigidity of permanent implants. Therefore, biodegradable magnesium (Mg) implants would constitute a promising alternative for elderly patients: on the one hand rendering a second removal surgery unnecessary, and on the other hand functionally supporting bone formation. At least in children the need for implant removal implies bioresorbable implants that are mandatory to improve treatment options. Applying a rare earth element-free alloy could overcome unknown long-term reactions assumed in recent studies. Recently we showed that the lean Mg-0.45% wt Zn-0.45% wt Ca (ZX00) implant material can be successfully implanted into femoral bone of juvenile rats and tibial bone of juvenile sheep, thereby supporting bone formation with adequate gas evolution.

Aim

In this study we compared the degradation and osseointegration of ZX00 after bicortical implantation into the metaphyseal tibia of old, osteoporotic and juvenile, growing rats. The primary outcomes measured included implant volume and surface as well as gas evolution over a time period of 24 weeks.

Methods

One-year old female Sprague Dawley® (SD) rats underwent ovariectomy to induce osteoporosis. After three months, osteoporotic and six-weeks old juvenile rats underwent bilateral, bicortical implantation of ZX00 pins into metaphyseal tibiae. *In vivo* micro-computed tomography (μCT) scans were performed 4, 8 and 12 weeks after ovariectomy and 2, 6, 12 and 24 weeks after surgical intervention at a resolution of 56 μm per voxel, respectively. After 24 weeks, all animals were euthanized and explanted bones were embedded in Technovit 9100 New and an *ex vivo* high-resolution μCT scan was performed at 18 μm per voxel.

Results

In vivo μCT imaging demonstrated osteoporosis progression over three months. Homogeneous ZX00 degradation was shown in juvenile rats over 24 weeks. Moreover, appropriate bone formation, osseointegration and bone in-growth were observed. However, in osteoporotic rats, ZX00 degradation was enhanced and induced more gas evolution when compared to juvenile rats.

Discussion

We compared ZX00 degradation in tibiae of osteoporotic and growing rats. ZX00 degradation markedly differed between both groups. Therefore, we suggest that differences in bone metabolism and changes in pH might influence the degradation behaviour, which needs further elucidation.

31: Echo Model Implementation Results on the Problem of HIV Infection in Kazakhstan

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Perspective view of WHO and UNAIDS at 2016–2030 yy is to achieve a zero level of new cases of HIV-infection, zero mortality associated with HIV, zero discrimination and, by 2030, put an end to the AIDS epidemic as a threat to public health. One of the key points is the provision of access to antiretroviral treatment for people from key vulnerable populations (KVP), in particular for injecting drugs consumers (IDC), who often have neurocognitive and mental disorders due to psychoactive substance use. ECHO® project (Extension for Community Healthcare Outcomes) is an innovative model of medical education and the improvement of the quality of medical services for patients through telementorism and a team approach to medical education and patient management. ECHO project promotes the provision of medical services to patients in the right amount, at the right time, in the right place. The project was developed by the University of New Mexico, USA in 2003 and with positive results is being implemented in more than 15 countries of the world. Aim: The aim of the study is to analyze the results of the ECHO model implementation in Kazakhstan on the problem of HIV infection in persons from KVP (IDC) on the "Clinical Case" component.

Materials

The clinical cases presented for analysis for the period 2016–2018 in the frame of the ECHO project. The theme of the training cycle "HIV infection", which includes 9 modules and 38 topics, each of which provides for the analysis of the clinical case in the form of Consilium of doctors of various specialties. The 142 clinical reviews were conducted in total. The documents developed for the presentation of clinical cases are the Patient Form and the Patient Recommendation Form. The main instrument of the project is a video session lasting 60–75 minutes (the duration can be determined according to need). For web conferencing/tele-sessions the video conference program ZOOM for 24 sites of the republic was used.

Results

During the sessions, in 46% of cases, the HIV infection stages were changed by HIV experts to a more advanced one, which corrected the diagnosis, in accordance with the HIV Classification and radically changed the tactics of patient management and treatment. The difficulties of practical doctors were due to the fact that 84% of patients had the manifestation of HIV, most patients had expressed and severe immunosuppression (88 %).

Conclusion

The "ECHO" project showed its advantage, not only in terms of the learning component but also provided real-time advice to patients (adults and adolescents) with complex and unclear and HIV-related diseases. It can be concluded that, with the use of low-cost technologies, in the form of video conferencing, the ECHO model contributes to the creation of professional networks of health professionals from various institutions and regions.

32: DKK1 – A novel biomarker for predicting risk of breast cancer metastasis to lung

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Rationale and hypothesis

Despite major progress in breast cancer treatment, metastatic breast cancer remains an incurable disease. Bones as well as lung are significant sites for breast cancer metastasis and the identification of biomarkers predicting risk of breast cancer spread would enable tailoring of treatment to those patients most likely to benefit, maximising therapeutic outcomes. To this end we have utilised immunocompromised mouse models of human breast cancer bone metastasis and proteomics methodologies to identify novel biomarkers predictive of breast cancer spread.

Objectives

Proteomic studies within our laboratory have identified the differential expression of the Wnt-family inhibitor Dickopf-1 (DKK1) within a lung metastatic variant of the MDA-MB-231 breast cancer cell line (LM) compared to the parental control cell-line (PCC) and bone homing variant (BM1). We tested this protein as a potential predictor for risk of metastasis to bone, lung and other sites in primary breast tumour samples from patients within the AZURE trial.

Methodology

Primary tumour cores from AZURE trial patients were stained with a DKK1 antibody. Cytoplasmic staining was assessed independently by two trained operators, blinded

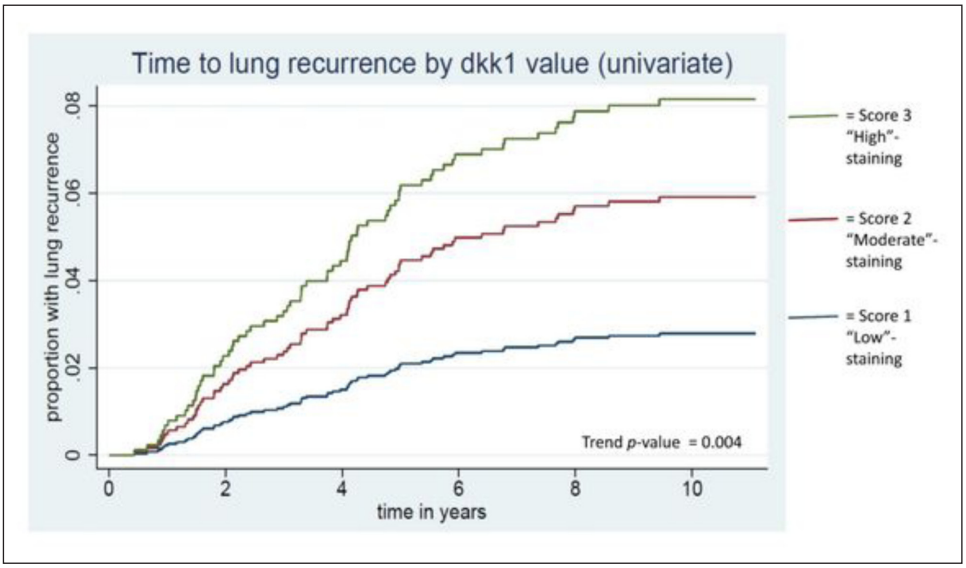

Figure 1.

