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Giving birth to active anticancer drugs from phase-I clinical trials and translational researches

Plenary 1

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Early preclinical researches and phase I trials are crucial to properly drive further steps of drug development with novel anticancer agents. Novel compounds require a delicate attention to accurately define drug-targets and potential biomarkers of activity. This process often occurs before or concomitantly to first-in-man trials allowing to personalise tumour targets and to restrict drug access to patients with a high likelihood of activity. However, early clinical evidences may also require some backward re-investigations of drug effects in preclinical models while trials are already ongoing. This bidirectional translational process of drug development may apparently slow down the drug development process but on the long run often provide a higher efficiency and minimise the risk of drug development failures. Phase I trials are far to be limited to define the dose-limiting toxicities, the maximum tolerated doses and the pharmacokinetic profile of new agents. It also offers the opportunity to test pharmacodynamics biomarkers of activity, optimal schedules and combinations that will be used in phase II/III trials to place the novel drug in current treatment algorithms. During the last few years, progresses in biotechnologies have offered the opportunity to revisit early drug development by incorporating sophisticated methods that describe the biology of tumour in individual patients.

During the last 15 years, my group has been deeply involved in the development of novel drugs. Experiences with drugs such as oxaliplatin, sunitinib, everolimus, cetuximab, and temsirolimus allowed us to better understand how phase I clinical trials could provide sufficient information to successfully drive further steps of drug developments in several tumour types. Antiangiogenic agents, such as Sunitinib and mTOR inhibitors, further demonstrated efficacy in renal cell, liver, and neuroendocrine carcinomas in phase III trials. Understanding tumour changes induced by those drugs also provided valuable information to identify biomarkers of resistance and define novel targets for therapeutic interventions. Counteracting primary and acquired resistance to targeted agents may also be obtained through drug combinations that should be tested early after first-in-man phase I trials. More recently, ERK1/2 inhibitors provided evidences of activity in tumour types such as melanoma and pancreatic adenocarcinoma displaying oncogenic Ras and Raf mutations.

In this review, we will provide an overview of our experience in drug development emphasising the role of translational data generated in the lab concomitantly to early phases of clinical trials. Recent translational experiences with novel anticancer agents will also be discussed.

Stem cell drug—Flk1$^+$MSCs hold great promise for regenerative medicine

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Mesenchymal stem cells (MSCs), a type of adult stem cell, have generated a great amount of interest over the past decade in the field of regenerative medicine. We have isolated and culture-medium-selected a subpopulation of MSCs (Flk1$^+$CD44$^+$CD29$^+$CD105$^+$CD166$^+$CD34$^-$ CD31$^-$Lin$^-$) named Flk1$^+$ MSCs from a variety of human tissues. These cells are multipotent cells with extensive proliferation ability and immunomodulatory function. To explore their potential application in disease treatment, we first evaluated their safety with both in-vitro and in-vivo experiments. Flk1$^+$MSCs did not form teratoma when injected in vivo and can even inhibit malignant cell proliferation by secreting DKK-1. We then carried out a preclinical trial to further evaluate the feasibility and safety of intravenous infusion of these cells after ex-vivo expansion. Rhesus monkey and human Flk-1$^+$MSCs were isolated from bone marrow, expanded and transplanted into rhesus monkeys and human volunteers, respectively. During the infusion of these cells, the life signs of the recipients were normal. Laboratory tests for blood, bone marrow, kidney, and liver function were conducted and no significant changes were observed before and after cell infusion. Flk1$^+$ MSCs was the first stem cell product that received approval for clinical trial from the State Food and Drug Administration (SFDA) of China.

After confirming the safety of Flk1+MSCs for clinical application, we began to evaluate their efficacy in treatment for several diseases. Two clinical trials were carried out. The first study found that a new transplantation strategy combining haploidentical peripheral blood stem cells (PBSCT) and Flk1$^+$ -MSCs could improve donor engraftment and prevent severe GVHD. The second one was an open-label, randomised phase II clinical trial to assess the outcome of MSC coinfusion ($3\text{-}5\times10^5$ cells/kg) during haploidentical haematopoietic stem cell transplantation. We also investigated the possible mechanisms underlying the immunomodulatory effects of Flk1$^+$MSCs which are mediated through interacting with a wide range of immune cells or secreting bioactive molecules. Flk1$^+$MSCs facilitate the immunosuppressive effect of cyclosporin A on T lymphocytes through Jagged-1-mediated inhibition of NF-κB signalling. In the presence of Flk1$^+$MSCs, the percentage of cells with cDC phenotype is significantly reduced whereas the percentage of pDC increased. Interestingly, Flk1$^+$MSCs could drive maDCs to differentiate into a novel Jagged-2-dependent regulatory DC population and escape their apoptotic fate, providing more evidence to support the role of Flk1$^+$MSCs in rejection prevention during organ transplantation and treatment of autoimmune disease. In BXSB mice that were born with an immunologic deficiency, Flk1$^+$MSCs can significantly down-regulate Th2 cells and further inhibit the abnormal activation of humoral immunity to maintain the original balance. Based on studies in our laboratory, we drew a schematic picture of the immunomodulatory effects of Flk1$^+$MSCs both in vitro and in vivo.

In conclusion, our studies demonstrate that Flk1$^+$MSCs represent a safe and effective treatment for some haematological disorders. We are now carrying out more clinical investigations to extend their potential applications in other conditions.

1

Adiponectin is protective against oxidative stress–induced cytotoxicity in amyloid-beta neurotoxicity

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Beta-amyloid (Aβ) neurotoxicity is important in Alzheimer's disease (AD) pathogenesis. Aβ neurotoxicity causes oxidative stress, inflammation, and mitochondrial damage resulting in neuronal degeneration and death. Oxidative stress, inflammation, and mitochondrial failure are also pathophysiological mechanisms of type 2 diabetes mellitus (T_2DM) which is characterised by insulin resistance. Interestingly, T_2DM increases risk to develop AD which is associated with reduced neuronal insulin sensitivity (central insulin resistance). We studied the potential protective effect of adiponectin (an adipokine with insulin-sensitising, anti-inflammatory, and anti-oxidant properties) against Aβ neurotoxicity in human neuroblastoma cells (SH-SY5Y) transfected with the Swedish amyloid precursor protein (Sw-APP) mutant, which overproduced Aβ with abnormal intracellular Aβ accumulation. Cytotoxicity was measured by assay for lactate dehydrogenase released upon cell death and lysis. Our results revealed that Sw-APP–transfected SH-SY5Y cells expressed both adiponectin receptor 1 and 2, and had increased AMP-activated protein kinase (AMPK) activation and enhanced nuclear factor–kappa B (NF-κB) activation compared with control empty-vector–transfected SH-SY5Y cells. Importantly, adiponectin at physiological concentration of 10 μg/mL protected Sw-APP–transfected SH-SY5Y cells against cytotoxicity under oxidative stress induced by hydrogen peroxide. This neuroprotective action of adiponectin against Aβ neurotoxicity-induced cytotoxicity under oxidative stress involved (1) AMPK activation mediated via the endosomal adaptor protein APPL1, and possibly (2) suppression of NF-κB activation.

2

Association between age and rehabilitation outcome in frail older adults

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Background: The association between age and rehabilitation outcome in frail older adults is controversial.

Methods: Patients from 2004 to 2012 of the geriatric day hospital (GDH) of Fung Yiu King Hospital were reviewed in this retrospective cohort study. Age, gender, place of residence, co-morbidities, blood test results (serum creatinine, albumin, haemoglobin), functional status using functional independence measure (FIM), cognitive status, body mass index, and referred diagnosis were collected. Age was stratified into groups (<70, 71-75, 76-80, 81-85, 86-90, and >90 years). Outcome measurement was change of FIM after receiving rehabilitation in GDH.

Results: A total of 833 GDH patients (503 women, 330 men; mean age 80.0±7.1 years) of age 65 years and above were included. Median change of FIM was 4, interquartile range (IQR) was 0-9. There was no significant difference in FIM across different age-groups (age <70: median 5, IQR 2-11; age 71-75: median 5, IQR 0-10; age 76-80: median 4, IQR 0-9; age 81-85: median 3, IQR 0-9; age 86-90: median 3, IQR 0-8; age >90: median 4, IQR 0-8.5; P=0.42).

Conclusion: Age has no influence on rehabilitation outcome in frail Chinese older adults. Older patients should not be excluded from rehabilitation programmes because of increasing age.

3

Association between functional status of Chinese nursing home older adults and long-term mortality

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Background: There is little research into the functional status of nursing home older adults and long-term mortality.

Methods: It was a 3-year prospective cohort study in nine nursing homes in Hong Kong. Functional statuses of participants were assessed by Barthel index (BI) and participants were stratified into different groups according to their BI scores: 100, 75-95, 45-70, 15-40, and 0-10. Other covariates included age, gender, co-morbidities, score of abbreviated mental test, serum albumin, serum creatinine, serum haemoglobin, and hospitalisation in the preceding year. The outcome measures were the 1-, 2-, and 3-year all-cause mortality.

Results: A total of 672 nursing home older adults (224 men; 448 women) with a mean age of 85.0±7.4 years were recruited. Older adults with lower BI score had significantly higher all-cause mortality and this trend persisted in 1-, 2-, and 3-year mortality (P<0.001). After multivariate analysis, there was a dose-response relationship in hazard ratio (HR) between BI score and 3-year all-cause mortality—compared with BI score 100, BI score 75-95: HR=1.38 (confidence interval [CI]: 1.00-2.56; P<0.05); BI score 45-70: HR=1.80 (CI: 1.04-3.11; P<0.001); BI score 15-40: HR=2.12 (CI: 1.21-3.70; P<0.001); BI score 0-10: HR=3.13 (CI: 1.82-5.41; P<0.001); trend test P<0.05. Similar relationships were found in 1-year and 2-year mortality.

Conclusion: Impaired functional status is associated with higher short-term and long-term mortality with a dose-response relationship in Chinese nursing home older adults. Appropriate advance care planning should be made according to functional status of nursing home older adults.

4

Prognostic factors for frail Chinese older adults: is cognitive function an important predictor?

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Background: There is controversial evidence regarding the effect of cognitive function on mortality for frail Chinese older adults.

Methods: This was a retrospective cohort performed in Geriatric Day Hospital (GDH) of Fung Yiu King Hospital. Patient data from 2004 to 2012 were reviewed. The following data were collected: age, gender, smoking status, place of residence, co-morbidities (quantified into Charlson comorbidity index [CCI]), blood test result (serum creatinine, albumin, haemoglobin), functional status using functional independence measure (FIM), cognitive status using Mini-Mental State Examination, body mass index (BMI), and referred diagnosis. Age was stratified into groups: <70, 71-80, 81-90, and >90. BMI was stratified into groups: >25, 20-25, and <20. Baseline characteristics were used to predict survival in univariate and multivariate Cox proportional hazard models.

Results: A total of 1657 GDH patients (976 women, 681 men; mean age, 80.7±7.3 years) of age 65 years and above were included. The median follow-up was 48 months. Overall mortality rate was 35.3%. Increased age (hazard ratio [HR]=1.17; confidence interval [CI]: 1.06-1.29; P<0.01), increased CCI (HR=1.39; CI: 1.27-1.51; P<0.001], and presence of any arrhythmia (HR=1.90; CI: 1.35-2.66; P<0.001) increased risk of death. Increased serum albumin level (HR=0.95; CI: 0.92-0.97; P<0.001), increased BMI (HR=0.79; CI: 0.66-0.96; P<0.05), and increased FIM (HR=0.99; CI: 0.98-0.99; P<0.001) decreased risk of death. Impaired cognitive status was not associated with increased mortality.

Conclusion: Impaired cognitive status has no influence on survival after controlling for major confounding factors in frail Chinese older adults.

Is residency in nursing home an independent risk factor for mortality in Chinese older adults?

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Background: Different studies suggested that nursing home older adults have a higher mortality than older adults living at home. However, there is little research investigating whether residency in nursing home is an independent risk factor for mortality.

Methods: This was a retrospective cohort study among patients discharged from Geriatric Day Hospital (GDH) of Fung Yiu King Hospital from 2004 to 2012. Data on residency (nursing home versus home) upon discharge from GDH were retrieved. Other data retrieved included age, gender, co-morbidity quantified by Charlson comorbidity index, functional status quantified by functional independence measure, cognitive status by Mini-Mental State Examination, nutritional status by body mass index. Outcome measure was 1-year mortality. Any patient who had changed his or her place of residency during follow-up was regarded as censored during analysis.

Results: A total of 1642 patients (976 women, 666 men; mean age, 80.3±7.2 years) were included; 1035 (63%) and 607 (37%) patients lived in home and nursing home, respectively. At 1-year follow up, 13.6% patients lived in nursing home and 5.8% patients lived in home died (P<0.001). But residency in nursing home was found not to be an independent risk factor for death after multivariate analysis with consideration of other confounding factors (hazard ratio=1.28; confidence interval, 0.61-2.69; P=0.51).

Conclusion: Nursing home older adults have a higher mortality likely because of their underlying worse functional status, worse nutritional status, and more co-morbidity. Residency in nursing home is not an independent risk factor for mortality in Chinese older adults.

Glomerular filtration rate estimated by different equations in Chinese older adults and its importance in medication prescription

6

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Background: Currently, many drug prescription guidelines are dependent on estimated glomerular filtration rate (eGFR) by Cockcroft-Gault (CG) equation. We aimed to study the relationship between serum creatinine level and eGFR by different equations.

Methods: This was a retrospective cross-sectional study among out-patients followed by the Geriatric Day Hospital (GDH) of Fung Yiu King Hospital from 2004 to 2012. Data on the serum creatinine levels, age, and body mass index were retrieved. eGFR was calculated by Modification of Diet in Renal Disease Study (Chinese-adjusted) [MDRD] equation and CG equation.

Results: A total of 1341 GDH patients (810 women, 531 men; mean age, 80.1±7.3 years) were included—17.3% of them had serum creatinine levels above upper limit; 30.4% and 99.1% of them had eGFR of less than 60 mL/min/kg estimated by MDRD equation and CG equation respectively; 4.6% and 62.3% of them had eGFR of less than 30 mL/min/kg estimated by MDRD equation and CG equation respectively. GFRs estimated by MDRD equation were consistently higher than GFR estimated by CG equation. The mean difference of GFR (GFR-diff) was 47.1±23.0. The GFR-diff increased with decreasing body mass index (BMI) of patients (adjusted Pearson coefficient: -0.35; P<0.001). There was no correlation between GFR-diff and age.

Conclusion: Underdetection of renal impairment in older adults is possible if we depend on serum creatinine level alone. However, the MDRD equation may also overestimate GFR in older adults, especially in those with lower BMI.

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PR prolongation strongly predicts new-onset myocardial infarction, ischaemic stroke, heart failure, and cardiovascular death in coronary patients or risk equivalent: a 5-year clinical-pathophysiological study

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Objectives: To investigate the role of PR prolongation in new-onset adverse cardiovascular (CV) events in high-risk CV patients, and the underlying pathophysiological mechanisms in terms of vascular phenotypes.

Methods: We prospectively followed up 597 high-risk CV out-patients (mean age 66±11 years; male 67%; coronary disease 55%, stroke 22%, diabetes 52%) for new-onset ischaemic stroke, myocardial infarction (MI), congestive heart failure (CHF), and CV death. Vascular phenotypes were assessed by high-resolution ultrasound for mean carotid intima-media thickness (IMT), and Vascular Profiling System (VP-2000; subgroup n=338) for pulse wave velocity (PWV). Impaired left ventricular ejection fraction (LVEF) from transthoracic echocardiography (subgroup, n=194) was defined as <35%. PR interval was determined from 12-lead electrocardiogram.

Results: PR prolongation >200 ms was present in 79 (13%) patients at baseline, and was associated with a higher mean carotid IMT (1.05±0.37 mm vs 0.94±0.28 mm, P=0.010), higher PWV (1144±142 cm/s vs 1091±143 cm/s, P=0.024), and impaired LVEF of <35% (16% vs 5%, P=0.027). Adjusted for potential confounders, PR prolongation was independently associated with increased carotid IMT by +0.073 mm (95% confidence interval [CI]: 0.003-0.143, P=0.041). After a mean follow-up of 63±11 months, increased PR interval significantly predicted new-onset ischaemic stroke (P=0.006), CHF (P=0.040), CV death (P<0.001), and combined CV endpoints (P<0.001) at cut-off >200 ms, and new-onset MI at >162 ms (P=0.008) [C-statistic 0.70, P=0.001], with K-M analyses showing significantly reduced event-free survival (all P<0.05). Adjusting for potential confounders by multivariable cox regression, PR prolongation independently predicted increased risk of new-onset ischaemic stroke (hazard ratio [HR]=5.1, 95% CI: 1.3-19.1, P=0.017), CV death (HR=16.4, 95% CI: 4.0-67.5, P<0.001), combined CV endpoints (HR=2.3, 95% CI: 1.3-4.3, P=0.007) at cut-off >200 ms, and increased new-onset MI (HR=8.1, 95% CI: 1.7-39.1, P=0.010) at cut-off >162 ms.