Table 1.

DKK1 lung	UNIVARIATE COX MODEL					
	CONTROL (N = 698)		Zol (N = 713)		ALL (N = 1411)	
Endpoint	Chi-square	P-value	Chi-square	P-value	Chi-square	P-value
Lung recurrence only	4.3	.04	5.1	.02	8.2	.004
Any lung recurrence	3.2	.07	1.7	.19	3.6	.06
	MULTIVARIATE COX MODEL					
	CONTROL (N = 698)		Zol (N = 713)		ALL (N = 1411)	
Endpoint	Chi-square	P-value	Chi-square	P-value	Chi-square	P-value
Lung recurrence only	6.3	.012	4.1	.04	8.91	.003
Any lung recurrence	3.5	.06	1.3	.26	2.6	.11

to outcome data, under supervision of an experienced breast histopathologist. DKK1 levels were correlated with local and distant recurrences by multivariate analysis.

Results
Immunohistochemical analysis of primary tumour sections from 1773 patients from within the AZURE trial for DKK1 Levels revealed statistically significant association with first distant recurrence in lung. Patients with high DKK1 levels (TMA score = 3) had a higher cumulative incidence of first recurrence in lung compared to patients with low DKK1 levels (TMA score = 1), p-value = 0.004 (for combined Control + zoledronate arms). Correlation of DKK1 levels with potential confounding factors such as ER status, menopausal status, stage, grade and metastatic spread to lymph nodes did not reveal any obvious correlation (e.g. r = .06). DKK1 levels did not correlate with distant spread to bone (p = 0.88), with distant spread to liver (p = 0.38), overall survival (p = 0.27) or survival after recurrence (p = 0.055) – all p-values quoted are within the control + zoledronate arms. The predictive effect of DKK1 levels upon risk of lung metastatic spread was confirmed by gene expression analysis within publicly available databases. Biomarker validation of DKK1 is currently underway utilising patient serum samples from the AZURE trial as well as serum samples derived from the immunocompromised mouse model of breast cancer bone metastasis.

Conclusion
DKK1 is a potential biomarker predictive of risk of lung metastasis within early breast cancer patients.

36: Impact of metabolic factors on hepatic steatosis in patients with chronic hepatitis B

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Background
The prevalence of hepatic steatosis in patients with chronic hepatitis C and the mechanisms associated with fatty liver have been well-described. However, little is known about the coexistence of hepatic steatosis and chronic hepatitis B (CHB).

Objectives
The objectives of this study were to determine the prevalence of hepatic steatosis in patients with CHB; determine the factors related to the presence of fatty liver; and investigate whether interleukin 28B (IL28B) genetic polymorphism is associated with hepatic steatosis in CHB.

Methods
Patients with CHB who underwent a liver biopsy were enrolled in this study. Liver biopsies were assessed using the Metavir and Knodell Histology Activity Index (HAI). Peripheral blood samples of CHB patients with or without steatosis and normal controls were genotyped for IL28B using the 5'nuclease assay.

Results
Fasting blood glucose of CHB patients with liver steatosis was found to be significantly higher than patients without steatosis (P = 0.017). High density lipoprotein cholesterol of patients without steatosis was observed to be higher than patients with steatosis (P = 0.008). Although not statistically significant, body mass index, triglyceride levels, low density lipoprotein cholesterol and HAI in CHB patients with steatosis were found to be higher than those without steatosis. There was no significant association between the stage of fibrosis and severity of steatosis. No significant differences in IL28B allele frequencies between CHB patients and controls were noted.

Conclusions
The prevalence of liver steatosis among patients with CHB is 41%. Fatty liver in CHB patients was associated with metabolic factors such as diabetes and dyslipidemia. IL28B polymorphism is not associated with hepatic steatosis.

Acknowledgements
This work was supported by a research grant (Project No. 12-002c) to the St. Luke's Liver Diseases Study Group through the Research and Biotechnology, St. Luke's Medical Center-Quezon City, The Philippines.

37: METTL21B, one of the non-histone lysine methyltransferase, regulates gastric cancer growth and survival ability through methylation of MKK7

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METTL21B is a non-histone protein lysine methyltransferase. The recently discovered that METTL21B has reported little of its function and methylated substrates. Based on the low survival rate of patients with gastric cancer at the time of overexpression of METTL21B, additional experiments were conducted to designate the new role of METTL21B in enhancing tumorigenesis. MKN1, which overexpressed METTL21B, was found to have increased cell proliferation and migration permeability, and increased mRNA of tumor-associated factors such as IL-6, IL-12 and TNF-α. Besides, the activity of MKK7, p38 related to the AP-1 signalling pathway was increased. In contrast, METTL21B$^{-/-}$ and shMETTL21B MKN1 cells inhibited cell growth and viability. This inhibition confirmed the cell cycle G2/M phase arrest through inhibition of Cyclin B in METTL21B$^{-/-}$ and shMETTL21B. In addition, the JNK signalling pathway leading to MKK7, JNK, and c-Jun was inhibited. Interestingly, MKK7 and METTL21B were co-expressed, the activity of AP-1 including MKK7 and JNK increased synergistically. Surprisingly, these synergistic effects were similar in Cyclin B mRNA expression in MKK7 and METTL21B transfected condition.

METTL21B and MKK7 domain mutants were identified and the binding ability of METTL21B and MKK7 was not changed when D, DVD, D and DVD were deleted. Five additional Kinase domain deleted mutants were constructed. As a result of confirming the difference of binding affinity using the prepared mutants, The ability of KD1 and KD4 to binding affinity with METTL21B was lowered. Consistent with this, expression of the AP-1 promoter luc gene was also decreased in the KD1 and KD4 regions. Finally, methylation of K152 and K312 residues of MKK7 was first identified through *in vitro* and *in vivo* methylation assays. Oncogenic genes such as MMP-2, IL-6, IL-1β and cyclin B were reduced in MKK7 K152A and MKK7 K312A, respectively, which were mutated at the methylation sites of MKK7 with alanine, compared with wild type MKK7.

Taken together, METTL21B-mediated MKK7 stimulates the AP-1 signalling pathway and increases Cyclin B expression leading to tumorigenesis. The presentation of this new mechanism will contribute to the study of various diseases in the future.