Conclusions: PR prolongation of >200 ms strongly predicts new-onset ischaemic stroke, MI, and CV death, and combined CV endpoint including CHF in high-risk CV patients. Increased risk of MI was observed at >162 ms. Abnormal vascular function may represent intermediate phenotypes or a mediating mechanism to clinical events.

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First year of 24/7 Acute Stroke Unit. Part 2: Outcome of stroke thrombolysis using telemedicine

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Objective: To evaluate the safety and efficacy of intravenous recombinant tissue plasminogen activator (IV-rtPA) for acute ischaemic stroke through telemedicine consultation.

Methods: Records of stroke patients treated with IV-rtPA over the first 12 months of 24/7 thrombolytic service were reviewed. Outcomes of patients treated outside and during office hours, with the former through telemedicine consultation, were compared.

Results: Of the 45 patients given IV-rtPA, 25 (55.6%) cases were treated during non-office hours and 20 (44.4%) cases during office hours by the on-site neurologists, with mean door-to-needle time of 102 minutes in the former and 98 minutes in the latter (P=0.64). The number of symptomatic intracranial haemorrhage and other complications in each group of patients was 3 or 4 (12% and 20%, P=0.68), respectively. Median modified-Rankin score (mRS) was 3 in patients treated during non-office hours with 28% achieving functional independence. There was no significant difference as compared to those treated during office hours (median mRS=3.5, P=0.3; proportion achieving functional independence=45%, P=0.35). The mortality rates in the two groups were 20% and 10% (P=0.44), respectively.

Conclusion: Rapidity, safety, and outcome of IV-rtPA patients treated after office hours through telemedicine consultation are comparable to those managed during office hours by an on-site neurologist. Our data support that the current 24/7 IV-rtPA protocol is a feasible model for the effective administration of thrombolytic therapy to acute stroke patients.

Exchange protein directly activated by cAMP 1 is involved in beta3-adrenergic induction of brown adipose tissue in white adipose tissue

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Introduction: Exchange protein directly activated by cAMP (Epac1, Epac2a and Epac2b) were identified as cAMP-regulated guanine nucleotide exchange factors. Previously Epac1-deficient mice were shown to have slightly heavier body weight, higher respiratory exchange ratio, and develop more severe high-fat-diet–induced obesity and hyperglycaemia than the wild type mice, suggesting the role of Epac1 in energy expenditure and lipid metabolism. The beta3-adrenergic signalling in white adipocytes is reported to be important in lipid metabolism and energy homeostasis by induction of brown adipose tissue (BAT) in white adipose tissue (WAT).

Methods: To investigate the role of Epac1 in beta3-adrenergic induction of BAT in WAT, a beta3-adrenergic receptor agonist (CL 316,243, CL) or saline was administrated to wild type ($Epac1^{+/+}$) and homozygous Epac1 knockout ($Epac1^{-/-}$) mice. Peri-uterine WAT was collected for histology, immunocytochemistry, Western blots, and real time quantitative polymerase chain reaction (qPCR). Lipolytic activity was determined by glycerol released from WAT explants with or without CL treatment.

Results: After CL (1 mg/1 kg body weight/day, i.p.) treatment for 10 days, peri-uterine WAT exhibited a BAT-like phenotype with smaller eosinophilic adipocytes with multilocular lipid droplets in both $Epac1^{+/+}$ and $Epac1^{-/-}$ mice. WAT of CL-treated $Epac1^{-/-}$ mice showed less typical BAT morphology compared to that of CL-treated $Epac1^{+/+}$ mice. In addition, CL-induced up-regulation of uncoupled protein 1 (UCP1) seen in WAT of $Epac1^{+/+}$ mice was not observed in $Epac1^{-/-}$ by immunocytochemical, Western blot and real-time qPCR analyses. Inductions of other genes shown to be critical for thermogenesis (Cidea, PGC1 alpha) and adipocyte differentiation (CEBP alpha and PPAR gamma) with CL treatment were also not observed in WAT of CL-treated $Epac1^{-/-}$ mice by real-time qPCR analysis. Concomitantly, Epac1 expression was increased in WAT of $Epac1^{+/+}$ mice after CL treatment, whereas no Epac1 and no compensation of Epac2 expressions were observed in $Epac1^{-/-}$ WAT. Interestingly, CL-induced lipolysis is increased in a dose- and time-dependent manner but it is significantly less from $Epac1^{-/-}$ compared to $Epac1^{+/+}$.

Conclusion: Epac1-mediated lipolytic activity may play an important role in beta3 adrenergic induction of UCP1 in WAT.

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APPL1 regulates insulin secretion in pancreatic beta cells

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Introduction: Pancreatic beta cell dysfunction, characterised by defective glucose-stimulated insulin secretion, is a major contributor to the progression of type 2 diabetes. APPL1 has been suggested as an insulin-sensitising molecule in various insulin-responsive tissues. In this study, we aimed to investigate whether APPL1 regulates beta cell functions using genetically engineered mouse models.

Methods: APPL1 knockout and transgenic mice and their wild-type littermates were fed with standard chow or high-fat diet for a period of 24 weeks. Basic metabolic parameters and beta cell functions were examined in the above animals.

Results: In dietary obese and genetically inherited diabetic mice, expression of APPL1 in pancreatic islets is dramatically decreased, which is associated with defective insulin secretion. Genetic deletion of APPL1 leads to glucose intolerance and impaired insulin secretion, the latter due to defective exocytosis of insulin granules. By contrast, transgenic expression of APPL1 protects mice from dietary-induced glucose intolerance partly by enhancing insulin secretory capacity. Ex-vivo analysis revealed that APPL1-deficient islets exhibit a significant reduction of number of docked insulin granules accompanied with decreased exocytosis of insulin as a result of significantly decreased expression of SNARE proteins, the core components of the exocytotic machinery of eukaryotic cells. In molecular level, APPL1 enhances insulin-mediated Akt activation by suppressing the binding of Akt to TRB3, thereby up-regulating expression level of SNARE protein and insulin secretion in pancreatic beta cells.

Conclusion: APPL1 integrates the effects of insulin in peripheral target tissues and pancreatic beta cells to maintain glucose homeostasis in the body.

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Serum beta-2 microglobulin predicts mortality in patients with diabetes

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Introduction: Predictor of mortality in diabetes is lacking. We aimed to evaluate the relationship between beta-2 microglobulin (B2M) and mortality in participants with diabetes.

Methods: A total of 897 diabetes participants of the Third National Health and Nutrition Examination Survey were included in the analysis. Serum B2M level was used in multivariate Cox regression analysis to predict all-cause and cardiovascular mortality.

Results: During a median follow-up of 11.8 years (range, 0.1-18.2 years) and 9222 person-years, 207 (16.8%) and 542 (42.4%) participants died from diabetes causes and all causes, respectively. Tertile 3 of B2M was significantly associated with all-cause mortality (hazard ratio [HR]=3.28, 95% confidence interval: 1.54-7.00) and diabetes mortality (HR=4.03, 95% CI: 1.20-13.59).

Conclusions: Serum B2M level is a novel independent predictor of diabetes and all-cause mortality in participants with diabetes.

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High ferritin and low transferrin saturation are associated with pre-diabetes among adults

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Introduction: Iron overload is known to cause diabetes. However, the underlying mechanism is poorly understood. We therefore studied the association of different markers of iron metabolism, namely ferritin, erythrocyte protoporphyrin and transferrin saturation (TSAT, as defined by a percentage of transferrin that is saturated with iron) with pre-diabetes (preDM) in US adults without chronic kidney disease, anaemia, and iron deficiency.

Methods: Data on 2575 participants of the National Health and Nutrition Examination Survey (NHANES) 1999-2002 who were free of diabetes, chronic kidney disease, iron deficiency, and anaemia were analysed. Data on 3876 participants of the NHANES III (1988-1994) were used as replication. Homeostasis model assessment of insulin resistance (HOMA-IR), blood glycosylated haemoglobin level (HbA1C), fasting glucose, insulin, and preDM (defined as a fasting plasma glucose level of 100-125 mg/dL or an HbA1C value of 5.7-6.4%) were measured as the outcomes.

Results: Logistic regression analyses indicated independent associations of high ferritin (Ptrend=0.028) and low TSAT (Ptrend=0.029) with preDM after multivariable adjustment. The NHANES III data showed similar associations. Combining the results showed a more significant association for high ferritin (Pmeta=0.016) and low TSAT (Pmeta=0.002). Moreover, TSAT was associated with HbA1C, fasting glucose, insulin, and HOMA-IR (Pmeta ≤0.001).

Conclusions: Higher ferritin and lower TSAT are associated with higher risk of preDM in a general population without confounding diseases. Further research is needed to examine the underlying mechanism of these two indices, especially TSAT, in the pathophysiology of preDM.

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Serum beta-trace protein is independently associated with metabolic syndrome in US adults

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Introduction: β-Trace protein (BTP) is a novel renal marker. It is belonged to the lipocalin protein family that binds and transports lipophilic biomolecules. BTP converts prostaglandin (PG)H2 to PGD2. It is subsequently metabolised into PGJ2, which is a potent peroxisome proliferator activated receptor (PPAR) ligand. However, the role of BTP in humans has not been clearly defined.

Objectives: To study the relationship of serum BTP with diabetes and metabolic syndrome (MetS) in the US population.

Methods: Data on 3136 participants aged ≥20 years (50.1% women) of the National Health and Nutrition Examination Survey (NHANES) III were examined. Logistic regression was used to assess the association between BTP and diabetes and MetS.

Results: In multivariable logistic regression analysis, BTP levels in quartile 1 were significantly associated with diabetes (odds ratio [OR]=2.61; 95% confidence interval [CI], 1.34-5.09; Ptrend=0.013) when compared with quartile 4. BTP levels in quartile 1 were significantly associated with MetS, when compared with quartile 3 (OR=2.54; 95% CI, 1.47-4.41) and quartile 4 (OR=1.76; 95% CI, 1.02-3.03) [Ptrend=0.009]. Further adjustment of estimated glomerular filtration rate attenuated the association with diabetes but not MetS. The associations of serum BTP with diabetes or MetS were significantly modified by age, body mass index, and smoking status.

Conclusions: A reduced serum BTP level is independently associated with MetS in humans. Our findings raise the possibility that BTP can be used as a biomarker or even a new therapeutic target for MetS.

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Serum beta-2 microglobulin concentration predicts cardiovascular and all-cause mortality

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Introduction: Beta-2-microglobulin (B2M) is expressed in all nucleated cells and shed from cell surfaces. Serum B2M level predicts mortality in multiple myeloma and chronic kidney disease. We hypothesised that it would predict cardiovascular and all-cause mortality in the general population.

Methods: A total of 6609 adult participants of the Third National Health and Nutrition Examination Survey were included in the analysis. Serum B2M level was used in multivariate Cox regression analysis to predict all-cause and cardiovascular mortality. Reclassification of mortality was assessed using integrative discrimination index (IDI) and category-less net reclassification improvement (NRI).

Results: During a median follow-up of 13.5 years (80 039 person-years), 2559 and 1165 participants died from all causes and cardiovascular causes, respectively. Serum B2M level increased with cardiometabolic and inflammatory risk factors. In unadjusted analysis, quartile 4 of B2M was significantly associated with all-cause (hazard ratio [HR]=21.51, 95% CI: 14.84-31.16) and cardiovascular mortality (HR=30.06, 95% CI: 16.31-55.4). After multivariate adjustment, the HRs remained significant (2.43, 95% CI: 1.75-3.37, for all-cause mortality and 2.01, 95% CI: 1.09-3.72, for cardiovascular mortality). Similar results were obtained in the subgroup with normal estimated glomerular filtration rate level. The area under receiver operating characteristics curve of B2M was 0.806 and 0.766 for all-cause and cardiovascular mortality, respectively. Serum B2M, when added to Framingham Risk Score, showed significant reclassification in terms of IDI and category-less NRI.

Conclusion: Serum B2M level is a powerful independent predictor of cardiovascular and all-cause mortality in the general population.

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Whole genome sequencing of NK leukaemia

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Introduction: Natural killer (NK)–cell leukaemia is common in Asia and South America populations. This is a fatal disease with the patients' median survival time of around 2 months. Little is known about the molecular characteristics and pathogenetic mechanisms of the illness. The aim of this study was to identify novel pathogenic mutations in NK bone marrow by whole genome sequencing.

Methods: An adult NK leukaemia patient bone marrow sample sorted with CD56+ together with the skin biopsy were captured for whole genome sequencing. The somatic sequence changes identified will be further validated in other NK bone marrow samples by Sanger sequencing.

Results: The whole genome in both samples has an average coverage at 40X. A total of 42 single-nucleotide variations (SNVs) were identified in the NK bone marrow sample but absent in the skin biopsy. However, no recurrent SNVs were identified in three other NK bone marrow samples.

Conclusion: The identified SNVs need to be further validated with an increased number of NK bone marrow samples.

The psychosocial burden of psoriasis and barriers to biologic therapy in Hong Kong: patients' perspectives

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Introduction: Psoriatic skin lesions are often visible and have negative impact on patients' quality of life (QoL). The introduction of biologic therapies has revolutionised the treatment of psoriasis. They are highly efficacious in clearing skin lesions and relieving joint symptoms. The use of biologics in psoriasis patients is associated with improved QoL and patient satisfaction. In Hong Kong, the psychosocial impacts of psoriasis on patients and their satisfaction towards various treatment modalities have not been well studied. Moreover, the ways local patients come to access the information on biologics and their concerns are unclear.

Methods: We conducted a survey of 85 psoriasis patients from a patient self-help group to discern the disease impacts on QoL and patient attitudes towards biologics. The patients' self-perceived disease severity and the disease impacts on physical, psychological, and social well-beings were assessed by a 10-point scale (1=minimal; 10=maximal).

Results: The results show that 29% of patients (n=25) had mild (body surface area [BSA] <3%), 36% (n=31) had moderate (BSA 3-10%), and 34% (n=29) had severe (BSA >10%) psoriasis. The male-to-female ratio was 1.4:1. The mean age was 52.6±13.6 years. The median duration of disease was 8 years (3-13 years). The self-perceived disease severity was 7.68±2.70. Patients with moderate-to-severe psoriasis was associated with significantly higher self-perceived disease severity ($P<0.01$). The perceived disease impacts on six thematic issues were analysed: emotion (7.36±2.74); social life (6.92±2.81); general physical health (6.76±2.76); economy (5.67±3.20); job opportunity and work (5.61±3.30); and family life (4.76±2.64). Nearly half (48%) of patients was unsatisfied with current treatments. The main reasons were lack of treatment efficacy (58%), side-effects (29%), and high cost (20%). Only six (7%) patients had received biologic therapies; 46% of patients had never been informed or aware of biologic treatments in psoriasis. Most patients learned biologics through media (78%), medical personnel (20%), self-help groups (13%), and internet (11%).

Conclusion: Psoriasis has negative impacts on patients' QoL, especially on psychosocial aspects. Majority of patients were unsatisfied with current treatments due to suboptimal disease control. Biologic therapy is underutilised in management of skin psoriasis and there is a lack of public awareness of such treatment modality. Medical professional has a potential pivot role in patient education of biologic therapy.

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Relevance of short-range connectivity to brain compensation and cognitive efficiency in healthy and pathological ageing: a combined functional magnetic resonance imaging and tractography study on prospective memory

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Introduction: Cognition and its efficiency are related to the activities of specific brain regions and their interactions. The brain function and structure are vulnerable to both healthy and pathological ageing, and these processes may underlie the impaired cognitive functions in daily life.

Methods: In this functional magnetic resonance imaging (fMRI) study on prospective memory, fMRI and diffusion tensor imaging were used to investigate the neural mechanism underlying prospective memory (PM) impairment in ageing and Alzheimer's disease (AD). Three groups of young, older adults, and patients with AD were recruited. The fMRI data were analysed with SPM8.

Results: Increased brain activations were found in healthy older adults and patients with AD. Furthermore, the PM-specific frontal activations in three groups were found distributed along the rostrocaudal axis in the frontal lobe. These findings indicate that subjects with different cognitive capacity may encounter different levels of conflict during the PM task and they may need different strategies to deal with the task. Analysis on structural connectivity of the brain network revealed that the short-range fibres were impaired in both healthy and pathological ageing. This may underlie their low cognitive efficiency and higher compensatory brain activation during PM task performance. In contrast, the long-range fibre tracts are only impaired in AD. It may be related to the slightly lower PM accuracy in AD patients.

Conclusion: For the first time, this combined fMRI and tractography study showed complementary evidence that the compensative brain activations of healthy and pathological ageing may be more related to the impairment of short-range fibre tracts and regional disconnectivity.

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Assessment of autonomic function in patients with a history of neuropsychiatric lupus

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Introduction: Neurological involvement is common in patients with systemic lupus erythematosus (SLE) and can involve both central and peripheral nervous systems. We hypothesise that patients with neuropsychiatric involvement (NPSLE) may be more prone to autonomic dysfunction as a result of focal insult to structures of the central autonomic network. We aimed to study if autonomic dysfunction is more common in NPSLE patients compared to those without NPSLE.