38: Immune complexome analysis to identify disease-associated immune complex antigens for biomarker discovery and treatment development

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Immune complexes (ICs) are formed upon noncovalent interaction between foreign antigens or autoantigens and antibody proteins. They are produced during an immune response and may reflect some aspects of an ongoing immune response. Antigens in ICs (IC-antigens) are target of immune system. An increase of some ICs leads to autoimmune diseases, or results from infections or cancers. Therefore, the identity of antigens incorporated into ICs provides insights into pathophysiology. Moreover, the identity provides the information that in the future may aid in the development of diagnosis and treatment strategies for these diseases, and this information might be more relevant than information on free antigens. However, such studies have been limited to date because tools for screening of ICs are lacking.

We have developed a method, designated "immune complexome analysis", to catalogue IC-antigens. In this method, ICs are isolated from biological fluids, such as serum and cerebrospinal fluid, by using Protein G- or Protein A-coated beads that bind the fragment of crystallization domain of antibodies. The ICs are then subjected to papain-digestion and elution. Papain selectively cleaves immunoglobulin (Ig) at the heavy chain hinge region into three fragments: one Fc and two identical Fab fragments, resulting in selective dissociation of the antigens from ICs without eluting the proteins bound non-specifically to the beads. The eluted IC-antigens are tryptically digested and the resulting peptide digests are analyzed using nano-liquid chromatography-tandem mass spectrometry (nano-LC-MS/MS). We have used this method to identify specific IC-antigen(s) in biological fluids, such as serum or cerebrospinal fluid, for autoimmune diseases, infectious disease and cancers and have found some these antigens valuable as a research target. For example, we found thrombospondin-1 or suprabasin to trigger disease-specific inflammation in rheumatoid arthritis or neuropsychiatric systemic lupus erythematosus, respectively. We are planning to use this method to understand other diseases, such as infertility, that are relevant to immunological disorder. In this paper, we will show the potential of immune complexome analysis in biomarker discovery as well as treatment development.

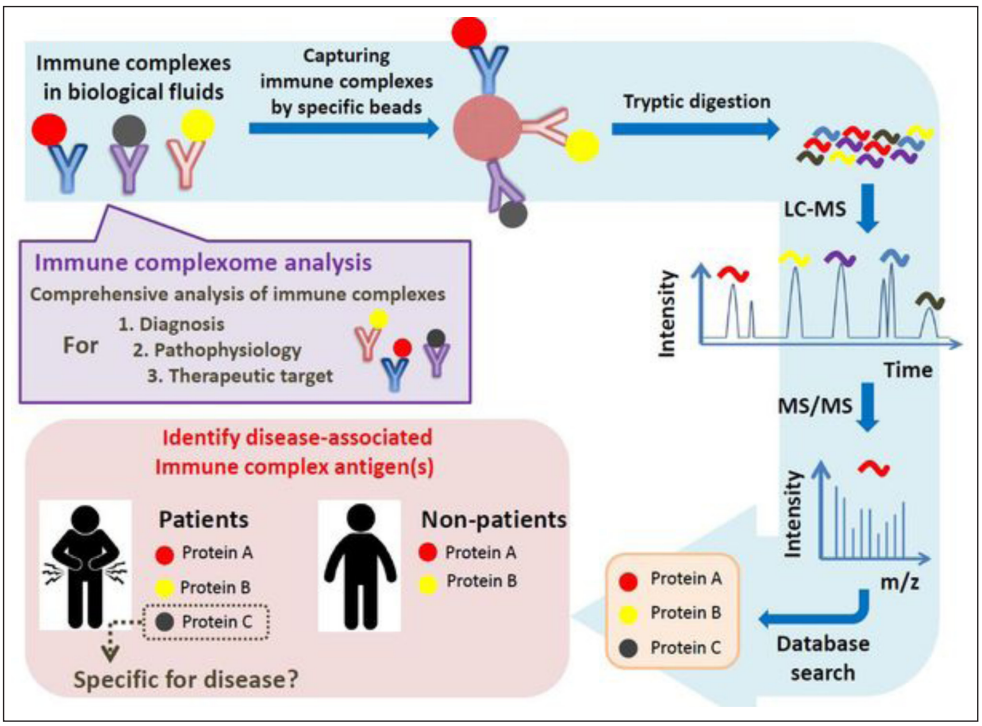

Figure 1.

41: A new polylipoic acid-based nano-platform for heart diseases treatment

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Background
Nanomedicine, that is the application of nanotechnologies to medicine, attracts an enormous interest. The aim of this study is to evaluate the safety, feasibility and biocompatibility of a new polylipoic acid-based nano-platform (NPs) for heart diseases treatment.

Material and Methods
The lipoic acid polymerization reaction has been used to create highly cross-linked polymeric nanoparticles. Dimeric and trimeric derivatives of lipoic acid with different spacers have been synthesized and used to produce surfactant stabilized nanoemulsions. Thiol-initiated polymerization of the microemulsions have been lead to the formation of a highly reticulated polymeric nanostructure. Macrophages and red blood cells were incubated with different concentrations of nanoparticles (up to 200 μg/ml) for 24h hours, the vitality was assessed by MTS assay and the association of NPs to the cells was assessed by cytofluorimetry.

After that, 18 male health Sprague-Dawley rats were injected with 10 mg/Kg of NPs via vein tail. 7 male health rats were taken as controls. Rats were sacrificed 1hour, 3 hour, 24 hours 3 and 7 days after NPs injection and blood and organs were collected. Section of 2mm of lung, heart, liver, kidney and spleen were analyzed by Alliance 2.7 3D software to identify and quantify NPs-rhodamine conjugated. NPs localization in tissues was identify by confocal microscopy.

Results
We found that in vitro this new polylipoic acid-based nano-platform didn't exert any toxicity towards all the different cell types we used, even at high concentrations (200 μg/ml); NPs associate at low levels to all the tested cells, but they are captured by human macrophages at high levels. Moreover, NPs didn't induce red blood cell lysis. In vivo, NPs fluorescence was identify immediately after injection in the heart. Moreover, the heart was able to retain the NPs until 7 days after injection. The heart demonstrated to have a low clearance of NPs. Liver and kidney showed NPs fluorescence clearance at 3h after injection. Confocal microscopy showed that NPs are localize in the interstitium of organs and in the endothelial cells. NPs did not show any toxicity in the rats.

Conclusion
Heart is able to retain NPs injected in blood flow for up to 7 days without evident negative side effects. These preliminary data suggest that this new nano-platform formulation could be used to target heart diseases and for therapeutic drug delivery.

43: Posterior reversible encephalopathy syndrome, preeclapmsia or stroke? A diagnostic dilemma

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Introduction
Posterior reversible encephalopathy syndrome (PRES) is characterised by a symptom constellation of headaches, seizures, visual disturbances and encephalopathy. A diagnosis can be made based on clinical presentation, in which there may be significant heterogeneity, and radiological evidence of focal vasogenic edema most commonly seen bilaterally in the posterior parietooccipital cortices. There are several predisposing factors to PRES including hypertension, infection, renal disease, autoimmune conditions and preeclampsia, of which the latter is quite common.