Methods: NPSLE patients with a history of acute confusional state, seizure, stroke and transverse myelitis and age- and sex-matched non-NPSLE patients and healthy subjects were recruited. Symptoms of autonomic dysfunction were screened by questionnaire. Standardised tests on sympathetic and parasympathetic functions of the cardiovascular system were performed.

Results: Autonomic symptoms were reported in 96.6% of all SLE patients (29 NPSLE and 29 non-NPSLE). Although SLE patients, in particular NPSLE patients, were found to have impaired heart rate response to active standing (ratio, 30:15) compared to healthy controls (n=16) [NPSLE 1.13±0.11, non-NPSLE 1.16±0.11, and controls 1.26±0.11; P=0.006 by Kruskal-Wallis test), no difference was observed between SLE and controls and between NPSLE and non-NPSLE patients regarding autonomic function according to Ewing's criteria.

Conclusions: NPSLE patients were found not to have higher autonomic dysfunction compared to non-NPSLE patients and healthy subjects.

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Acquired FMS-like tyrosine kinase 3 internal tandem duplication (FLT3 ITD) during leukaemic transformation from underlying myelodysplasia: successful rescue with sorafenib

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Introduction: Identifying molecular targets during leukaemic progression may provide new treatment options for patients with secondary acute myeloid leukaemia (sAML) from antecedent myelodysplastic syndrome (MDS). In this study, the mutational status of a gene encoding for FMS-like tyrosine kinase 3 (FLT3) for MDS and leukaemic transformation as well as the clinical response to the FLT3 inhibitor sorafenib were evaluated.

Methods: Eighty patients with MDS according to the World Health Organization classification were prospectively evaluated. Patients who subsequently developed sAML were identified. Bone marrow (BM) samples at the diagnosis of MDS and sAML were collected and genomic DNA (gDNA) was extracted. Internal tandem duplication (ITD) of FLT3 at the juxtamembrane domain was identified by polymerase chain reaction (PCR). To increase the sensitivity so that small MDS clones with FLT3-ITD could be detectable, an ITD-specific PCR was designed. Patients with FLT3-ITD + sAML were treated with sorafenib at 400 mg twice daily. Bone marrow aspiration and biopsy was performed at 3 weeks following treatment.

Results: Between August 2009 and August 2012, 12 patients with sAML from antecedent MDS were identified, of whom four were FLT3-ITD + sAML. ITD-specific PCR performed on the marrow/blood samples collected at the MDS phase before leukaemic transformation was negative in all cases. All four patients received sorafenib 400 mg twice daily, of whom three achieved complete remission (CR) 3 weeks after treatment. There was no overt dysplasia at morphological remission. Two of these patients underwent subsequent allogeneic haematopoietic stem cell transplantation (HSCT) and had remained in remission. The third patient received maintenance azacitidine. The fourth patient achieved significant blast count reduction and subsequently received maintenance treatment with azacytidine.

Conclusion: Our observation showed that acquisition of FLT3-ITD is associated with leukaemic progression in MDS, and the use of FLT3 inhibitor effectively induced remission that could be further consolidated with allogeneic HSCT or demethylating agents. The study of such patients provides an opportunity whereby MDS clonal heterogeneity and evolution during leukaemic transformation can be biologically evaluated.

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Relapsed acute promyelocytic leukaemia with central nervous system involvement: a 12-year prospective follow-up study on 169 patients treated with oral arsenic trioxide

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Introduction: Multiple relapses and central nervous system (CNS) involvement are associated with a poor outcome in patients with acute promyelocytic leukaemia (APL). There is a paucity of such data in the era of arsenic trioxide (As_2O_3).

Methods: We prospectively followed 169 patients treated in our unit with oral As_2O_3 between year 2000 and 2012. Patients with relapses and CNS involvement were identified. Clinicopathologic features were summarised and risk factors for relapse and CNS involvement were determined. Prognostic factors for overall survival (OS) and relapse-free survival were also determined.

Results: A total of 79 patients had one or more relapses. Non-As_2O_3–based maintenance regimens at first complete remission and high peak white blood cell (WBC) count were independently associated with increased risk of relapse (P<0.001 and P=0.008, respectively). Three different patterns for relapse were compared: medullary relapses (n=65), medullary and CNS relapse (n=8), and isolated CNS relapse (n=6). High WBC count on presentation was associated with a higher risk of subsequent isolated CNS relapse (P=0.007). Patients with two or more relapses also had a higher risk of concurrent medullary and CNS relapse as well as isolated CNS relapse (P=0.003). Relapse while on arsenic maintenance was also associated with CNS involvement at relapse (P=0.001). Relapse while on oral As_2O_3 was also associated with a worse OS (risk ratio [RR]=9.43, P<0.001, 95% confidence interval [CI]: 4.01-21.32). Concurrent bone marrow and CNS relapse was associated with the worst survival (RR=22.84, P<0.001, 95% CI: 5.88-88.74). Patients with three or more relapses had the worst OS (RR=16.45, P<0.001, 95% CI: 4.49-60.34).

Conclusion: Multiple relapses, high WBC count, and relapse while on oral As_2O_3 were associated with a higher risk of CNS involvement and in turn a worse OS. Identification of such risk factors is important in formulating effective treatment and prophylactic protocols for high-risk APL patients.

Myelodysplastic syndromes: a 10-year follow-up study

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Introduction: Myelodysplastic syndromes (MDS) are a group of heterogenous clonal disorders characterised by ineffective haematopoiesis, cytopenia and the propensity to leukaemic transformation. Risk stratification of patients with MDS is essential when considering various treatment options such as demethylating agents or haematopoietic stem cell transplantation (HSCT).

Methods: A total of 229 patients with MDS were followed between 2002 and 2012. Clinicopathologic characteristics, cytogenetics, transformation to acute myeloid leukaemia (AML) and overall survival (OS) were determined.

Results: The median international prognostic scoring system (IPSS) score and the revised IPSS (R-IPSS) score was 1 (range, 0-3) and 4 (1-8.5), respectively; 58 patients (25.3%) transformed into secondary AML. The 5-year OS was 27% (95% confidence interval, 60-97 months). Refractory anaemia with excess blasts-1 (RAEB-1), RAEB-2, refractory cytopenia with multilineage dysplasia (RCMD), and therapy-related MDS were significantly associated with a worse OS (P<0.001). Other factors associated with worse OS were intermediate and high-risk cytogenetics by IPSS (P<0.001) and secondary AML (P<0.001). The use of demethylating agents was associated with a better progression-free survival (P=0.012) but not OS. The use of HSCT was associated with a superior OS (P<0.001).

Conclusion: High-grade MDS, therapy-related MDS, and intermediate/high-risk karyotype were best predictors of worse OS in patients with MDS.

Whole genome sequencing on donor cell leukaemia in a patient with multiple myeloma identified gene mutations that may provide insights to leukaemogenesis

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Introduction: Donor cell leukaemia (DCL) is leukaemia referring to donor origin in patients who have received allogeneic haematopoietic stem cell (HSC) transplantation. We have presented before a patient who has myeloma and developed subsequent DCL. We hypothesised the comparison between donor HSC before transplantation and subsequent relapse acute myeloid leukaemia (AML) on genomic level, and will provide insights on the pathogenesis of AML.

Methods: DNA was extracted from donor mobilised peripheral blood mononuclear cells (mPBMNC) frozen before transplantation and unfractionated and $CD34^+$ myeloblasts of the patient's bone marrow at diagnosis and subsequent relapse of AML. Whole genome sequencing (~40X) was performed on DNA from donor mPBMNC and relapse AML. Single nucleotide variants (SNVs) and insertion and deletion (indels) were detected in bioinformatics analysis. Sanger sequencing was used to validate the mutations found by bioinformatics analysis. Gene expression was checked on cDNA made from RNA extracted from patient MNC at relapse AML by conventional or quantitative polymerase chain reaction.

Results: A total of 128 752 and 56 330 SNVs and indels were detected exclusively in relapse DCL. Mutations outside the gene region, within introns, non-pathogenic SNP and synonymous mutations were filtered. 142 Non-synonymous SNVs were identified, 25 were considered as statistically highly confident and 17 of them could be confirmed by Sanger sequencing in the relapse DCL sample. Twelve were also identified from the diagnostic DCL sample and six of them have gene expression in relapse DCL. These candidates include ion channel protein (*ACCN5*), cytoskeleton protein (*MYH10*), ribonucleoprotein (*RAVER1*), secreted protein (*WNT7A*), protein involved in DNA damage repair (*APLF*), and pre-mRNA splicing complex (*PRPF8*). Twenty-six indels were identified in the coding region, of which five were considered as statistically highly confident, however, only XBP1 could be confirmed by Sanger sequencing in the relapse sample and was not present in the diagnostic sample.

Conclusions: The whole genome sequencing performed in paired pre-leukaemic (donor HSC) and leukaemic (DCL) human samples in this study has provided us a platform to dissect the genetic changes in HSC that may contribute to the initiation of AML with complex karyotype.

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Anti-dsDNA antibodies induce cell activation and fibrotic processes in human proximal tubular epithelial cells

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Introduction: Anti-dsDNA antibodies are implicated in the pathogenesis of lupus nephritis. We investigated whether anti-dsDNA antibodies can contribute to epithelial-to-mesenchymal transition (EMT) and fibrotic processes in human renal proximal tubular epithelial cells (PTEC).

Methods: Primary human PTEC were incubated with human polyclonal anti-dsDNA antibodies or control immunoglubolin G for 24 hours. Markers of cell activation and fibrogenesis, and activation of signalling pathways were assessed by Western blot analysis. Renal specimens obtained from NZBWF1/J mice at different stages of disease were assessed for the same markers using immunohistochemical staining.

Results: Anti-dsDNA antibodies induced fibroblast specific protein-1 (FSP-1), fibronectin and laminin expression after 24-hour incubation, which was accompanied by MAPK activation. Renal specimens from lupus-prone mice showed increased expression of FSP-1, fibronectin, and laminin with increasing severity of disease.

Conclusion: Anti-dsDNA antibodies can induce EMT and fibrogenesis in human PTEC through the activation of MAPK signalling pathway, which may contribute to renal fibrosis in patients with lupus nephritis.

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Pharmacological intervention of adipocyte fatty acid binding protein alleviates both endotoxin-induced acute liver injury and non-alcoholic steatohepatitis in mice

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Introduction: Adipocyte fatty acid binding protein (A-FABP) is a key mediator of inflammatory response in macrophages. Elevated hepatic expression and circulating levels of A-FABP have been observed in patients with non-alcoholic fatty liver disease (NAFLD). Here, we investigated the role of A-FABP in lipopolysaccaride (LPS)–induced acute liver injury and high-fat-high-cholesterol (HFHC) diet–induced NAFLD in mice.

Methods: Mice were injected with LPS to induce acute liver injury or fed with HFHC diet to induce NAFLD. After treatment with the A-FABP inhibitor BMS309403, the liver tissues of the mice were analysed by immunohistological chemistry, Western blot or real time polymerase chain reaction.

Results: A-FABP expression in hepatic macrophages was significantly elevated in mice with LPS-induced acute liver injury and HFHC diet–induced obese mice as compared to their healthy controls. Pretreatment with the A-FABP inhibitor BMS309403 led to a reduced LPS-induced elevation in serum levels of alanine transaminase, and reduced hepatic production of pro-inflammatory cytokines. Chronic treatment of HFHC diet–induced obese mice with BMS309403 ameliorated hepatic steatosis, macrophage infiltration, and cellular ballooning of hepatocytes. Such improvements in liver function and morphology by BMS309403 were accompanied by significantly decreased activation of both C-Jun N-terminal kinase and nuclear factor–kappa B. Furthermore, pretreatment with BMS309403 suppressed both LPS- and palmitate-induced pro-inflammatory responses in isolated rat Kupffer cells, but not in hepatocytes. On the other hand, adenovirus-mediated ectopic expression of A-FABP alone was sufficient to induce liver injury and inflammation in mice.

Conclusions: These findings suggest that A-FABP is an important contributor to both LPS-induced acute liver injury and diet-induced NAFLD by potentiating inflammation in Kupffer cells. Pharmacological inhibition of A-FABP may represent a promising modality for obesity-related non-alcoholic steatohepatitis.

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Genetic analysis of occult hepatitis B virus infection

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Introduction: Occult hepatitis B infection (OBI) is characterised by absence of serum HBsAg and persistence of hepatitis B virus (HBV)–DNA in serum or liver. The HBV-DNA levels are usually extremely low. The mechanisms underlying OBI remain to be clarified. To determine if specific sequence variations of HBV genome may be associated with OBI, we used rolling circle amplification (RCA) method to overcome the difficulties in amplification of the full-length HBV genome (3.25 kb) from occult samples.

Methods: A total of 185 serum samples and 60 liver biopsies from subjects with OBI were analysed. Ten serum samples from subjects with overt HBV infection were used as controls.

Results: Twenty-two full-length HBV genomes were amplified from subjects with OBI and 10 from overt samples. The full-length HBV DNA was sequenced and aligned. Generally, occult HBV cases showed higher genetic changes than overt controls and sequence variations led to coding amino acid changes occurs through the viral genomes. We found point mutations and deletions led to abolishment of PreS2 start codon (ATG) in four occult samples that disrupt PreS2 protein synthesis. In addition, mutations turned 16 amino acid codons to stop codons that resulted in early termination of viral protein synthesis in 11/22 (50%) occult samples and 2/10 (20%) overt samples. We next explored the effect of mutation unique to OBI by studying the level of transcription activity driven by preS1 and preS2/S promoters, and core promoter using luciferase assays. The promoter activity on preS1 and S constructs were relatively similar to overt controls. However, the transcription activity of core promoter constructs with mutation at nucleotide 1677 and 1726 where transcription factor binds was only 72% and 40% of the control overt constructs, respectively.

Conclusion: Although some unique genetic changes were only detected in occult HBV samples, the findings from this study do not readily distinguish occult HBV from overt HBV.

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Effects of functional transient receptor potential channels on proliferation and migration in human cardiac c-kit$^+$ cells

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Introduction: Human adult c-kit$^+$ cardiac stem cell are characterised by the expression of c-kit in the absence of lineage markers such as Nkx2.5. They are self-renewing, clonogenic and multipotent, giving rise to a minimum of three differentiated cell types: myocytes, smooth muscle, and endothelial vascular cells. These cells, although not specifically programmed for myocardial differentiation, have been shown to improve cardiac function in a myocardial injury/reconstitution assay. However, cell biology is not fully understood. The present study was to investigate the expression of transient receptor potential (TRP) channels in human cardiac c-kit$^+$ cells, and their role in regulating migration and proliferation.

Methods: Whole-cell patch voltage-clamp, RT-PCR, and Western blot approaches were used to determine functional expression of TRP channels in cultured human cardiac c-kit$^+$ cells. ShRNA targeting TRP channels were constructed to silence the related TRP channels. Wound healing and transwell assay were applied to observe the effect of the TRP channels on cell migration. Cell proliferation assay was made with MTT and ^{3}H-thymidine incorporation approaches.

Results: A small background current was inhibited by the TRPC channel blocker La^{3+}. Removal of Mg^{2+} of pipette solution or bath solution induced a Mg^{2+}-sensitive current, and the current was suppressed by the TRP channel blocker 2-aminoethoxydiphenyl borate. RT-PCR revealed significant mRNA expression of TRPC1, TRPC3, TRPC4, TRPV2, TRPV4, and TRPM7 channels in human preadipocytes. Western blot analysis confirmed the protein expression of these TRP channels. ShRNAs targeting TRPV2, TRPV4 and TRPM7 suppressed the corresponding gene and protein expression. Interestingly, TRPV2-shRNA and TRPM7-shRNA significantly reduced proliferation of human cardiac c-kit$^+$ cells. Migration of human cardiac c-kit$^+$ cells was reduced by TRPV2-shRNA, TRPV4-shRNA.

Conclusion: Our results demonstrate for the first time that multiple TRP channels, TPC1/3/4, TRPV1/2/4, and TRPM7 are present in human cardiac c-kit$^+$ cells. TRPV2, TRPV4, and TRPM7 channels participate in regulating migration and proliferation.

Dose sparing intradermal trivalent influenza (2010/2011) vaccination overcome reduced immunogenicity of the 2009 H1N1 strain*

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Background: We hypothesised that low-dose intradermal (ID) vaccination of the trivalent influenza vaccine (TIV) delivered by the MicronJet600™ (NanoPass Technologies, Israel) would be non-inferior to the full-dose intramuscular (IM) and mid-dose Intanza® vaccination in the elderly and the chronically ill adults.

Methods: We performed a prospective randomised trial on elderly and chronically ill adults. Subjects were randomly assigned into four groups. Groups ID3 and ID9 received reduced dose ID TIV (3 µg and 9 µg of haemagglutinin per strain respectively) delivered by MicronJet600™ (NanoPass Technologies, Israel). Group INT9 received reduced-dose ID TIV (9 µg) delivered by Becton Dickinson's Soluvia™ device (Intanza®9, Sanofi-Pasteur, France). Control group IM15 received a full-dose IM TIV (15 µg). We measured antibody titres by haemagglutination inhibition (HAI) and microneutralisation (MN) assays at baseline and day 21.

Results: Baseline characteristics for all groups were similar (group and sample sizes: ID3=63; ID9=68; INT9=65; IM15=66). At day 21 post-vaccination, the GMT ratio and the seroconversion rates difference for all three strains of the ID vaccine groups were non-inferior to the IM vaccine group. The seroconversion rate, seroprotection rate, and the GMT of the H1N1 strains by HAI and MN assays were significantly higher in the ID groups compared with the full-dose IM vaccine group. The seroconversion rates of the H3N2 strain by HAI assay were also significantly higher in the ID groups when compared with the full-dose IM group. Direct comparison among the three ID devices showed no significant differences. No serious adverse events related to vaccination were reported.

Conclusion: Dose-sparing ID TIV can overcome reduced immunogenicity of the H1N1 strain, and according to some measures, for the H3N2 strain. At-risk subjects indicated for the TIV should be considered for intradermal immunisation to compensate for reduced immunogenicity.

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Short-term influence of bariatric surgery on obesity-associated diabetes mellitus

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Introduction: Whilst bariatric surgery (BS) to combat obesity confers risks, it can also result in remission of diabetes mellitus (DM). Clinicians managing such patients should appreciate the relative and absolute extent of this effect. We therefore set out to derive the relative chance (RC) and number needed to treat (NNT) for such an effect.

Methods: Based on the results of two recently published, non-blinded, randomised clinical trials comparing DM remission rates in severely obese diabetic patients having BS versus conventional/intensive anti-diabetic therapy[1,2] as previously described,[3] we calculated respective unadjusted values for RC (equivalent to relative risk) and NNT.

Results: The patients in these trials did not suffer any serious short-term complications from their BS. Respective RC and NNT values and corresponding 95% confidence intervals (CIs) are shown in the Table.

Conclusion: In the short term (1 to 2 years), BS was a highly effective means of eliminating DM in these patients. The perceived success rate depended on the precise criteria used to define diabetes and the type of BS.

Trial (No. of patients)	Primary endpoint	Active treatment§	RC (95% CI)	NNT (95% CI)
Mingrone et al,[1] (n=60)*	HBA_1C <6.5% at 2 years‡	GB & PD	8.5 (2.0 to 36.4)	1.33 (1.08 to 1.74)
		GB	7.5 (1.7 to 32.8)	1.54 (1.13 to 2.41)
		BPD	9.5 (2.1 to 42.0)	1.18 (0.98 to 1.46)
Schauer et al,[2] (n=150)†	HBA_1C <6.0% at 1 year	GB & SG	3.2 (1.2 to 8.4)	3.68 (2.42 to 7.68)
		GB	3.4 (1.3 to 9.3)	3.36 (2.12 to 8.00)
		SG	3.0 (1.1 to 8.3)	4.08 (2.40 to 13.54)

* Assuming no DM in 2 controls lost to follow-up
† No intention-to-treat analysis
‡ With fasting glucose <100 mg/dL and no DM pharmacotherapy
§ GB denotes gastric bypass, BPD biliopancreatic diversion, and SG sleeve gastrectomy

References

1. Mingrone G, Panunzi S, De Gaetano A, et al. Bariatric surgery versus conventional medical therapy for type 2 diabetes. N Engl J Med 2012;366:1577-85.
2. Schauer PR, Kashyap SR, Wolski K, et al. Bariatric surgery versus intensive medical therapy in obese patients with diabetes. N Engl J Med 2012;366:1567-75.
3. Kumana CR, Cheung BM, Lauder IJ. Gauging the impact of statins using number needed to treat. JAMA 1999;282:1899-901.

First year of 24/7 Acute Stroke Unit. Part 1: eligibility and utilisation of intravenous thrombolysis

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Introduction: Intravenous recombinant tissue plasminogen activator (IV-rtPA) is the standard therapy for acute ischaemic stroke. Because of its narrow therapeutic time window, eligibility and utilisation rates of this treatment remained low. Our IV-rtPA programme was enhanced to a 24/7 protocol since September 2011.

Methods: Records of patients admitted to Acute Stroke Unit (ASU) over the first 12 months were reviewed for the utilisation pattern of IV-rtPA.

Results: A total of 447 patients were admitted to the ASU during the study period, in whom 384 (86%) were diagnosed to have stroke or transient ischaemic attack (TIA) upon discharge. Of these, 188 (49%) presented within 3 hours of symptom onset, in whom 122, 34, or 32 (65%, 18%, or 17%) had ischaemic stroke, TIA or intracranial haemorrhage, respectively. For the 122 ischaemic stroke patients, assessment for the suitability of IV-rtPA could be completed in 113 (93%) of them before the 3 hours therapeutic time-window expired. Sixty-six (58%) of them were considered not suitable candidates for intravenous thrombolysis because of presence of contra-indications. In the remaining 47 patients, IV-rtPA was given in 44 cases (94%, one additional case was treated with an extended therapeutic time-window of 4.5 hours).

Conclusion: The 24/7 ASU protocol enabled the timely assessment of acute stroke patients, with 93% having their essential workup completed within 3 hours of stroke onset, and a 94% utilisation rate of IV-rtPA in those who were eligible for treatment. With this model, the routine administration of thrombolytic therapy for acute ischaemic stroke can be facilitated.

Variants, clinical characteristics and prognostic factors of Guillain-Barré syndrome in Chinese

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Introduction: The variants, clinical characteristics, and prognostic factors of Guillain-Barré syndrome (GBS) in Hong Kong Chinese has not been widely studied previously.

Methods: We performed a retrospective review of adults with GBS admitted to Queen Mary Hospital, Hong Kong during the period 1997-2011.

Results: Mean age of the patients was 57±17 years and the mean length of hospital stay was 36±69 days. Male-to-female ratio was 1.5:1. Of the 63 patients with GBS during this period, 3 (4.8%) patients had acute motor axonal neuropathy, 11 (17.5%) with Miller Fisher syndrome and 50 (77.8%) had acute inflammatory demyelinating polyneuropathy together with other unspecified subtypes. Of the patients, 31 (49.2%) had preceding upper respiratory tract illness, 3 (4.76%) had preceding gastrointestinal illness, whilst 5 (7.94%) received vaccination during the 6 weeks preceding onset of neurological symptoms; 14 (22.2%) patients were admitted to Intensive Care Unit and 8 patients (12.7%) required mechanical ventilation; 12 (19.0%) patients were treated conservatively, 25 (39.7%) received intravenous immunoglobulin only, 19 (30.2%) received plasmapheresis only and 7 (11.0%) received both intravenous immunoglobulin and plasmapheresis. Of the patients, 36.1% of patients were associated with poor functional recovery (requiring walking with aid at 6 months after admission). Two (3.18%) patients died during hospital stay (one due to nosocomial pneumonia and the other due to cardiac arrhythmia). Multivariate analysis revealed that necessity of mechanical ventilation during hospitalisation (odds ratio=43.3; 95% confidence interval: 1.2-1539.4; P=0.039) was an independent predictor of poor functional recovery at 6 months after admission. Receiver operating characteristics curve analysis also showed that an Erasmus GBS Outcome Score of >4 was associated with good functional recovery with a c statistic of 0.87 (P<0.0001).

Conclusions: The proportion of patients with Miller Fisher syndrome is significantly greater in the Chinese population compared to the West. Necessity of mechanical ventilation during hospitalisation is an independent predictor of poor functional recovery 6 months after admission and the Erasmus GBS Outcome Score is a useful score in predicting functional recovery.

Soluble receptor for advanced glycation end-products in Chinese type 1 diabetic patients

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Objectives: The receptor for advanced glycation end-products (RAGE) plays an important role in the pathogenesis of diabetic complications. Interfering with the activation of RAGE by using a soluble form of the receptor (sRAGE) ameliorates the vascular complications of diabetes in animal models. sRAGE in the human circulation is partly derived from ectodomain shedding of the membrane-associated receptor. We have investigated the effect of insulin on the generation of sRAGE in vitro and evaluated serum levels of sRAGE in a group of Chinese type 1 diabetic subjects.

Methods: THP-1 cells were incubated with insulin in vitro and sRAGE in the medium measured by Western immunoblot. Cell-surface biotinylation and immunoprecipitation was performed to investigate ectodomain shedding. Serum level of sRAGE in diabetic patients was measured by ELISA.

Results: Insulin increased sRAGE in cell conditioned-media in a time- and dose-dependent manner. Pretreatment of THP-1 cells with GM6001, a general metalloproteinase inhibitor which blocked cellular shedding events, significantly reduced the production of sRAGE. This would suggest that the increase in sRAGE upon incubation with insulin was partly due to the shedding of sRAGE by cleavage of full-length cell surface RAGE. In type 1 diabetic patients (n=120), serum sRAGE was higher compared to a group of age-matched non-diabetic healthy subjects (1021.6 pg/mL [724.8-1212.4.0] vs 812.9 pg/mL [548.0-1202.2.5] respectively, P=0.004).

Conclusions: Chinese type 1 diabetic patients have higher serum levels of sRAGE and we have shown that insulin can stimulate the ectodomain shedding of cell surface RAGE.

Suppression of thymidylate synthase by arsenic trioxide in mesothelioma cell lines

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Introduction: Malignant pleural mesothelioma (MPM), mostly caused by asbestos exposure, has been a global health problem with high mortality and morbidity for the past decades. Multi-targeted anti-folate agent, which inhibits thymidylate synthase (TS), is now the cornerstone chemotherapy for MPM. Arsenic trioxide (ATO), an established treatment for acute promyelocytic leukaemia, has recently been shown to suppress TS in colorectal cancer. This study aimed to investigate the suppression of TS induced by ATO in mesothelioma cell lines.

Methods: Five mesothelioma cell lines (H28, 211H, H266, H2052, and H2452) were studied. Standard MTT assay was used to determine cell viability. Protein and mRNA expressions were analysed by Western blot and qPCR, respectively. TS activity was assayed using 2'-deoxycytidine [5-3H]. TS siRNA and TS plasmid was applied to knockdown and over-express TS protein expression, respectively.

Results: ATO reduced the viability of mesothelioma cell lines with IC50 values below clinically reachable concentration. After treating with ATO, the TS protein expressions in H28, H2052 and H2452 cells were suppressed, but otherwise up-regulated in H266 cells. The TS mRNA level was detectable and repressed in H28 cells only. The pRb gene expression was detectable and decreased in H28 cells only. The E2F-1 gene expression was suppressed in H28, H266 and H2052 cells, while remained unchanged in H2452 cells. Consequently, TS down-regulation was mediated through pRb E2F-1 pathway. Total TS activity was decreased but specific TS activity (per unit cell) was unchanged in H28 cells. Interestingly, total TS activity and specific TS activity were increased in H266 cells. The total TS activities and specific TS activities were inhibited in H2052 and H2452 cells. In H28 and H266 cells, knockdown of 90% TS protein expression caused 25% and 20% of cell death, respectively. In H2052 cells, knockdown of more than 95% TS protein expression did not cause significant cell death. TS over-expression increased ATO resistance in H28 cells.

Conclusion: Arsenic trioxide can suppress protein expression, mRNA expression and activity TS in mesothelioma cell lines, which partially accounts for cell death.

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35 Small molecule inhibitors screening platform for optimised design of personalised treatment for chemo-refractory acute myeloid leukaemia patients

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Introduction: Treatment failure for acute myeloid leukaemia (AML) is mainly attributable to the relapse and subsequent resistance after standard treatment. Relapsed leukaemia clones may survive on patient-specific multiple signalling pathways. We hypothesise that a personalised high throughput drug discovery platform may enable us to identify possible treatments at real time for these patients with hitherto dismal outcome.

Methods: A drug library comprising 25 therapeutic agents, mostly small molecules and kinase inhibitors and FDA approved, was used. They were plated into 96-well plates at 1 μmol/L either singly or in dual combinations, giving rise to 325 items in each screen. The screening assay included a 3-day culture of AML cells (at 10^6 cells/mL in IMDM with 10% FBS) from bone marrow (BM) or peripheral blood (PB) of AML samples. Drug effects on cellular proliferation were measured by the PrestoBlue® fluorometric assay.

Results: Validation of the platform to ensure its feasibility for clinical application has been done. The average turn-around time (sample-to-results) of the screen was within 7 days. There were significant correlations between experimental replicates (r=0.98), fluorescence intensities and the viable cell count after culture (r=0.86), fresh and thawed aliquot of the same leukemic sample (r=0.72) as well as BM and PB leukaemic cells of the same patient (r=0.79) [P<0.0001 for all]. Importantly, our preliminary data suggest a correlation between the effects of sorafenib (a multi-kinase inhibitor) on patients' leukaemic cells in vitro and the clinical response of these patients.

Conclusion: Our custom drug library screening platform would facilitate personalised treatment with targeted therapy for chemo-refractory AML patients.

36 Hepatitis B surface antigen level, viral load, and liver biochemistry after 5 years of continuous entecavir treatment in chronic hepatitis B patients

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Introduction: Entecavir is effective in suppressing hepatitis B (HBV) DNA level. The kinetics of hepatitis B surface antigen (HBsAg) level with long-term antiviral therapy is not well-defined.

Methods: From 2002 to 2007, 222 patients with chronic hepatitis B were recruited. Serum quantitative HBsAg level, hepatitis B e antigen (HBeAg) status, viral load and liver biochemistry were determined at baseline and 5th year of treatment.

Results: A total of 174, 170 and 71 patients had continuous entecavir therapy for 3, 4 and 5 years respectively. The median age of commencement of antiviral therapy was 47.5 (range, 21-73.9) and 77% patients were male. The cumulative rates of HBeAg seroconversion, alanine aminotransferase (ALT) normalisation and HBV DNA undetectability for years 3, 4 and 5 were 38.9%, 93.1%, 94.3%; 48.9%, 88.8%, 97.1%, and 55.6%, 90.1% and 97.2% respectively. One patient developed signature entecavir resistance and one patient achieved HBsAg seroclearance. The median annual HBsAg titre reduction at year 4 and year 5 of entecavir therapy were 0.11 log IU/mL/year (range, -0.77 to 1.28) and 0.142 log IU/mL/year (range, -0.26 to 0.55) respectively. When comparing HBeAg-positive versus HBeAg-negative patients, there was no significant difference in the median annual HBsAg titre decline (0.19 log IU/mL/year and 0.15 log IU/mL/ year, P=0.45).

Conclusion: Long-term entecavir therapy is highly effective in reducing hepatitis B viral load. Rate of HBsAg decline was not associated with duration of therapy or HBeAg status.

Association of a HLA-DP locus (rs3077) with hepatitis B viral activity: an interim analysis

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Introduction: Genetic variants along the human leukocyte antigen (HLA) were shown to be associated with chronicity of hepatitis B infection and disease outcomes in chronic hepatitis B. Its association with viral replication in chronic hepatitis B is not known.

Methods: We recruited 100 treatment-naive hepatitis B e antigen (HBeAg)–negative chronic hepatitis B patients who have undetectable HBV DNA (DNA level <20 IU/mL) and another 100 treatment-naïve age- and sex-matched HBeAg-negative controls with high HBV DNA levels (DNA level >2000 IU/mL). Genotyping of SNP rs3077 was performed by using TaqMan SNP genotyping assay (Life Technologies, Carlsbad, CA). Genotype results were expressed and analysed in biallelic manner (major allele G, minor allele A).

Results: The median age at recruitment was 47.9 (range, 20.4-77.4) years and 62% were male patients. The median HBV DNA level for patient in high HBV DNA group was 17460 IU/mL (range, 2502-3 596 760) At the time of writing, genotyping was performed for 49 patients with low viral activity and 86 patients with high viral activity. Genotyping for the rest of the patients is expected to be completed by December 2012. The genotypic frequencies of rs3077 are as follows:

patients with low DNA: GG vs GA vs AA: 31 (63.2%) vs 14 (28.6%) vs 4 (8.2%);
patients with high DNA: GG vs GA vs AA: 45 (52.3%) vs 33 (38.4%) vs 8 (9.3%).

There was no statistically significant association between HBV viral replication and allelic frequency (P=0.28), dominant gene action (P=0.82), recessive gene action (P=0.22), and genotypic frequency (P=0.46).

Conclusion: This interim analysis showed no association between rs3077 with hepatitis B viral activity. Genotyping for the remaining patients is currently still ongoing.

Surveillance colonoscopy for patients with serrated lesions of the colon: an interim analysis

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Introduction: Serrated lesions of the colon, once thought to be of low malignant potential, are now believed to be the precursors of about one-third of colorectal cancer. However, the optimal surveillance interval for patients who had serrated lesions of the colon is not well defined.

Methods: From the local colonoscopy database, 186 patients were found to have serrated lesions (including sessile serrated adenoma, traditional serrated adenoma, and hyperplastic polyps) from January 2008 to June 2010. These patients were invited to repeat colonoscopy for polyp surveillance.