Case Description
A fit and well 20-year-old primigravid female of 32 weeks gestation, presented to obstetric triage with a 2-day history of headache and epigastric discomfort, with a more recent 1 day onset of blurred vision, dizziness and diplo-

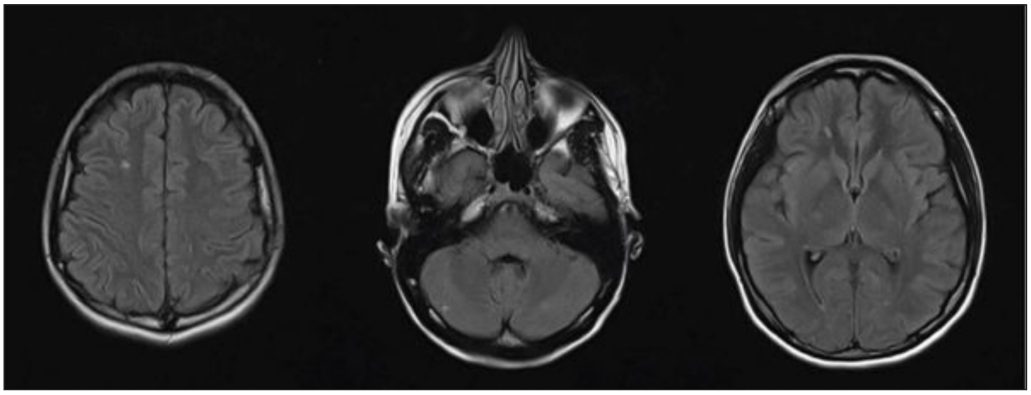

Figure 1: T2 weighted MRI axial sequences demonstrating multiple supra- and infra-tentorial hyperintensities in the right cerebral and cerebellar hemispheres.

pia at the extremes of lateral gaze. General examination was unremarkable and, in particular, reflexes were normal and clonus was absent. She was investigated for possible preeclampsia and a serial blood pressure profile recorded consistently normal blood pressures below 140/85 mmHg, with a stable heart rate of 70–75 bpm.

Results

The patient was normotensive, had no proteinuria and normal preeclampsia bloods, which included full blood count, liver function tests, urea and electrolytes, coagulation screen and C-reactive protein. However, due to worsening symptoms, medical and surgical reviews were sought, prompting further investigations. An abdominal ultrasound was arranged, and amylase levels were checked, both of which returned normal. A MRI of the brain was advised, which excluded dural venous sinus thrombosis but showed multiple supra- and infra-tentorial hyperintense lesions with restricted diffusion in the right cerebral and cerebellar hemispheres (**Figure 1**). This was reported as representative of either an acute stroke or an atypical presentation of PRES, a rare neurological syndrome first described in 1996. Both conditions represent diagnoses which are seldom seen or even considered in modern obstetric practice, and created a significant diagnostic dilemma. Multi-disciplinary team working between neurologists, obstetricians, radiologists and stroke physicians were key to managing the patient, who ultimately went onto make a full recovery and deliver a healthy baby.

Conclusions

PRES is a rare clinicoradiological syndrome, which demonstrates significant heterogeneity in its presentation making its diagnosis challenging. The most widely accepted theory explaining the pathophysiological changes involves dysfunction of the endothelium, which may occur in the presence or absence of hypertension. This case demonstrates that PRES may be considered in obstetric patients who present with symptoms of pre-eclampsia, but without the more typical PRES features of encephalopathy or seizures. While our MRI findings in this case are also atypical, no gold standard radiological diagnostic criteria exist, again demonstrating the wide spectrum in clinicoradiological presentation.

44: Relationship between Mucosal Healing and Symptomatic Relief in Erosive GERD – A four Arm Randomized Trial involving Omeprazole, Ranitidine, Antacids and a Barrier Therapy – Cross-linked Polymeric Sucralfate (Esolgafate)

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Background

Barrier therapy has become an acceptable approach to manage heartburn from erosive gastro-esophageal reflux disorder (GERD). Esolgafate is a cross-linked polymeric formulation of standard sucralfate (CLPS) that self-anneals following administration to achieve a mucosal surface concentration of sucralfate that is 2400% greater than is possible using equimolar amounts of standard non-polymeric sucralfate. Sucralfate, omeprazole and ranitidine accelerates healing of mucosal erosions, but the relationship between symptomatic relief and healing is not well known.

Objective

Use a seven day efficacy study to examine the relationship between heartburn relief and healing of erosive GERD by comparing three acid controlling therapies to cross-linked polymeric sucralfate (Esolgafate).

Design

Multi-center parallel randomized controlled, open label trial, in two university medical centers in Bangladesh using a protocol approved by the Medical Research Council.

Participants

Of the 77 patients evaluated for severe dyspepsia, 41 were found to have erosive GERD and were randomized into four treatment arms. Two patients were excluded due to failure to return on day 8 for re-evaluation, thus leaving 39 (28 males & 11 females with mean age of 38+/–9.4 yrs) for data analysis.

Intervention

Seven days of treatment with either CLPS containing 1.5 gram of sucralfate bid or one of three acid-controlling therapies – omeprazole 20 mg bid, ranitidine 150 mg bid or aluminum/magnesium hydroxide antacid 30 ml qid.

Outcome measures

(1) Endoscopic healing of erosions, (2) symptomatic relief of heartburn and/or painful acid regurgitation, (3) comparative relationship between healing and relief and (4) reports of any adverse events.

Results

For CLPS, omeprazole, ranitidine and antacids symptomatic relief and healing were 80% and 80%, 90% and 30%, 66% and 0%, 80% and 0%, respectively. No adverse reactions were reported.

Conclusions

Symptomatic relief from CLPS barrier therapy was high and comparable to that of acid-controlling therapies. However, mucosal healing was divergent, with a high degree of relief from acid-controlling therapies being inversely correlated with a low degree of mucosal healing. However, relief from CLPS barrier therapy tracked positively with the degree of mucosal healing. Thus, CLPS barrier therapy seemed to synchronize meaningful symptomatic relief with significant mucosal healing of erosive GERD, a correlation that is positive and biologically intuitive.

46: Raman spectroscopy and surface enhanced Raman spectroscopy as a promising approach for assessment of platelet function

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The task of the study was to assess the feasibility of assessing platelet characteristics of patients with cardiovascular diseases using Raman spectroscopy. In our study, for the first time successfully implemented a technique based on the use of Raman spectroscopy or Surface Enhanced Raman spectroscopy (SERS) for the study of a single human platelet. This approach is based on the interaction of laser radiation with the surface of the platelet and is a fast, non-invasive, highly sensitive and accurate method.