Results: A total of 112 (60.2%) patients agreed to have surveillance colonoscopy (male, 63%). The median age was 62 (range, 34-89) years. Thirty-six (32.1%) patients have concomitant adenoma at baseline colonoscopy. At the time of writing, 45 patients had surveillance colonoscopy performed and the rest of surveillance colonoscopy is expected to be completed by November 2012. The median time to repeat colonoscopy was 34 (range, 6-54) months. For those who had surveillance colonoscopy performed, 29 (64%) patients were found to have new colonic polyps (metachronous polyps).Fourteen (31.8%) patients had adenomatous colonic polyp and 3 (6.81%) patients had advanced adenoma on surveillance colonoscopy at 3 years. By Kaplan-Meier analysis, the cumulative rate of polyp recurrence is 49.5% at 3 years. There is no statistically significant difference in the rate of metachronous colonic polyps between those with serrated adenoma at baseline and those with hyperplastic polyp at baseline (73% and 54.5%, P=0.203).

Conclusion: This interim analysis showed that metachronous colonic polyp is found in a large proportion of patients with serrated lesions at baseline.

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Garlic intake is an independent predictor of endothelial function in patients with ischaemic stroke

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Objectives: To investigate the effects of garlic on endothelial function in patients with ischaemic stroke (ISS).

Methods: A total of 125 Chinese patients with prior ISS due to athero-thrombotic disease were recruited from the out-patient clinics during July 2005 to December 2006. Daily allium vegetable intake (including garlic, onions, Chinese chives and shallots) was ascertained by means of a validated food frequency questionnaire for Chinese and brachial artery flow-mediated dilatation (FMD) was measured using high-resolution ultrasound in all subjects.

Results: The mean age of the study population was 65.9±11.1 years and 69% were males. Mean allium vegetable intake and garlic intake of the study population was 7.5±12.7 g/day and 2.9±8.8 g/day, respectively. Their mean FMD was 2.6±2.3%. Daily intake of total allium vegetable (r=0.36, P<0.01) and garlic (r=0.34, P<0.01) significantly correlated with FMD. Using the median daily allium intake as cut-off (3.37 g/day), patients with a low allium intake <3.37 g/day was noted to have a lower FMD compared to those with a normal allium intake (2.1±2.1% vs 3.0±2.4%, P<0.05). After adjusting for confounding factors, multivariate analysis identified that daily allium vegetable (B=0.05, 95% confidence interval [CI]: 0.02-0.09, P<0.01) and garlic (B=0.07, 95% CI: 0.02, 0.12, P<0.01) intake, but not onions, Chinese chives and shallots were independent predictors for changes in FMD in patients with ISS.

Conclusions: Daily garlic intake is an independent predictor of endothelial function in patients with ISS and may play a role in the secondary prevention of atherosclerotic events.

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Roles of the $CHADS_2$ and CHA_2DS_2-VASc scores in post-myocardial infarction patients: risk of new occurrence of atrial fibrillation and ischaemic stroke

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Introduction: Patients with myocardial infarction are at risk of development of atrial fibrillation (AF) and ischaemic stroke. We sought to evaluate the prognostic performance of the $CHADS_2$ and CHA_2DS_2-VASc scores in predicting new AF and/or ischaemic stroke in post-ST segment elevation myocardial infarction (STEMI) patients.

Methods: A total of 607 consecutive post-STEMI patients without previously documented AF were studied.

Results: After a follow-up of 63 months (3184 patient-years), 83 (13.7%) patients developed new AF (2.8% per year). Patients with a high $CHADS_2$ score and/or CHA_2DS_2-VASc score were more likely to develop new AF. The annual incidences of new AF were 1.18%, 2.10%, 4.52% and 7.03% in patients with $CHADS_2$ of 0, 1, 2, and ≥3; and 0.39%, 1.72%, 1.83%, and 5.83% in patients with the CHA_2DS_2-VASc score of 1, 2, 3 and ≥4. The test discrimination of the CHA_2DS_2-VASc score (C-statistic=0.676) was superior to the $CHADS_2$ (C-statistic=0.632) for new AF. Furthermore, 29 patients developed ischaemic strokes (0.9% per year). Likewise, the incidences of stroke increased with increasing $CHADS_2$ (0.41%, 1.02%, 1.11% and 1.95% with $CHADS_2$ of 0, 1, 2, and ≥3) and CHA_2DS_2-VASc scores (0.39%, 0.49%, 1.02%, and 1.48% in patients with the CHA_2DS_2-VASc score of 1, 2, 3 and ≥4). The C-statistic of the CHA_2DS_2-VASc score as a predictor of ischemic stroke was 0.601, which was superior to that of $CHADS_2$ score (0.573).

Conclusion: The $CHADS_2$ and CHA_2DS_2-VASc scores can identify post-STEMI patients at high risk of AF and stroke, enabling close surveillance and prompt anticoagulation for stroke prevention.

Prognosis and functional outcome after ischaemic stroke in Chinese

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Introduction: Ischaemic stroke (ISS) is a significant cause of disability and mortality worldwide. Here, we studied the subtypes and long-term prognosis of ISS in our locality.

Methods: A total of 1214 patients with ISS receiving rehabilitation at Tung Wah Hospital during 2004-2008 were prospectively followed-up for a mean of 76±18 months. Presence of recurrent stroke and all-cause mortality during the follow-up period was documented. The modified Rankin Score (mRS) at 1-year follow-up was delineated.

Results: It was found that 49% of ISS were due to small artery or penetrating artery atherosclerosis, 19% due to cardio-embolism, 9% due to large artery atherosclerosis, and 22% were of undetermined aetiology. Of the patients, 8.8%, 13.1% and 21.8% had a recurrent stroke and 8.7%, 15.5% and 31.0% passed away at 1-year, 2-year & 5-year follow-up. 45% of patients had mRS >3 at 1 year after stroke. Advanced age was an independent poor predictor for all adverse clinical outcomes (P<0.05). Underlying diabetes and stroke of undetermined cause were also independent predictors of recurrent stroke whilst underlying renal disease, stroke due to large artery atherosclerosis, and of undetermined cause were independent predictors of a mRS >3 at 1-year follow-up (all P<0.05). Underlying congestive heart failure, carotid atherosclerosis >50% stenosis, stroke due to large artery atherosclerosis or cardio-embolism were independent predictors of mortality at 5 years (P<0.05). Statin use was an independent predictor protective against all-cause mortality at 5 years (P<0.0001).

Conclusions: The prognosis after ISS remains poor in Hong Kong. Statins are protective against all-cause mortality after ISS and should be considered in all patients suffering from ISS.

Prognostic implications of surrogate markers of atherosclerosis in low-to-intermediate risk patients with type 2 diabetes

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Introduction: Type 2 diabetes mellitus (T2DM) patients are at increased risk of developing cardiovascular events. Unfortunately traditional risk assessment scores, including the Framingham Risk Score (FRS), have only modest accuracy in cardiovascular risk prediction in these patients.

Methods: We sought to determine the prognostic values of different non-invasive markers of atherosclerosis, including brachial artery endothelial function, carotid artery atheroma burden, ankle-brachial index, arterial stiffness and computed tomography coronary artery calcium score (CACS) in 151 T2DM Chinese patients who were identified low-intermediate risk from the FRS recalibrated for Chinese (<20% risk in 10 years). Patients were prospectively followed up and presence of atherosclerotic events documented for a mean duration of 61±16 months.

Results: A total of 17 atherosclerotic events in 16 (11%) patients occurred during the follow-up period. The mean FRS of the study population was 5.0±4.6% and area under curve (AUC) from receiver operating characteristic curve analysis for prediction of atherosclerotic events was 0.59±0.07 (P=0.21). Among different vascular assessments, CACS >40 had the best prognostic value (AUC 0.81±0.06, P<0.01) and offered significantly better accuracy in prediction compared with FRS (P=0.038 for AUC comparisons). Combination of FRS with CACS or other surrogate vascular markers did not further improve the prognostic values over CACS alone. Multivariate Cox regression analysis identified CACS >40 as an independent predictor of atherosclerotic events in T2DM patients (hazard ratio=27.11; 95% confidence interval, 3.36-218.81; P=0.002).

Conclusions: In T2DM patients identified as low-intermediate risk by the FRS, a raised CACS >40 was an independent predictor for atherosclerotic events.

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Elevated circulating adipocyte-fatty acid binding protein levels predict incident cardiovascular events in a community-based cohort: a 12-year prospective study

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Introduction: Obesity is closely associated with various cardiovascular diseases (CVD). Adipose tissue inflammation and perturbation of adipokines secretion may contribute to the pathogenesis of CVD. This study aimed to evaluate whether the two most abundant adipokines, adipocyte-fatty acid binding protein (A-FABP) and adiponectin, are independent risk factors predisposing to CVD.

Methods: We investigated prospectively the 12-year development of CVD in relation to the baseline levels of A-FABP and adiponectin in a population-based community cohort, comprising 1847 Chinese subjects recruited from the Hong Kong Cardiovascular Risk Factors Prevalence Study 2 (CRISPS 2) cohort without previous CVD. Baseline serum levels of A-FABP, adiponectin, and C-reactive protein (CRP), an established biomarker predictive of CVD, were measured.

Results: Of the 1847 Chinese subjects, 182 (9.9%) developed CVD during a median follow-up of 9.4 years. The CVD group had more traditional risk factors, higher baseline levels of A-FABP and CRP (both P<0.001), but similar adiponectin levels (P=0.881), compared to the non-CVD group. In Cox regression analysis including both biomarkers, the adjusted hazard ratio for A-FABP and CRP for subjects above the optimal cut-off values were 1.57 (95% confidence interval: 1.14-2.16, P=0.006) and 1.60 (1.12-2.27, P=0.01) respectively, after adjustment for traditional risk factors. Likelihood ratio test showed that elevated levels of either A-FABP (P=0.026) or CRP (P=0.002) could enhance the prediction of incident CVD by traditional risk factors.

Conclusions: Circulating A-FABP level predicts the development of CVD, independent of traditional risk factors, in a community-based cohort. Its clinical use for CVD prediction warrants further validation.

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Central nervous system inflammatory demyelinating disorders in Hong Kong Chinese

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Background: Classical multiple sclerosis (CMS) must be differentiated from neuromyelitis optica (NMO) as treatments are different. Serum aquaporin-4 autoantibodies (AQP4 Ab) are specific for NMO spectrum disorders (NMOSD). We aimed to study the diagnoses of CNS inflammatory demyelinating disorder (IDD) patients presenting to a hospital over 29 years.

Methods: Chinese patients presenting with CNS IDD to our hospital from 1981 to 2009 were studied. Patients referred from other centres were excluded. Since 2008, patients had yearly magnetic resonance imaging (MRI) brain and cord for 3 years even without relapse, and 3 or more yearly sera for serial AQP4 Ab assay by cell-based immunofluorescence assays. CMS was diagnosed by revised McDonald criteria (2005), NMO by revised Wingerchuk's criteria (2006). Other NMOSD was diagnosed in restricted forms of NMO seropositive for AQP4 Ab.

Results: Among a total of 181 Chinese patients studied, 61 (33.7%) had CMS (45 female, mean onset age 29.8 years), 40 (22.1%) NMOSD—24 NMO (all relapsing, 21 female, mean onset age 43.1 years, 19 [79.2%] AQP4 Ab seropositive, 20 [83.3%] with LETM), 16 other NMOSD (14 female, 8 recurrent AM with LETM, 3 recurrent ON without AM, 2 single LETM attack, 1 recurrent brainstem encephalitis , 1 AM and brainstem encephalitis, 1 single ON attack), 30 (16.6%) single AM attack, 20 (11.0%) single ON attack, 8 (4.4%) relapsing myelitis (4 with and 4 without LETM), 8 (4.4%) ADEM, 7 (3.9%) relapsing ON, 4 (2.2%) OSMS, 3 (1.7%) signal brainstem encephalitis attack. One of 61 (1.6%) CMS patients had LETM clinically. He responded well to beta-interferon. Two (5%) NMOSD patients had MRI brain at presentation fulfilling Barkhof's criteria, both had LETM and AQP4 Ab. All seropositive patients had AQP4 Ab detected in first assay. None turned seronegative.

Conclusions: CMS and NMOSD are the most common relapsing CNS IDD in Hong Kong Chinese at a ratio of 3:2. At least 22.1% patients had NMOSD.

Long-term prognosis of stroke survivors in a regional rehabilitation hospital

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Introduction: The long-term prognostic data of stroke patients who have survived in the acute phase and gone through the rehabilitation training programme at Tung Wah Hospital (TWH) are scarce. The data of the TWH stroke registry of a group of first-ever stroke patients at 18 months after the onset of stroke are described.

Methods: A retrospective cohort of 62 first-ever stroke patients who were admitted into TWH in the first quarter of 2010 was identified from the TWH stroke registry. Data on stroke recurrence, mortality at 12 and 18 months after stroke onset were described and determinants of the prognosis were analysed.

Results: There were 38 female (61%) and 24 male (39%) patients with an overall mean age of 74 years. Majority suffered from ischaemic strokes (82%) and haemorrhagic strokes contributed the rest of 18 %. Mortality at 12 months was 11/62 (17.7%) and at 18 months was 15/62 (24.2%). Patients who died at 12 and 18 months were significantly older (81.6 vs 72.4), suffering from cortical strokes, and had background hypertension and atrial fibrillation. Subtype and side of stroke as well as diabetes mellitus (DM) were not associated with increased mortality. Seven (11%) of 62 suffered from recurrent stroke by 18 months after onset. Elder age, presence of DM, hypertension, and atrial fibrillation were significantly associated with stroke recurrence.

Conclusion: The mortality and recurrence rates after stroke by 18 months were not low among stroke survivors. Prognostic determinants identified in this study may help to better manage this group of patients.

Hay fever and hypertension in the US adult population

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Objective: Hypertension is associated with inflammation. Whether the inflammation caused by allergic diseases such as allergic rhinitis can predispose to hypertension is controversial. Therefore, we studied the association between hay fever and hypertension in the United States National Health and Nutrition Examination Survey (NHANES).

Methods: We analysed data on 1883 men and 2029 women in NHANES 2005-2006. We included participants aged 20 years or older who had valid data on hay fever and hypertension.

Results: Of the participants, 13.5% had a previous diagnosis of hay fever and 36.2% had hypertension. There were ethnic differences in the prevalence of previous hay fever diagnosis ($P<0.001$) and hypertension ($P<0.001$). There was no significant association between previous hay fever diagnosis and hypertension in men in any age-group. The association between previous hay fever diagnosis and hypertension in women was significant in those aged 20 to 39 years (odds ratio [OR]=2.59, 95% confidence interval [CI]: 1.26-5.30, P=0.013). The association between previous hay fever diagnosis and hypertension in women aged form 20 to 39 years remained significant after adjustment for age, race, and body mass index (OR=2.74, 95% CI: 1.48-5.06, P=0.003). After further adjustment for physical activity, alcohol consumption and smoking, the association was not attenuated (OR=2.68, 95% CI: 1.38-5.18, P=0.006). Further adjustment for liver enzymes, C-reactive protein and immunoglobulin E level attenuated the association slightly (OR=2.72, 95% CI: 1.19-6.22, P=0.021).

Conclusions: In this nationally representative population-based survey, previous hay fever diagnosis is not significantly associated with hypertension in adults except for young women aged 20 to 39 years. Further work is needed to confirm that this is a true association.

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Effects of induced pluripotent stem cell– and bone marrow–derived mesenchymal stem cells on cigarette smoke–induced lung damage in rats

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Introduction: Cigarette smoking (CS) is the major cause of chronic obstructive pulmonary disease (COPD) which is the fourth leading cause of death and predicted to be the third by 2030. Administration of bone marrow–derived mesenchymal stem cells (BM-MSC) was reported to attenuate CS-induced emphysema in murine model. This study aimed to investigate the effects of induced pluripotent stem cell–derived MSC (IPSC-MSC) on CS-induced lung damage in comparison to BM-MSC treatment in rats.

Methods: Male Sprague-Dawley rats were randomly divided into four groups. The sham air (SA) group was exposed to fresh air while the other three groups to 4% cigarette smoke (CS) 1 hour per day for 56 days. During the second half of the smoking period, two doses of 3×10^6 of IPSC-MSC or BM-MSC cells were injected intravenously via tail vein to the two groups, ie IP/CS group or BM/CS group at day 29 and day 43. The SA and CS groups were injected with phosphate-buffered saline of the same volume. All rats were sacrificed 24 hours after the last CS exposure. Morphological changes were examined in paraffin-embedded lung sections. Levels of inflammatory markers were measured by ELISA.

Results: Both IPSC-MSC and BM-MSC were able to reside in the lung for as long as 14 days with significant higher density of resided IPSC-MSCs. Both treatments shared similar efficacy to attenuate CS-induced lung cell apoptosis to restore CS-induced reduction of lung IL-10 and to alleviate CS-induced elevation of systemic TGF-β1.

Conclusion: Our findings suggest that treatment of IPSC-MSC or BM-MSC might be able to slow down CS-induced disease progression, possibly through anti-oxidant, anti-inflammatory and anti-apoptotic properties.

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MDM2 inhibitor nutlin-3a impairs glucose homeostasis and insulin secretion in mice

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Introduction: Mdm2, an E3 ubiquitin ligase, regulates the tumour suppressor p53 by enhancing its proteasome-mediated degradation through the protein-protein interaction. A small molecule Nutlin-3a has been shown to disrupt the interaction between p53 and MDM2, thereby up-regulating expression of p53 in cancer cells. Therefore, this small molecular represents a promising therapeutic agent for cancer. However, the effect of nutlin-3a on glucose homeostasis has never been explored so far. In this study, we aimed to investigate whether nutlin-3a affects glucose metabolism using both in-vivo and in-vitro approaches.

Methods: Twelve-week male C57BL/6 mice were treated with nutlin-3 or DMSO (as a vehicle control) by oral gavage 2 hours before the experiment. Glucose tolerance and insulin sensitivity were assessed by intraperitoneal glucose tolerance test (GTT) or insulin tolerance test (ITT). Insulin secretion during GTT was examined. Effect of nutlin-3a on glucose or non-glucose secretagogues-stimulated insulin secretion was determined in mouse pancreatic islets and beta cell lines.

Results: The mice treated with nutlin-3 exhibited glucose intolerance which is accompanied with blunted insulin secretion, but similar insulin sensitivity compared to the DMSO control group. Further analysis revealed that beta cells treated with nutlin-3a exhibited impairment of glucose-stimulated insulin secretion (GSIS), which was associated with diminished ATP production and calcium influx. Furthermore, nutlin-3a treatment also suppresses insulin secretion induced by non-glucose secretagogues, including the mitochondria activators, the potassium channel blockers and the cell membrane depolarisation agents.

Conclusion: These findings demonstrated that disruption of the interaction between MDM2 and p53 by nutlin-3 leads to glucose intolerance, owing to defective insulin secretory capacity. Such inhibitory effects on insulin secretion may attribute to impaired glucose metabolism, mitochondria activation and/or calcium mobilisation.

The role of erlotinib-induced autophagy in non–small-cell lung carcinoma

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Introduction: Tyrosine kinase inhibitor (TKI, eg erlotinib) is commonly used in non–small-cell lung cancer (NSCLC). Autophagy is a catabolic process in response to deprivation of nutrients. This study aimed to investigate whether autophagy confers acquired resistance to erlotinib treatment in NSCLC.

Methods: Two NSCLC cell lines HCC827 and HCC4006 with EGFR mutations (exon 19 deletions) were selected. MTT and annexin-V assay were performed to determine cell proliferation and apoptosis. Autophagic molecules were monitored by Western blot. Acidic vesicular organelle (AVO) formation was determined by acridine orange staining. Autophagy inhibitor (chloroquine) and RNA interference were used to study the effect of induced autophagy.

Results: MTT confirmed that both cell lines were sensitive to erlotinib. Erlotinib treatment increased LC3II expression, ATG-5/ATG12 conjugation, formation of AVO and p62 degradation, compatible with induction of autophagy. Combination of chloroquine with erlotinib increased apoptotic events compared to either single treatment. Erlotinib combined with Atg-silence or Beclin-silencing significantly increase apoptotic cell death compared with single treatment in both cell lines.

Conclusion: Erlotinib can induce both apoptosis and autophagy in EGFR-mutated (exon 19 del) NSCLC cell lines. Inhibition of autophagy with chloroquine or siRNA can enhance erlotinib-induced cell death. Autophagy may serve as a protective mechanism for NSCLC upon treatment with erlotinib.

Acknowledgement: No funding support.

Fibroblast growth factor 21 maintains glucose homeostasis during fasting by mediating the crosstalk between liver and brain

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Introduction: Fibroblast growth factor (FGF) 21 is a metabolic hormone mainly secreted from hepatocytes and markedly induced by fasting/starvation under the control of PPAR alpha. As therapeutic administration of recombinant FGF21 has been shown to exert multiple metabolic benefits on obesity-related disorders, it has recently attracted a great interest in pharmaceutical industry. Physiologically, elevated FGF21 mediates metabolic adaptations to fasting/starvation, including ketogenesis and growth inhibition. However, the physiological role of FGF21 in regulating glucose metabolism remains unclear.

Methods: Fed and fasting blood glucose of FGF21 knockout (KO) mice and wild type (WT) littermates were determined. The plasma level of various hormones of hypothalamic-pituitary-adrenal (HPA) axis, including glucocorticoids and adrenocorticotropic hormone (ACTH), were determined by ELISA. Gene expression and protein abundance was determined by real-time polymerase chain reaction and Western blot respectively. To study the role of FGF21 in the brain, intracerebroventricular injection of recombinant FGF21 at a dose of 0.02 μg/g was performed in fasting FGF21-KO mice and the same parameters were determined as stated above.

Results: FGF21 deficiency causes a severe hypoglycaemia and impaired induction of hepatic gluconeogenic genes during prolonged fasting/starvation. Such a hypoglycaemic phenotype in fasted FGF21 mice is associated with a marked impairment in production of glucocorticoids from adrenal gland and its upstream hypothalamic regulator corticotropin-releasing hormone (CRH). Notably, central administration of FGF21 into the hypothalamic region reverses fasting hypoglycaemia and impairment in CRH/glucocorticoid production both in FGF21 and PPAR alpha KO mice.

Conclusion: We conclude that FGF21 is a key metabolic hormone for maintaining glucose homeostasis during fasting/starvation, by activating the HPA axis to produce glucocorticoids, which in turn enhances hepatic glucose production.

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51 The effect of human mesenchymal stem cell on cigarette smoke–induced alterations of cardiac function and lipid metabolism in rat

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Introduction: Cigarette smoking is recognised as a major risk factor for cardiovascular diseases. Mesenchymal stem cells (MSC) were reported to attenuate cardiac injury of myocardial infarction. The aim of this study was to investigate the effect of bone marrow–derived MSCs (BM-MSC) and induced pluripotent stem cell–derived MSC (IPSC-MSC) in heart on cigarette smoke–exposed rat model.

Methods: Male Sprague-Dawley rats (aged 6-7 weeks) were randomly divided into sham air (SA) group, cigarette smoke (CS) group, IPSC-MSC treatment (IP/CS) group, and BM-MSC treatment (BM/CS) group respectively. All the animals were exposed to 4% CS except SA group to fresh air for 1 hour each day for 56 days in ventilated chambers. Two doses of 3×10^6 of IPSC-MSC or BM-MSC cells were injected intravenously via tail vein in IP/CS or BM/CS group on the day 29 and day 43. Animals were anaesthetised 24 hours after the last smoking exposure for cardiac function examination by echocardiography. Animals were then sacrificed and lipid extraction of heart tissue was prepared for determining cholesterol, triglyceride, and free fatty acid (FFA) levels.

Results: From echocardiograph, IPSC-MSC reversed the CS-induced decrease in cardiac function by elevating left ventricular ejection fraction (LVEF) and fractional shortening (FS). Both IPSC-MSC and BM-MSC treatments were able to attenuate the CS-induced elevation of cholesterol and triglyceride level but could not reverse the reduction of FFA level in heart tissue.

Conclusion: Our findings suggest that IPSC-MSC treatment could alleviate the CS-induced cardiac dysfunction, which is likely due to the amelioration of lipid metabolism in heart.

52 Characteristics and prognosis of gastric cancer patients diagnosed within 5 years of prior negative gastroscopy

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Introduction: Gastric cancer diagnosed within 3 years of previous negative gastroscopy (OGD) is considered as missed cancers. Little is known about the characteristics and prognosis of these patients. This study aimed to compare the frequency, characteristics, and prognosis of gastric cancer patients with a previous negative OGD performed at different time intervals.

Methods: Consecutive patients with gastric adenocarcinoma diagnosed in our hospital between 2006 and 2010 were identified. All prior endoscopy records were retrieved from a centralised computer database. Patients were divided into three groups according to the intervals of previous "negative" endoscopy: between 6 and 36 months (Group A), between 3 and 5 years (Group B), and between 5 and 10 years (Group C).

Results: A total of 487 patients with gastric cancers were diagnosed in the study period and 48 (9.9%) of them had previous "negative" gastroscopy. There were 12 (2.5%) patients in group A, 15 (3.1%) in group B, and 21 (4.3%) patients in group C. The most common baseline endoscopy findings in these patients were gastric ulcer (31.3%). Patients who developed gastric cancer within 5 years of previous endoscopy had lower prevalence of intestinal metaplasia at baseline (P=0.039). Although stage I/II cancers were more common in Group A (58.3%), the median survival of this group was not superior to Group C (log rank test, P=0.035).

Conclusion: Gastric cancers that were diagnosed within 5 years of prior negative gastroscopy had lower survival rates, which cannot be explained by difference in tumour staging alone. Our findings may suggest a more aggressive behaviour of a subtype of gastric cancer that is not easily recognised by prior endoscopy. These findings may have implications on the optimal screening interval for patients at high risk of gastric cancer development.

53 Ten days quadruple versus sequential therapy as empirical first- and second-line treatment for *Helicobacter pylori* eradication: a randomised crossover trial

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Introduction: *Helicobacter pylori* (HP) is one of the commonest bacterial infections worldwide. The eradication rate of clarithromycin-based and bismuth-based therapy has been declining in the western world. The aim of the study was to compare the efficacy and tolerability of HP eradication with a 10-day sequential therapy versus quadruple therapy as empirical first- and second-line treatment.

Methods: Eligible HP-positive patients were randomised to receive either sequential (SEQ) therapy (esomeprazole 20 mg twice daily and amoxicillin 1 g twice daily for the first 5 days followed by esomeprazole 20 mg twice daily, clarithromycin 500 mg twice daily and metronidazole 400 mg four times daily for the subsequent 5 days) for 10 days or quadruple (QUAD) therapy (esomeprazole 20 mg twice daily, bismuth subcitrate 120 mg four times daily, tetracycline 500 mg four times daily, and metronidazole 400 mg four times daily) for 10 days. All patients returned 8 weeks after completing the treatment for a urea breath test (UBT) to confirm eradication. Patients who failed the initial UBT would crossover to receive the alternate treatment regimen.

Results: A total of 391 patients were recruited into the study with 213 in the SEQ group and 178 in the QUAD group. The baseline demographics and endoscopic diagnoses were similar in both groups. A total of 200 patients (93.9%) and 163 patients (91.6%) completed treatment in SEQ and QUAD group respectively. By per-protocol analysis, the eradication rate was 96% in SEQ group and 98.7% in the QUAD group (P=0.195). The eradication rate calculated by the intentional-to-treat was 91% (194/213) in SEQ group and 92.7% (165/178) in the QUAD group (P=0.348). The most common adverse events were bitter taste (1.9%) and nausea (2.2%) in SEQ and QUAD group respectively. There were seven cases from SEQ group crossover to receive QUAD therapy and two patients who initially failed QUAD therapy crossover to the SEQ therapy. All of the patients were successfully eradicated from HP after receiving the alternate therapy.

Conclusions: Both SEQ and QUAD therapy are highly effective in HP eradication as first- and second-line treatment with similar mild adverse events.

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54 Why patients fractured with higher bone mineral density?

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Objective: The aim of this cross-sectional study was to determinate the clinical risk factors associated with fractures in Chinese population with a higher bone mineral density (BMD) [T-score > –2.0].

Methods: All post-menopausal women with age of more than 50 years referred to Osteoporosis Clinic with low-traumatic fractures were included in the analysis. All subjects underwent a structured evaluation and BMD was measured by dual-energy X-ray absorptiometry. Common risk factors related to osteoporosis were identified and compared between patients with T-score ≤ –2.0 and > –2.0.

Results: A total of 450 patients with fracture were assessed. The mean age was 67±8 years. Of the patients, 65% (n=293) of the patients presented with T-score ≤ –2.0 (low BMD group) and 35% (n=157) had T-score > –2.0 (high BMD group). Subjects of high BMD group were younger (63±8 vs 69±7 years, P<0.001), had lower body mass index (14.32±3.12 vs 15.42±4.11 kg/m^2, P<0.05), shorter period of post-menopause time (11±8 vs 19±7 years, P<0.001), and lower oral diet calcium intake (643±272 vs 713±283 mg/day, P<0.05). More of them were current alcohol drinkers (11.5% vs 5.1% respectively, P<0.05) and smokers (8.3% vs 3.8% respectively, P<0.05). This group of patients had significantly higher serum parathyroid hormone (44.32±12.92 vs 40.41±11.73 pg/mL, P<0.05) but their serum calcium levels were similar to the lower BMD group (2.30±0.13 vs 2.30±0.11 mmol/L).

Conclusions: Our findings showed that a significant proportion of patients sustained fractures with higher BMD. Some clinical risk factors identified in this group are modifiable. Public health education for bone health promotion should focus not only on osteoporotic patients, but also on individuals with higher BMD.

The effect of ex-vivo rotenone intoxication on dopamine re-uptake of *LRRK2*-R1441G mutant mouse

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Introduction: Mutations in *LRRK2* are strongly associated with Parkinson's disease, and R1441G is the second most common mutation of *LRRK2*. In order to develop a mouse model for Parkinson's disease, we have generated a knock-in mouse carrying this mutation. However, the mutant mice remain healthy and do not demonstrate any behavioural or histological abnormalities until 18 months old. Since an important neurochemical defect in Parkinson's disease is decreased dopamine content in the striatum, and both genetic background and environmental stress make contribution to the disease progression, we investigated the effect of *LRRK2*-R1441G mutation and ex-vivo rotenone treatment on the ability of dopamine re-uptake.

Methods: Synaptosomes were isolated from mouse striatum and incubated with 3H-labelled dopamine with or without low-dose rotenone pre-treatment, and the radioactivity of synaptosomes was measured to assess the ability of dopamine re-uptake by dopaminergic neurons. Wild type and *LRRK2*$^{R1441G/R1441G}$ mutant mice at both 3 months and 18 months old were compared.

Results: There was no significant difference in the ability of dopamine re-uptake between wild type and mutant mice in either young (3 months old) or aged (18 months old) groups without rotenone treatment. However, dopamine re-uptake is significantly compromised in the mutant mice at 3 months old under rotenone treatment.

Conclusions: *LRRK2*-R1441G alone may not be sufficient to affect dopamine transmission even in aged mice, but the synergetic effect of *LRRK2* mutation and rotenone stimulus can lead to impaired dopamine uptake. Therefore, this study implies the importance of interaction between genetic and environmental factors in Parkinson's disease.

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A pilot study on the efficacy and safety of Prismocitrate 18/0 replacement solution in continuous venovenous haemodiafiltration (CVVHDF)

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Introduction: Regional citrate anticoagulation is efficacious for prolonging filter function, and confers less risk of bleeding and mortality compared to systemic anticoagulation. However, technical complexity, risks of sodium disturbance and citrate toxicity related to older citrate formulation limit its widespread use. Primocitrate 18/0 (Gambro), a new formulation of citrate with modified sodium content, could potentially ameliorate these side-effects. We aimed to develop a feasible protocol using this solution coupled with calcium-free dialysate (Prism0cal B22) for CVVHDF, and to determine its safety and efficacy for local critical care setting.

Methods: Asian medical patients admitted to the adult Intensive Care Unit (AICU) of Queen Mary Hospital who are indicated for continuous renal replacement therapy, without a history of advanced cirrhosis, or shock on high dose vasopressor, are recruited. The protocol adopts fixed flow rates of blood and Primocitrate 18/0 as pre-dilution at 120 mL/min and 1000 mL/min, respectively. Dialysate (Prism0cal B22) flow rate was adjusted according to body weight to achieve the ultrafiltration dose of 30-35 mL/kg/hour. CVVHDF is performed using Prismaflex® machine with high-flux filter (ST100, Gambro). Pre-filter and post-filter calcium levels target between 0.3 and 0.5 mmol/L. Calcium was replaced systemically via a separate central venous access. Systemic total calcium to ionised calcium ratio (target <2.5) was monitored for potential citrate toxicity.

Results: Fifteen eligible subjects (10 males, 5 females; age 49-85 years) were recruited so far. All of them fulfilled the AKIN definition of acute kidney injury and two of them were diagnosed metformin-associated-lactic-acidosis. Nine of them completed the CVVHDF therapy without filter clotting, and CVVHDF therapy last 12 to 72 hours. No electrolytes disturbance, citrate toxicity, or bleeding episode was reported after starting on the protocol. Filter clotting was reported in three subjects, after CVVHDF for 26, 38, 53 hours. Among the three subjects with filter clotted, catheter malfunction requiring A-V swapping was reported in two, which was believed to be a major reason predisposing to clotting of filter. The remaining subject had venous thrombosis elsewhere and required investigations for systemic thrombotic tendency. Three subjects were withdrawn from the study after CVVHDF for 5, 12 and 21 hours, subsequent to clinical deterioration with the development of contra-indications for citrate infusion. All subjects achieved pre-filter and post-filter calcium level within the targeted range. Systemic ionised calcium level was maintained within safe range (1.0-1.2 mmol/L) using the current protocol.