Two groups of subjects were examined – healthy volunteers (group 1, n = 9) and patients after acute myocardial infarction who received double anti-platelet therapy (group 2, n = 3). Prior to the beginning of the study, the approval of the Local Ethics Committee at the Clinical Research Center of the BFU. Kant was received. A procedure for obtaining the written informed consent of individuals was carried out with each subject of the study.

Whole blood sampling from healthy donors and patients will be conducted in vacuum tubes containing 3.2% sodium citrate. Peripheral blood platelets were isolated by phased centrifugation using an Eppendorf 5702R centrifuge at +18C. After centrifugation, platelet-rich plasma was collected, to which, to prevent cell aggregation, sodium citrate solution (106 mM, pH 5.5) was added in a plasma: citrate ratio of 3: 1. Plasma was centrifuged at +18°C at 400 g for 5 minutes. The supernatant was removed, the pellet was resuspended in 300 μl of Tyrode buffer.

A Centaur U Raman spectrometer was used, the Raman spectra of single platelets were obtained when excited with a DPSS solid-state laser at a wavelength of 632.8 nm and a power of 37 mW using an Olympus optical microscope. To realize the effect of SERS scattering, nanostructured silver Silmeco (Denmark) substrates were used. Spectra of SERS were recorded with a signal accumulation of 15–120 seconds. Spectral changes of amino acid tryptophan and amide groups were detected (**Figure 1**). For the first time, the possibility of obtaining Raman spectra and SERS of single platelets was shown. Large differences in the spectra of Raman and SERS scattering of isolated single platelets between healthy individuals and patients with myocardial infarction in the spectral range of amino acid tryptophan and amide groups were revealed. The obtained data confirm the reasoning for further study of the method of Raman spectroscopy and SERS for the evaluation of platelet function and other during antiplatelet therapy.

Funding Information
This work is supported by the Russian Science Foundation under grant 19-15-00132.

51: Association of gene polymorphisms of SLC22A1 (R61C, del420) and SLC47A1 (rs2289669) with metformin transport during T2D treatment

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Introduction
Type 2 Diabetes Mellitus (T2D) is a metabolic disorder characterized by high blood sugar levels over prolonged period due to insulin resistance, a condition in which cells fail to respond to insulin properly. Metformin is used as

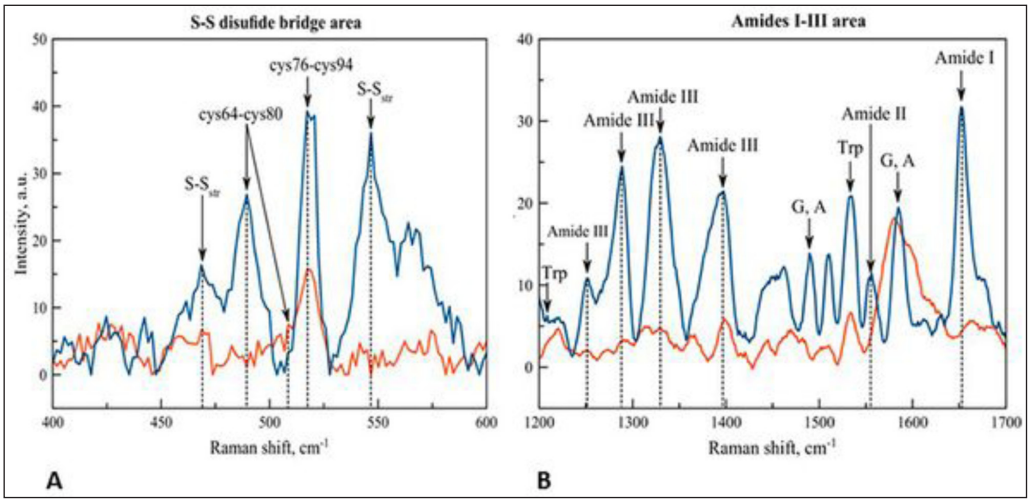

Figure 1: Examples of Raman spectra using low-efficiency substrates **(A)** and SERS spectra from the region of amide groups **(B)** of platelet membranes in healthy individual (blue line) and in patients after myocardial infarction (red line).

the first-line medication for the treatment of T2D, because of its ability to reduce the concentration of glucose in the blood by inhibiting the formation of glucose in the liver. Unfortunately, almost 50% of patients are not able to use metformin properly due to its side effects, such as gastrointestinal disease. Moreover, there are a lot of patients who do not have a positive effect after taking the medicine. It is known that these affects relate to polymorphisms in different protein carriers coding genes. Some of these polymorphisms are *R61C* and *del420* – dysfunctional polymorphisms of *SLC22A1* that is coding organic cation transporter 1 (OCT1) and rs2289669 – dysfunctional polymorphism of *SLC47A1* that is coding multidrug and toxin extrusion 1 (MATE1).

Aim
To analyze the polymorphisms of *SLC22A1* and *SLC47A1* genes in group of patients with T2D and population group in North-West region of Russia.

Material and Methods
DNA was extracted from whole venous blood samples using standard phenol-chloroform method. Analysis of gene polymorphism was performed by PCR-RFLP method.

Results
We elaborated the test systems based on PCR-RFLP approach for study of polymorphic variants *R61C, del420* (*SLC22A1*) and *rs2289669 (SLC47A1).* Genotypes and alleles frequencies of *R61C, del420* and *rs2289669* polymorphic variants were analyzed in population group, positive metformin treatment group of T2D patients and negative metformin treatment group of T2D patients.

54: Identification of a quaternary ammonium compound with antiviral activity against herpes simplex viruses type 1 and type 2 in human gingival fibroblasts

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Background
Herpes simplex viruses (HSV-1 & HSV-2) elicit numerous clinical manifestations in humans, such as skin lesions in the orofacial area and genitalia, as well as herpetic gingivostomatitis. Acyclovir, a nucleoside analogue commonly used to treat HSV infection is poorly effective for treating herpetic skin lesions when applied as a cream. Furthermore, acyclovir-resistant isolates have been isolated from immunosuppressed patients, thus requiring therapeutic alternatives. Cetylpyridinium chloride (CPC) is a quaternary ammonium compound with bactericidal properties that is frequently added to mouthwashes and deodorants; nevertheless, it was recently reported to have viricidal activity against influenza A virus.

Objectives
We sought to assess the potential antiviral effects of CPC against HSVs.

Methods
Epithelial cells and human gingival fibroblasts were infected with HSV-1 or HSV-2 strains that express a green fluorescent protein (GFP) and then treated with CPC. Infection and viral processes were then followed within the infected cells.

Results
We found that cells infected with HSV and then treated with CPC produced significantly less virus plaque forming units and displayed reduced expression of virus-encoded genes as compared to controls. Dissection of the viral step inhibited by CPC treatment indicates that CPC inhibits the transcription of viral gene early after infection by blocking the translocation of NF-κB to the nucleus of HSV-infected cells. Taken together, our results suggest that CPC has anti-HSV effects which is mediated by non-viricidal effects, but rather the modulation of cellular signalling events required by HSVs for replication.