Conclusion: The current CVVHDF protocol using Prismocitrate 18/0 as replacement solution is feasible and no adverse events were reported so far. Safety profile is to be observed further upon ongoing recruitment.

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57 Health care needs and mortality among older people living in residential care homes suffering from advanced cognitive impairment

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Introduction: In Hong Kong, studies looking at the mortality and health care needs of residential care homes (RCHEs) residents with advanced cognitive impairment are scarce.

Objectives: The objectives of the study were to study the clinical service utilisation, mortality, and the predictors of mortality among RCHE elderly who have advanced cognitive impairment.

Methods: A cohort longitudinal study was performed in RCHEs in Hong Kong West Cluster (HKWC). Elderly with age of ≥65 years receiving follow-up by Hong Kong West Community Geriatric Assessment Team (HKW CGAT) and had advanced cognitive impairment with abbreviated mental test (AMT) score of 0/10 were included.

Results: A total of 312 RCHE residents (71 men and 241 women) were studied. Their mean age was 87.5 (standard deviation, 8.2; range 65 to 111) years. Of them, 164 (52.6%) required enteral feeding; 306 (98%) had bowel incontinence and 309 (99%) had urinary incontinence. Apart from CGAT clinics, 119 (38%) residents needed to attend other clinics outside RCHEs. The three commonest clinics were the Surgical, Eye, and Medical clinics. During the 1-year follow-up, there were 519 Accident and Emergency Department (AED) attendance, 478 acute hospital admissions with 2091 bed-days, and 164 convalescence hospital admissions with 1816 bed-days; 107 (34%) died within 1 year. The independent risk factors for 1-year mortality were active pressure sores (odds ratio [OR]=2.66, confidence interval [CI]: 1.37-5.1, P=0.0037), enteral feeding (OR=2.0, CI: 1.2-3.4, P=0.008), use of indwelling urinary catheter (OR=3.2, CI: 1.46-7.2, P=0.0036) and suffering from chronic obstructive pulmonary disease (OR=3.4, CI: 1.3-8.8, P=0.011). History of receiving pneumococcal vaccine was an independent protective factor for 1-year mortality (OR=0.47, CI: 0.28-0.78, P=0.004).

Conclusion: RCHE residents with advanced cognitive impairment were frail and were frequent user of out-patient, AED, and in-patient services. The development of "end-of-life" care and "hospital-in-home" services in RCHE is important to meet the need of this specific group of older patients in future.

58 A novel role of tescalcin in acute myeloid leukaemia leukemogenesis via activation of sodium/hydrogen exchanger1

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Acute myeloid leukaemia (AML) is a lethal cancer in Hong Kong. Despite intensive chemotherapy and allogeneic hematopoietic stem cell transplantation, AML has a very low cure rate. Therefore, novel therapies is in need. Internal tandem duplication (ITD) of FLT3 (fms-like tyrosine kinase 3) occurs in 30% AML and has become a target of therapeutic intervention. A FLT3 inhibitor sorafenib is effective in re-inducing remission in patients with FLT3-ITD AML refractory to chemotherapy. However, the response is transient and leukaemia progression occurs after 2 to 3 months. Microarray studies demonstrated that at leukaemia progression, a gene encoding for *tescalcin* (*TESC*), which normally activates sodium/hydrogen exchanger (NHE1), was significantly up-regulated in the myeloblasts. We hypothesised that targeting tescalcin and NHE1 may overcome sorafenib resistance. *TESC* expression is significantly up-regulated in human AML cell lines compared with cord blood $CD34^+$ cells. *TESC* knock-down in K562 cells by siRNA significantly lowered intracellular pH and reduced cellular proliferation. The results were recapitulated by a NHE1 inhibitor amiloride K562. These observations provide novel information about the pathogenetic role of *TESC*-NHE1-pH in AML. The mechanistic link of intracellular pH to sorafenib resistance and the potential clinical efficacy of amiloride in sorafenib-resistant AML are being explored.

Metabolic syndrome in Rawalpindi and Islamabad population

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Objective: Metabolic syndrome (MS) is considered a combination of interrelated risk factors which may increase the risk of developing cardiovascular disease and type 2 diabetes (T2D). The aim of the present study was to find out the prevalence of different components of MS among out-patients of selected hospitals in Rawalpindi and Islamabad, Pakistan.

Methods: One thousand adult subjects (613 men and 387 women) visiting the diabetic and weight management clinics of selected hospitals in Rawalpindi and Islamabad, Pakistan, were screened for MS. The diagnosis was based on International Diabetes Federation's criteria. Written informed consent was obtained from all subjects. Anthropometric and blood pressure measurements were taken and 5 mL blood was drawn for the lipid profile and fasting blood glucose assays.

Results: MS was confirmed in 293 male and 207 female subjects. The prevalence of MS was 39.6% in the 30-40 years' age-group as compared to 60.4% in the 41-50 years' age-group. The prevalence of the components of the MS was: T2D 59.8%, hypertension 58.2%, hypertriglyceridemia 83.0%, low high-density lipoprotein 72.4%, and central obesity 70%.

Conclusion: Our study results demonstrate that MS was more common among male as compared to female subjects screened for the disease. The risk of MS increases with age, with 41-50 years' age-group being more at risk. Our data indicate that MS is associated with hypertension as much as it is with T2D. Attention to diet and physical activity is needed if the prevalence of MS in these major cities in Pakistan is to be reduced.

Using abbreviations in prescriptions: the perspectives of doctors, pharmacists and nurses

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Introduction: Using inappropriate abbreviations in prescriptions may lead to medication errors. We sought the attitudes of health professionals on using abbreviations in prescriptions. Awareness of the 'Do Not Use' list, which is a list of abbreviations that is inappropriate for use in prescribing, was also assessed.

Methods: An on-line survey was conducted among clinical doctors, pharmacists, and nurses attached to a university and a large tertiary care hospital in Hong Kong.

Results: A total of 121 responses were received from doctors (n=51), pharmacists (n=34), and nurses (n=36). Most doctors (82.4%) believed that using abbreviations saved prescribing time while most pharmacists (79.4%) and nurses (63.3%) felt that interpreting abbreviations took more time than reading the full name. However, nearly all doctors (96.0%), pharmacists (100%), and nurses (100%) believed that avoiding inappropriate abbreviations would reduce the chance of medication errors. As many as 86.3% of doctors were aware of the 'Do Not Use' list of inappropriate abbreviations used in the hospital but among them, only 65.1% always followed it. Among doctors who were aware of the 'Do Not Use' list, some only sometimes (30.2%) or never (4.7%) adhered to the list mostly because they were uncertain of its contents and did not have time (7/14), a place (5/14), or found it cumbersome (6/14), to refer. Some did not adhere to the 'Do Not Use' list, because other doctors did not adhere (4/14), they forgot that the list existed (2/14), there was no time to adhere (3/14), or they felt the list was unimportant (2/14). Only 56.0% of all doctors were aware of the list of standard approved abbreviations recommended in the hospital.

Conclusions: Although prescribers intend to save time by using abbreviations in prescriptions, pharmacists and nurses need more time to interpret them. However, most health professionals believe that using inappropriate abbreviations may lead to medication errors. Therefore, continuous reminders on avoiding inappropriate abbreviations in prescriptions are needed.

61 Perceptions of pharmacy and nursing staff on a pilot programme of bar-code–assisted medication administration

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Introduction: User acceptance is important for the success of a bar-code–assisted medication administration (BCMA) system. We aimed to assess the perceptions of pharmacy and nursing staff on a BCMA system that is not supported by computerised prescribing.

Methods: A 2-D BCMA system was piloted in the pharmacy and one medical ward of a teaching hospital. As the system was not supported by computerised prescribing, pharmacy staff had to label dispensed drugs with a bar-code. Therefore potential users of the system were pharmacy and nursing staff whose attitudes were assessed using questionnaires and interviews, 1 month before and 8 months after implementation. All items in the questionnaire were measured using the Likert scale.

Results: Twenty-one pharmacy staff completed the questionnaire. Eleven of them completed both pre- and post-implementation surveys (matched participants). The constructs, 'attitude on output and intention to use', 'perceived ease of use', 'external influences', and 'job relevance' did not change significantly but 'perceived usefulness' reduced significantly among matched participants after implementation and after correcting for 5% type I error (P=0.008; power=0.76). A total of 16 pharmacy staff and 10 nurses were interviewed. Pharmacy staff felt that the system was slower (14/16) and more complicated (13/16), but helped to improve safety in drug administration (7/16). They perceived that the system offered less benefit to the dispensing process (9/16). Nurses felt that the system was less efficient (8/10) but useful in improving the accuracy of drug administration (7/10). However, technical, infrastructural and system-related issues were highlighted as barriers to using the new system (9/10).

Conclusions: Pharmacy staff perceives the BCMA system less useful without computerised prescribing as it does not improve the dispensing process. Nurses perceive this system useful in improving the safety of drug administration although it is difficult to use.

62 Development and validation of a survey instrument to assess attitudes of health professionals on using 2-D bar-code technology

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Introduction: User attitudes can help to identify practical issues related to using a technology. We aimed to develop and validate an instrument to assess attitudes of nurses and pharmacy staff on using 2-D bar-code technology in dispensing and drug administration.

Methods: The study was conducted in a teaching hospital in Hong Kong that piloted a 2-D bar-code assisted medication administration system in the pharmacy and one medical ward. The Technical Acceptance Model (TAM) was used as the basis for developing the instrument. New items were added to address areas more specific to 2-D bar-code technology. All items were measured using a 5-point Likert scale. The validity and psychometric properties of the instrument were assessed using responses from 46 health professionals (30 nurses and 16 pharmacy staff).

Results: The resulting instrument contained 26 items. Factor analysis extracted six constructs that were not identical but generally conformed to the factors in the TAM. The six constructs measured 'perceived attitudes on output and intention to use', 'perceived usefulness', 'perceived ease of use', 'external influences', 'job relevance', and 'perceived adequacy of training'. The factor structure showed good construct validity. All correlations between hypothesised construct and item were above 0.4 after adjusting for overlap. The instrument showed good reliability with an overall Cronbach's alpha of 0.86.

Conclusions: A valid and reliable instrument to measure attitudes towards using 2-D bar-code technology among pharmacy and nursing staff was developed as an extension of the TAM. This instrument may be used to assess perceived user attitudes before implementing the 2-D bar-coding technology or for continuous improvement of the system.

63 A pilot programme of 2-D bar-coded medication administration system: direct observation of changes to the pharmacy process

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Introduction: Bar-code–assisted medication administration (BCMA) helps to reduce drug administration errors. BCMA can be used without computerised prescribing if pharmacists label dispensed drugs with a bar-code. We assessed whether a BCMA system used without the support of computerised prescribing may have unintended effects on the pharmacy process.

Methods: The 2-D BCMA system was piloted in a teaching hospital and involved the pharmacy and one medical ward. The timing, number of dispensing steps, staff role shifts, problems related to human factors and technical defects when using the system, workarounds and potential dispensing errors (PDEs) were directly observed 1 month before and 8 months after the intervention.

Results: The dispensing of 1291 and 471 drug items were observed before and after the introduction of BCMA, respectively. The number of dispensing steps increased from 5 to 8 and time (standard deviation) to dispense 100 drug items by one staff personnel increased from 0.8 (0.9) minutes to 1.5 (0.12) minutes. In addition to the drug name and dose, pharmacy technicians had to enter prescribing instructions to the new system in order to create 2-D bar-coded drug labels. Human or technical issues occurred when using the 2-D bar-code label printer (12/26 times) and automated dispensing machine (7/18 times). Workarounds were not observed. Among all drug items observed, PDEs increased significantly from 0.4% to 3.2% ($P<0.001$), mainly due to unanticipated errors (2.34%).

Conclusions: A 2-D BCMA system used without the support of computerised prescribing increases the time and number of steps in the dispensing process. Introduction of this technology and related changes to the dispensing process may introduce unanticipated dispensing errors.

64 Role of IL28B and ITPA polymorphisms in the treatment of chronic hepatitis C virus genotype 6 infection

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Objective: IL28B and inosine triphosphatase (ITPA) polymorphisms are able to predict treatment response and degree of ribavirin-related anaemia respectively in the treatment of chronic hepatitis C virus (HCV) infection. However, their roles in the treatment of chronic HCV genotype 6 remain undetermined.

Methods: Sixty patients who were infected with HCV genotype 6 were commenced on 48 weeks of combination pegylated interferon and ribavirin therapy. Response to therapy, profiles of haemoglobin changes and platelet counts during therapy and their associations with IL28B rs8099917 and ITPA rs1127354 polymorphisms were analysed.

Results: The overall sustained virologic response (SVR) rate was 91.7%. Eighteen (30.0%) patients required a reduction in ribavirin dosage. The distribution of IL28B rs8099917 TT/TG genotypes and ITPA rs1127354 CC/CA genotypes were in Hardy-Weinberg equilibrium. IL28B rs8099917 TT genotype, when compared to TG genotype, was significantly associated with an increased SVR rate (96.2% and 62.5% respectively), and was the only clinical parameter that predicted SVR ($P=0.014$). The same significant association was observed when analysing allelic frequencies (T vs G, $P=0.001$). ITPA rs1127354 CA genotype, when compared to CC genotype, was associated with lesser degree of anaemia throughout therapy ($P<0.05$ for all time points). ITPA polymorphisms showed no association with changes in platelet count throughout therapy ($P>0.05$ for all time-points), and was not associated with SVR ($P=0.640$).

Conclusion: In chronic HCV genotype 6 infection, IL28B polymorphisms were associated with response to therapy. ITPA polymorphisms influenced the degree of anaemia but not thrombocytopenia during therapy.

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HLA-DP and IL28 polymorphisms: influence of host genome on hepatitis B surface antigen seroclearance in chronic hepatitis B

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Background: The roles of single nucleotide polymorphisms (SNPs) at HLA-DP and IL28B locus on hepatitis B surface antigen (HBsAg) seroclearance in chronic hepatitis B (CHB) are unknown.

Methods: We compared the HLA-DP (rs3077, rs9277378, rs3128917) and IL28B (rs12979860, rs8099917) polymorphisms of 203 CHB patients achieving spontaneous HBsAg seroclearance with 203 age- and sex-matched CHB patients without HBsAg seroclearance (controls).

Results: The distribution of all five polymorphisms was in Hardy-Weinberg equilibrium. HLA-DP rs3077 was associated with HBsAg seroclearance in terms of allelic frequency (minor allele A vs major allele G, P=0.035, odds ratio [OR]=0.699, 95% confidence interval [CI]: 0.501-0.976) and genotypic frequency (AA vs GG/GA, P=0.014, OR=0.295, 95% CI: 0.106-0.822). Haplotype analysis of HLA-DP polymorphisms showed haplotype block GAT (rs3077/rs9277378/rs3128917) to be associated with HBsAg seroclearance (OR=2.17, 95% CI: 1.06-4.45, P=0.034). Influence of HLA-DP polymorphisms on HBsAg seroclearance was more pronounced in younger patients, with the OR for rs3077 minor allele A and haplotype block GAT being 0.560 and 2.68 respectively among patients <50 years (P=0.027 and 0.047 respectively). IL28B haplotype block CG (rs12979860/rs8099917) was associated with HBsAg seroclearance (OR=10.5, P=0.026). None of the five polymorphisms influenced anti-HBs positivity among patients achieving HBsAg seroclearance, or serum HBV DNA and HBsAg titres among controls (P>0.05).

Conclusions: Specific SNPs in HLA-DP and IL28B locus, through individual and haplotype analysis, were associated with a higher chance of HBsAg seroclearance in CHB. The associations were more prominent in patients with HBsAg seroclearance at a younger age.

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Hepatitis B surface antigen kinetics during 10 years of virologic suppression with nucleoside analogue therapy

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Background: The kinetics of serum hepatitis B surface antigen (HBsAg) levels during long-term nucleoside analogue therapy has not been described.

Methods: We recruited 70 patients achieving persistent virologic suppression (serum HBV DNA <2000 IU/mL) during lamivudine therapy for at least 10 years (10 patients for 15 years). Serum HBsAg (Elecsys HBsAg II) and HBV DNA levels (Cobas Taqman) were determined at baseline, 5 years, and 10 years.