60: Usefulness of ultrasonography for prostate cancer follow-up in a rodent model of cancer (chemically-induced)

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Prostate cancer (PC) is one of the most frequent cancers of male population [1]. Animal models have been used to study several diseases, including cancer [2]. This work addressed the usefulness of ultrasonography for PC follow-up in a rodent model of cancer (chemically-induced). Procedures were approved by Ethics Committee (no. 021326). Male Wistar Unilever rats of four weeks-old were divided into two groups: control (n = 8) and PC (n = 14). At 12 weeks of age, animals from PC group received an injection of flutamide (50 mg/kg) for 21 consecutive days. Twenty-four hours after the last administration, they received an injection of testosterone (100 mg/kg). Forty-eight hours later were intraperitoneally injected with the carcinogen *N*-methyl-*N*-nitrosourea (MNU) (30 mg/kg).

Two weeks later, syllastic tubes filled with crystalline testosterone were subcutaneously implanted. Prostate was examined by ultrasonography at 11, 15, 21, 32 and 61 weeks of age. A real-time scanner and a 12 MHz linear transducer were used. A complete transverse prostate scan using B-mode was performed. Prostate area was determined using the scanner calipers. Ventral prostate lobes appear as hypoechoic elongated structures with a hyperechoic capsule, ventrally to the urinary bladder. Dorsal prostate was observed close to the urinary bladder neck as a round hypoechoic structure with a hyperechoic capsule, dorsally to the urinary bladder. Prostate area of animals from control group was gradually increasing through the experiment. Prostate area of animals from PC group decreased between the first and the second examinations due to the anti-androgenic administration. Then prostate area increased in the fourth and fifth examinations due to the testosterone and MNU administrations and decreased unexpectedly in the last examination. Cystic lesions were also observed in the prostate of PC group at last examination. Ultrasonography allows for a comprehensive and detailed study of the rat prostate, being recommended for the PC monitoring.

Acknowledgements
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61: Cancer spread to bone and biomarker discovery: towards personalised medicine in a major disease complication

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The skeleton is a common site of metastatic spread in many cancers with an estimated half a million people worldwide with bone metastases. In advanced breast and prostate cancer, upwards of 70% of patients have or develop bone metastases and, once bone metastasis has developed, the cancer becomes incurable. Bone metastasis can cause major skeletal complications including severe bone pain, bone fracture, hypercalcaemia and spinal cord injury. Treatments have been and continue to be developed, both to help manage metastatic bone disease once it has developed and to prevent development of bone metastasis. In both of these clinical situations, prognostic biomarkers are required to identify patients at greatest risk and predictive biomarkers to assess likely response to treatment.

In normal bone turnover, there is a balance between the removal of old bone (bone resorption) and new bone synthesis (bone formation). This balance is disrupted in bone metastasis, driven by increased bone resorption. A range of serum and urinary bone biomarkers has been developed to assess bone formation and bone resorption activity levels and strong correlations have been observed in patients with bone metastasis between elevated bone biomarkers and occurrence of skeletal complications. Most of these biomarkers are products of collagen breakdown (resorption markers) or collagen synthesis (formation markers) and have been particularly useful in monitoring response to treatment and in optimum dose selection.

In early (non-metastatic) breast cancer, modern omics techniques have been applied to the identification and clinical validation of biomarkers for assessing risk of developing bone metastasis and predicting response to treatment. Genomic analysis in animal models of breast cancer spread to bone and bone homing cell lines has identified the MAF transcription factor as well as the gene encoding interleukin-1β (IL-1β), which have been correlated with patients who later develop bone metastasis. Proteomic analysis of these bone homing cell-lines has identified several protein-based markers of spread to bone, including macrophage-actin-capping protein (CAPG), the PDZ domain-containing scaffolding protein GIPC1) and the cell motility regulator Dedicator of Cytokinesis – 4 (DOCK4), all of which have been clinically validated in our group, using large patient datasets, as prognostic for future development of bone metastases and predictive of benefit from adjuvant treatment to prevent bone metastases.

Advanced molecular techniques in prostate cancer are also beginning to yield encouraging results in the search for prognostic biomarkers for development of bone metastasis, including CXCR4 and IL-1β and a regulatory microRNAs which play key roles in priming bone for the arrival of metastatic prostate cancer cells. Studies on cancer spread to bone are entering an exciting era in which the outputs of omic technologies have considerable potential not only to predict which patients are at high risk, but also to inform patient treatment options.

63: Ectonucleotidase CD39 expression on circulating CD4+ T lymphocytes of patients with rheumatoid arthritis and cancer

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Introduction

Regulatory T cells (Tregs) are important for the maintenance of self-tolerance. They are implicated in the origin of autoimmunity and involved in the suppression of antitumor immune response in cancer patients. CD39 defines a subset of regulatory T cells (Tregs) with high suppressive capacity. We aimed to study frequency of CD39 T cells in the patients with rheumatoid arthritis, liver cancer and pancreatic cancer in comparison with healthy donors.

Methods

Blood samples were obtained from the patients with rheumatoid arthritis, liver cancer and pancreatic cancer. Percentages of $CD39^{+}$ cells were separately quantified within $CD4^{low}$ and $CD4^{high}$ populations. Data were acquired on a FacsArray cytometer and analyzed with FlowJo® v7.5.6 software.

Results
Our results demonstrate that the frequency of total circulating $CD4^+$ T lymphocytes was comparable between the cancer patients and controls. Whereas the percentages of $CD39^+$ cells showed 2-fold increase in the patients $CD4^+$ T cells compare to healthy donors. Patients with rheumatoid arthritis showed increased frequency of CD4+CD39+ cells in comparison with healthy controls. Percentage of these cells significantly correlated with DAS-28 score.

Conclusion
Ectonucleotidase CD39 expression by regulatory T lymphocytes is responsible for the suppressive activity of these cells and can be proposed a new target for therapy in many diseases.

65: A lateral flow strip in combination with microfluidic device and polymerase chain amplification for rapid and visual detection of lymphatic filarial infection

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Lymphatic filariasis (LF) is a neglected tropical disease caused by the filarial nematode parasites which is transmitted by the bites of infective mosquitoes. The diagnosis of LF traditionally relies on the detection of circulating microfilariae (mf) using Giemsa-stained thick blood smears which has several limitations. In the present study, we developed a lateral flow (LF) strip to be used in combination with a novel microfluidic device and polymerase chain reaction (PCR) for a rapid and visual detection of lymphatic filariae, *Brugia malayi/Wuchereria bancrofti* infection in human blood samples. The assay targets *B. malayi Hha I* gene and *W. bancrofti Ssp* gene. Prior to perform the assay, no DNA product clean up step required, thus, it can shorten time and reduce cost. The LF strip can detect as low as 10 pg of DNA product and no cross-reactivity with DNA of other parasites such as *Gnathostoma spinigerum*, *cysticercisis solium* nor with DNA of other filariae i.e. *Brugia pahangi*, *Dirofilaria immitis* and *D. repens.* The developed LF strip shows high sensitivity and specificity, in combination with a novel microfluidic device and PCR, can be used as an alternative tool for the diagnosis of lymphatic filariasis.

67: Potential longitudinal biomarkers for assessing therapeutic responses to systemic cyclosporine A treatment in patients with atopic dermatitis

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Objectives
Cyclosporine A (CsA) is an immunosuppressant agent widely used in severe atopic dermatitis (AD). However, responses to CsA treatment is largely different among AD patients and some patients gain smaller benefit compared to its risk. Thus, it is important to assess the therapeutic response of each patient in early phase of treatment to decide whether to continue the therapy. Currently, only clinical skin scoring methods such as SCORAD are used to monitor the response to CsA in AD, but some other index that systemically describe the condition of the patients and are more universally evaluable are needed. In this study, we sought the potential of blood TARC and LDH as longitudinal biomarkers for evaluating the therapeutic responses to CsA in AD patients.

Method
Medical records of 26 patients with severe AD who underwent CsA therapy between 2012 and 2018 were retrospectively evaluated. Association between the longitudinal course of SCORAD and blood TARC or LDH were analyzed up to 1 year after the initial prescription.

Results
Among 26 patients, 6 showed good response, 10 showed moderate response, 5 showed poor response and 5 manifested acute exacerbation and remission. The transition of combined index of TARC and LDH were well associated with the transition of SCORAD.

Conclusions
The combined index of TARC and LDH were suggested to be helpful for assessing the therapeutic response to CsA in AD patients. Further investigation with lager size of samples are needed for the practical utilization of the index.

69: MCO-multiple criteria optimization-based gene selection: A deterministic tool for the analysis of genomic data

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The current crisis in reproducibility in the health sciences makes it necessary to emphasize objectivity and repeatability in experimental design and analysis techniques. In the past, our group has proposed the use of multiple criteria optimization (MCO) to approach gene selection from microarray datasets. This method does not require

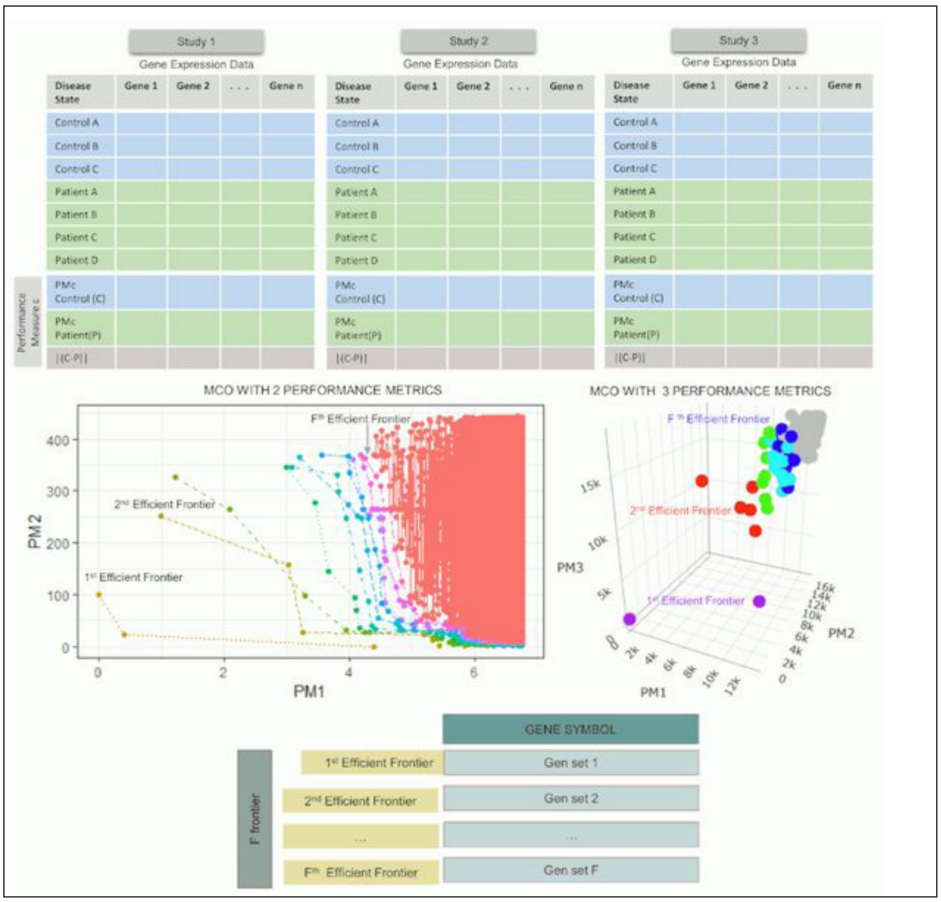

Figure 1.

for the user to manipulate neither informatics nor statistical parameters. Furthermore, the user does not have to choose a neither a preference structure among multiple measures of differential expression nor a predetermined quantity of genes to be deemed significant a priori. This implies that, by using the same datasets and the same performance measures (PM) the method will converge to the same set of selected differentially expressed genes (repeatability) in spite of who the analyst is (objectivity). The present work describes the development of a suite of an open-source software program in RStudio to enable both: 1) individual analysis of datasets with two and three PM and 2) meta-analysis with up to four PM selected from different datasets. The capabilities afforded by the code include license-free portability and the possibility to carry out analyses using modest computer hardware, such as personal laptops. To illustrate how MCO works, a case study using publicly-available microarray datasets with evidence of Parkinson Disease (PD) from the Gene Expression Omnibus (GSE99039, GSE19587, GSE72267, and GSE7621) is presented. The total cohort databases used for this study contained 271 control patients and 273 PD patients. The goal of the analysis was to detect relevant genes and to characterize and infer their biological meaning in PD through MCO implementation. MCO meta-analysis identified 13 significant genes: CRYAB, PTGDS, RPS4Y1, TUBB2A, HBA1/2, PTPRO, SEPP1, XIST, HBB, TUBA1A, PAQR6, RPS15, and TUBA1B. Using the PubMed engine search database, we observed that 8 out of the 13 identified genes have been mentioned in published articles related to Parkinson. For instance, alpha B-crystallin (Cryab) has been mentioned in 3 published papers directly associated with Parkinson, where 2 of them reveal that this gene was markedly upregulated in pathologies like PD. Also, the prostaglandin D2 synthase (PTGDS) gene was identified as an optimal PD biomarker with great diagnostic value. This result supports the sensitivity of our methodology to detect genes that are currently known to play a key role in PD. Furthermore, we found genes (TUBB2A, PTPRO, TUBA1A, PAQR6, and TUBA1B) with no published articles related to PD; however, 3 of them, TUBB2A, PTPRO, and TUBA1A, have 2, 1, and 7 published studies respectively, linked to neurodegenerative disease as of Aug 29, 2019. These genes are strong and promising candidates for in-depth exploration regarding their implications in PD.

72: Dietary natural products for the treatment of neurodegenerative disorders

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Neurodegenerative disorders, such as Parkinson's disease (PD) and Alzheimer's disease (AD), are characterized by debilitating motor dysfunction and cognitive symptoms. Currently, there are limited treatment options for these disorders. Oxidative stress is associated with the

accumulation of harmful free radical species and is likely a major mechanism contributing to neurodegenerative disease. When cells are overloaded with free radicals this results in cell damage, including dysfunction of the mitochondria and aggregation of insoluble proteins, such as amyloid-beta and alpha-synuclein. Chronic neuroinflammation is also associated with the pathology of neurodegeneration, where microglia become over-activated in response to the abnormal aggregation of proteins and release of inflammatory mediators. We have analyzed the potential protective effects of natural dietary products against the pathology of neurodegeneration for several years. For example, we have found that the fruits and leaves of berries protect cells against toxins associated with neurodegenerative disease, such as glutamate, alpha-synuclein and amyloid-beta protein. Polyphenols are prevalent compounds found in berries and possess high antioxidant and free radical scavenging activity, which may underly the protective effects. We have also found that extracts of sea cucumber, which are rich in compounds called saponins, can also protect brain cells from toxicity. We have recently conducted animal studies analyzing the potential protective effects of berries and sea cucumber extracts against neurodegeneration and will soon be initiating a clinical study in PD patients in order to test the potential of these dietary natural products to inhibit the progression of this disease. Therefore, dietary products or compounds derived from them, such as specific polyphenols or saponins, may hold promise in the treatment of neurodegenerative disorders.

74: Evolutionary learning for predicting survival after transarterial chemoembolization for BCLC stage B hepatocellular carcinoma

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Transarterial chemoembolization (TACE) is a standard treatment for Barcelona Clinic Liver Cancer (BCLC) stage B hepatocellular carcinoma (HCC). A simple to use Albumin-bilirubin (ALBI)-based model (ALBI-TAE) was newly proposed to predict overall survival after TACE for BCLC stage B HCC using three independent parameters, ALBI grade, alpha-foetoprotein (AFP) level, and Up-to-11 criteria. An accurate overall survival estimator can identify BCLC stage B HCC patients who may benefit most from TACE as initial treatment. This study aims to develop an accurate estimator called SVR-TACE for predicting the overall survival time after TACE in the HCC patients. SVR-TACE used an inheritable bi-objective combinatorial genetic algorithm (IBCGA) to identify a small set of factors with support vector machine (SVM) using an evolutionary learning approach. Evolutionary learning

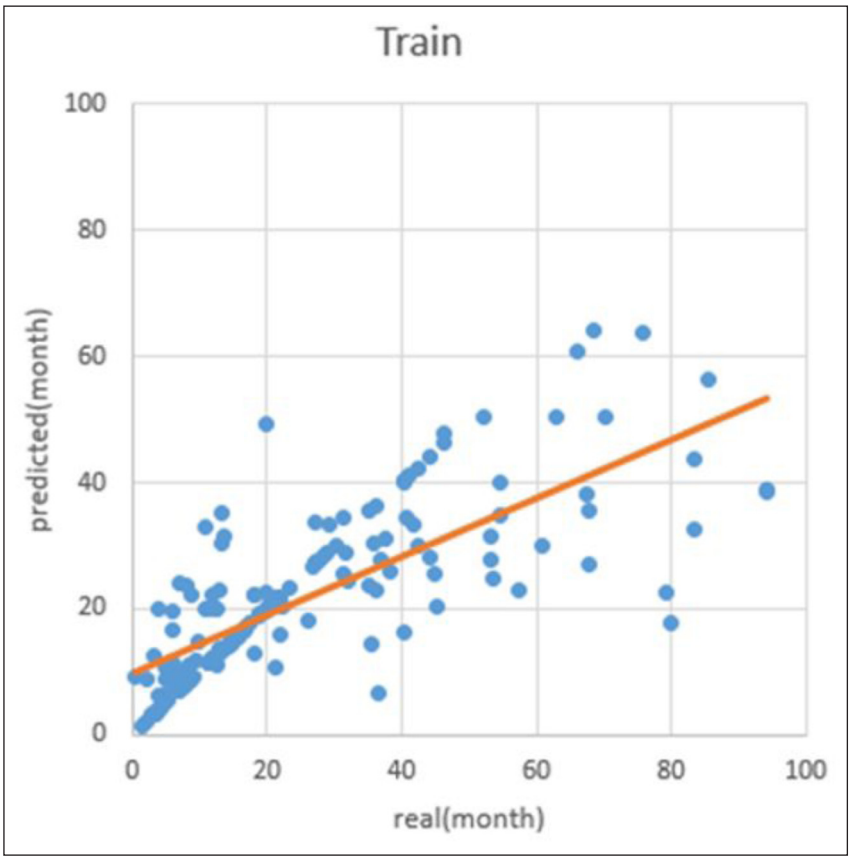

Figure 1.

aims to optimize the parameters in the machine learning methods. The IBCGA uses an intelligent evolutionary algorithm that can efficiently solve the large combinatorial optimization problems for feature selection. SVR is another version of SVM for solving regression problems. SVR-TACE is developed based on ν-SVR by optimizing feature selection and parameter settings of ν-SVR simultaneously while maximizing prediction accuracy. To avoid the overtraining, we used 10-fold cross-validation (10-CV) to evaluate the performance of the model. There were 570 treatment-naïve BCLC stage B HCC patients undergoing TACE as the initial treatment from 2007 to 2016 who were retrospectively enrolled. After the filtration process, there are 204 patients with overall survival time and 46 factors without missing values. The survival time was ranged from 1 to 95 months. The training and test datasets consisting of 159 and 45 patients, respectively. Another dataset of 197 HCC patients after TACE who are alive was used as an independent test cohort. SVR-TACE achieved a correlation coefficient of 0.76 and a root mean absolute error of 17.47 months using 10-CV on the training dataset. SVR-TACE achieved a correlation coefficient of 0.51 for test performance. SVR-TACE identified a set of 16 factors from 46 parameters: Age, Gender, PS, Child-pugh score, ALBI score, Alcoholism, WBC, PTINR, K, BUN, AAR(AST/ALT), AFP, HBsAg, BCLC-B sub-stage, TACE Response new, and AFP level. Not like the simple model ALBI-TAE, SVR-TACE obtained a more accurate model using a larger number of factors. The factors can be ranked based on the main effect difference (MED). The larger the MED value, the larger the contribution to the prediction. This study proposed an evolutionary method for identification and characterization of the risk factors associated with overall survival in patients with BCLC stage B HCC. Because the difference between the validation and test performance is still large, the increase of training patient number for SVR-TACE would significantly improve the test accuracy.

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