Results: The median age at lamivudine commencement was 38.6 (range, 13.1-66.7) years. Of the patients, 57 (78.1%) were male and 43 (58.9%) were hepatitis B e antigen (HBeAg)–positive, with all 43 patients achieving HBeAg seroconversion after a median period of 2.82 (range, 0.13-10.85) months. There was no significant difference in the median annual HBsAg decline rate from baseline to 5 years and from 5 to 10 years (0.350 and 0.359 log IU/mL/year respectively, P=0.749). There was no difference in median annual HBsAg decline when comparing baseline HBeAg-positive and negative patients (0.111 and 0.064 log IU/mL/year respectively, P=0.165), genotypes B and C (0.081 and 0.125 log IU/mL/year respectively, P=0.170), and persistent undetectable viremia versus intermediate levels of viremia of 20-2000 IU/mL (0.081 and 0.124 log IU/mL/year respectively, P=0.232). Seven patients (9.6% 4 HBeAg-positive and 3 HBeAg-negative) achieved HBsAg seroclearance after a median period of 7.59 (range, 3.59-12.15) years. The seven patients achieving HBsAg seroclearance, when compared to the remaining 64 patients, had a lower median baseline HBsAg level (2.72 and 3.67 log IU/mL respectively, P=0.029) and a significantly larger HBsAg decline (0.401 log IU/mL/year vs 0.081 log IU/mL respectively, P<0.001).

Conclusion: HBsAg reduction remained relatively stable during long-term lamivudine therapy. A low baseline HBsAg level and an increased HBsAg decline rate would be a prerequisite for subsequent HBsAg seroclearance.

Serologic and virologic outcomes of tenofovir in Asian chronic hepatitis B patients with prior nucleoside analogue exposure

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Objective: The efficacy of tenofovir in Asian chronic hepatitis B (CHB) patients with prior exposure to nucleoside analogue (NA) therapy has not been thoroughly investigated.

Methods: We determined the cumulative virologic, serologic, and biochemical outcomes of 142 Asian CHB patients, with at least 6 months exposure to other NAs (including lamivudine, adefovir, telbivudine and entecavir), taking tenofovir with or without lamivudine for up to 3 years.

Results: The median age was 46.9 years (83.1% male). Of the patients, 97 (68.3%) were HBeAg-positive; 142, 103 (71.8%) and 39 (27.5%) were followed up for 1, 2, and 3 years respectively. Sixty-one (42.9%) patients continued concomitant lamivudine. In patients with detectable viremia, baseline lamivudine, adefovir and entecavir signature mutations were present in 69.1%, 5.6% and 1.6% respectively. Cumulative rates of HBV DNA undetectability for all patients were 73.9%, 89.0% and 95.9% at years 1, 2 and 3, respectively. The cumulative 3-year HBeAg seroconversion rate among HBeAg-positive patients was 32.2%. Patients with concomitant lamivudine therapy, compared to the group on monotherapy, had significantly higher cumulative rates of virologic suppression (P=0.037). One patient achieved HBsAg seroclearance after 2 years of tenofovir monotherapy. There was no statistical difference in the annual HBsAg reduction rate when comparing HBeAg-positive and -negative patients (0.166 and 0.092 log IU/mL/year respectively, P=0.368). HBV genotype and HBeAg seroconversion among HBeAg-positive patients did not significantly influence the rate of HBsAg reduction (P=0.306 and 0.708, respectively).

Conclusion: In patients with prior exposure to NA, tenofovir, with or without lamivudine, was able to achieve high rates of virologic suppression up to year 3. Serum HBsAg levels declined slowly, and the rate of reduction was not influenced by HBV genotype or HBeAg seroconversion.

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Fractional 1435-nm diode laser system for skin rejuvenation in Chinese patients

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Background and objectives: This is a laser system that is commercially available. The objective of the study is to assess the efficacy and adverse effects of a 1435-nm laser system for skin rejuvenation in Chinese patients.

Methods: A fractional diode laser with a wavelength of 1435 nm and a maximum pulse energy of 9 mJ was used for the treatment of skin rejuvenation. Ten subjects were recruited. Four treatment sessions over an interval of 14 days were provided. The physician selected a use-selectable setting of low (4 mJ) / medium (7 mJ) / high (9 mJ) intensity on the hand piece and eight passes were delivered. Standardised photographs were taken at baseline, 2 weeks' post-treatment, and 1 month after the last treatment. Any pain related to the treatment was assessed by the patient by a visual analogue scale (0-10), redness, swelling and heat sensation with a severity scale of 0-3. Two independent physicians assessed the clinical photos for efficacy and adverse effects.

Results: Skin texture improved after two treatments with P value of 0.007, 0.0114, 0.006 at each follow-up, respectively. Improvement in pigmentation was also significant after two treatments with P values of 0.007, 0.020, 0.010. Improvement in wrinkles was observed at 1 month after the last treatment (P=0.046). Mild erythema was the most common adverse effect with a percentage of 50 one month post 4th treatment.

Conclusions: Fractional 1435-nm laser system is effective for the improvement of skin texture, pigmentation, and wrinkles in Chinese patients after a course of four treatments.

69 The efficacy of a dual-filter handpiece (500-670 nm and 870-1200 nm) of an intense pulse light device for the treatment of facial telangiectasia

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Background and objectives: The dual-filter handpiece with a wavelength of 500-670 nm and 870-1200 nm, pulse width 5-100 ms, and spot size 10 x 15 mm can deliver energy of up to 80 J/cm^2. The objective of the study was to assess the safety and efficacy of the MaxG handpiece for the treatment of facial telangiectasia.

Methods: Ten subjects were recruited into the study. Up to four treatments with an interval of 4 to 6 weeks were given. Only areas with facial telangiectasia were treated and the parameter used was 34-40 mJ/cm^2, 10 ms. A total of 25 treatment sessions have been carried out so far. At each visit, patient questionnaires were given for objective assessments and standardised clinical photographs were taken for two independent physicians' review.

Results: This is an ongoing study with one subject completing all treatment sessions. Preliminary results showed that mild pain and erythema are common adverse effects that resolve spontaneously. At 1-week post-treatment, one out of 18 treatment sessions developed crusting at the nasal region and seven out of 18 treatment sessions developed crusting at the cheeks. Hypopigmentation was noticed in one out of 18 treatment sessions and hyperpigmentation was noticed in three out of 18 treatment sessions at the cheeks. In terms of efficacy, statistically significant improvement was found after the second and third treatments. More comprehensive data are to be generated when all subjects complete the study.

Conclusion: Dual-filter handpiece with a wavelength of 500-670 nm and 870-1200 nm seems to be effective for the treatment of facial telangiectasia. Mild cases of crusting was observed which may be overcome by adjusting the parameters used.

70 Safety and efficacy of a minimally invasive radiofrequency device for skin tightening in Chinese patients

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Background and objectives: This is a minimally invasive radiofrequency device for the treatment of ageing skin. It stimulates the regeneration of new collagen, elastin, and hyaluronic acid. This study assessed the safety and efficacy when used for the treatment of skin tightening in Chinese patients.

Methods: Fifteen subjects were recruited (mean age, 53 years) and were offered one treatment. The cartridge consists of five pairs of 32°, bi-polar radiofrequency needles 1-2 mm which was inserted into the skin. Radiofrequency energy was delivered for a specific period of time at predetermined temperature settings. Patients' subjective assessment was collected by means of a questionnaire. Standardised clinical photographs were taken at baseline, immediately after treatment, 1 day, 3 days, 1 week, 1 month, 3 months, and 6 months post-treatment. These photographs were assessed by two independent physicians.

Results: Pain, swelling, redness and bruising were reported by the subjects which slowly resolved over time. There was one case of atrophic scar noticed at 3 months' and 6 months' follow-up. The level of satisfaction was 73% at 1-month follow-up and increased to 89% at 6-month follow-up. Physician assessment showed similar findings in terms of adverse effects. Both physicians observed statistically significant ($P<0.05$) skin tightening when assessing the jaw line, cheeks, nasolabial fold as well as oral commissures at 1-, 3-, and 6-month follow-up. Improvement in fine lines in the lower face was also noticed 3 and 6 months post-treatment (both $P=0.024$), whilst improvement in skin texture was seen at 6-month follow-up ($P=0.046$).

Conclusion: Minimally invasive radiofrequency device for skin tightening is effective in Chinese patients. The common adverse effects are pain, oedema, erythema, and bruising.

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The efficacy of a 915-nm laser, 650-nm LED light source with mechanical massage device for the treatment of cellulite and circumferential reduction in Chinese patients

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Objective: To assess the efficacy of a 915-nm laser, 650-nm LED light source with mechanical massage device, Smoothshapes®, for the treatment of cellulite and circumferential reduction of the thigh after a course of eight treatment sessions.

Methods: Ten subjects with cellulite stage 1 or above based on the Nurnberger-Muller Scale were recruited for the study. A series of eight treatment sessions were offered to one thigh of the subjects where two sessions per week at least 1 day apart. The non-treated thigh is the control. Standardised clinical photos, the stage of the cellulite, and circumference of the thigh were recorded at each visit as well as 1 and 3 months after the last treatment. At each visit, the subjects would fill out a patient questionnaire and two independent physicians assessed the standardised photographs.

Results: Preliminary results for four subjects are available at this point of this ongoing study. There was no improvement in cellulite grading according to the physicians' assessment of the clinical photos. There are insufficient data for the statistical analysis of any circumferential reduction yet. Subjects rated the results better with two reporting a 1-24% improvement and one 25-49% improvement after four treatments. Only one subject completed all eight sessions and she rated her improvement the best, 50-74%. Two cases of mild bruising were observed: one after the first treatment session, the other after the third treatment session. Both subsided spontaneously.

Conclusion: The preliminary result demonstrates that patient satisfaction was achieved. All treatment sessions are to be completed for assessment.

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The role of isocitrate dehydrogenase in zebrafish primitive haematopoiesis

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Introduction: Isocitrate dehydrogenase (IDH) is an enzyme that participates in the citric acid cycle. Recurrent mutations in IDHs, which have been discovered in many tumours, including gliomas, acute myeloid leukaemia and chondrosarcomas and so on, carry a novel enzymatic activity of 2-hydroxyglutarate production from alpha-ketoglutarate. However, the role of IDHs in normal haematopoiesis is still unknown. In this study, we have clarified the sequence homology, gene expression pattern, and haematopoietic functions by using the excellent animal model zebrafish.

Methods: Multiple alignments and phylogenetic tree of the full amino acid sequences of the IDH proteins in different species were generated with ClustalW (http://align.genome.jp/) and MEGA5 (http://www.megasoftware.net/), respectively. Spatial expression of zebrafish idh1 and idh2 genes were analysed by whole-mount in-situ hybridisation (ISH) during early development. Knockdown idh1 gene by morpholino technique was assessed by real-time reverse transcription polymerase chain reaction (RT-PCR) and whole-mount ISH.

Results: Zebrafish idh1 and idh2 shared 83% and 84% amino acid sequence identity, respectively, with the human ortholog. ISH results showed that idh1 gene mainly expressed in enveloping layer and olfactory vesicle during early development, later in pharynx, intestinal bulb, intestine, and liver. The idh2 gene was not expressed as early as 6 hpf, but appeared predominantly in eye, brain, pronephric duct and the intersomitic region from 24 hpf to 36 hpf, then gradually lost its expression in the intersomitic region and restricted in pectoral fin. Idh1 knockdown experiment showed disturbance in myeloid but not erythroid development during primitive haematopoiesis. Both the RT-PCR and ISH data suggested the increase of myeloid progenitor marker (spi1) and decrease of macrophage marker (l-plastin) and neutrophil marker (mpo).

Conclusion: The zebrafish IDH proteins were highly conserved among species. Knockdown idh1 resulted in myeloid lineage disturbance, but had no effect on erythroid development during zebrafish primitive haematopoiesis.

A novel C-type lectin receptor, CLEC16A, and systemic lupus erythematosus

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Introduction: C-type lectin receptors (CLR) are pattern recognition receptors that sense microbial pathogens and other forms of danger, leading to the induction of inflammatory responses in the host that link innate to adaptive immunity. Abnormalities in expression of CLRs can lead to various immune complications, such as autoimmune diseases. In several independent genome-wide association studies, polymorphisms of the C-type lectin domain family 16 member A (CLEC16A) gene were found to be associated with different autoimmune diseases, including systemic lupus erythematosus (SLE). Until now, two encoding isoforms of CLEC16A have been found in peripheral blood mononuclear cells (PBMC) in Caucasians, while none have been reported in Chinese.

Methods: CLEC16A expressed in Chinese PBMC was cloned and sequenced. To compare the expression levels of CLEC16A in normal versus SLE individuals, PBMC was collected from normal and SLE individuals for "real-time polymerase chain reaction quantification".

Results: Sequence differences have been found in both CLEC16A isoforms expressed in Chinese PBMC compared to Caucasians': a 6-bp deletion that was consistently present in isoforms 1 and 2, and an additional 54-bp deletion that has been detected in isoform 2. It has also been observed that expressions of both isoforms 1 and 2 are significantly lower in SLE than in normal individuals ($P=0.025$ and $P<0.0001$ respectively; n=43 normal vs 39 SLE).

Conclusion: Additional isoforms of CLEC16A are expressed in Chinese PBMC, which differ from the ones reported. Further experiments will be performed to clone any other possible isoforms expressed in Chinese. From expression comparison study, results suggest that the presence of less CLEC16A in PBMC is correlated with SLE. However, whether expression level is correlated with disease severity remains unknown, which will be studied by segregating patient samples according to disease status and evaluating the corresponding expression levels of both isoforms. The molecular and cellular functions of CLEC16A will also be studied in future, which would shed light on the importance of this receptor in SLE.

A new prognostic staging system with treatment guidelines for patients with hepatocellular carcinoma: Hong Kong Liver Cancer Prognostic Classification Scheme

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Introduction: Despite its wide use in western countries, the Barcelona Clinic Liver Cancer (BCLC) staging classification and treatment schedule may not fit in the management of hepatocellular carcinoma (HCC) patients in Asia where disease is mostly hepatitis B–related. This study aimed to develop a new prognostic classification scheme, namely Hong Kong Liver Cancer (HKLC) prognostic classification scheme, for HCC with treatment guidance based on an Asian patient population.

Methods: Between 1995 and 2008, 3856 HCC patients registered at a single institution with data collected prospectively were studied. The data were randomly separated into a training set and a test set for scheme development and performance assessment respectively. Four established prognostic factors, namely performance status, Child-Pugh grade, liver tumour status, and presence of extrahepatic vascular invasion/metastasis, were used. The scheme was proposed by clinicians and aided with Cox regression, and the treatment guidance was elicited by classification and regression tree analysis and then reconciled with clinical judgement to become the final scheme. This new prognostic classification was compared with BCLC classification in two aspects: discriminatory ability of the staging system and effectiveness of treatment guidelines.

Results: HKLC staging stratified patients to stages I to V with distinct overall survival outcomes. HKLC staging showed significantly better discriminatory ability (area under receiver operating characteristics curve [AUROC] around 0.84; concordance index 0.74) vis-à-vis BCLC staging (AUROC around 0.8; concordance index 0.70). HKLC treatment guidelines had wider indications for more aggressive treatments than the BCLC treatment schedule, and demonstrated significant survival benefit in selected patients.

Conclusion: The HKLC prognostic classification may be more effective in identifying HCC patients suitable for more aggressive treatments and hence yields a better survival outcome.

75 The effect of inhaled corticosteroids on the risk of diabetes mellitus, prediabetes and glucose regulation in adults with asthma (ICSD Study)

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Introduction: We investigated the risk of long-term inhaled corticosteroids (ICS) on diabetes mellitus (DM), prediabetes, and glucose tolerance in Chinese adult asthmatics in a population-based matched-controlled study.

Methods: A total of 691 asthmatics, aged ≥35 to 74 years, who used ICS regularly for ≥6 months were recruited from four asthma clinics in Hong Kong, excluding those with exacerbation in recent 4 weeks or had taken systemic steroid (SS) in recent 6 months. Each asthmatic was matched 1:1 on age, gender, and body mass index (BMI) with controls from the Hong Kong Cardiovascular Risk Factors Prevalence Study II based on a random population sample. All subjects underwent a 75-g oral glucose tolerance test. The lifetime cumulative budesonide dipropionate equivalent dose of ICS and numbers of SS prescriptions were ascertained. Cumulative median ICS dose was used as the cut-off for high-dose (>2000 mg) and low-dose (1-2000 mg) ICS. DM and prediabetes were defined by American Diabetes Association criteria. The risk of ICS and its dose-response association on DM and prediabetes were evaluated with multivariate regressions adjusting for potential confounders including lifetime SS prescriptions and asthma severity factors. In otherwise healthy ICS users and controls (those without physician-diagnosed cardiometabolic diseases), the dose association of ICS on glucose tolerance and insulin resistance (HOMA-IR) were also studied.

Results: The mean age was 52.8±10.4 years and mean BMI was 23.7±3.7 with 41% males. The median (interquartile range) dose of ICS, duration of ICS use, and numbers of SS prescriptions were 2226 (991-3983) mg, 9 (5-13) years, and 4 (1-11) respectively. ICS users had a significantly lower risk for DM (adjusted OR=0.41, 95% CI: 0.28-0.59), with significant protective associations present in both low-dose (adjusted OR=0.45, 95% CI: 0.22-0.91) and high-dose ICS groups (adjusted OR=0.38, 95% CI: 0.17-0.86); Likewise for prediabetes (adjusted OR=0.35, 95% CI: 0.27-0.47), protective associations were seen in both low-dose (adjusted OR=0.40, 95% CI: 0.28-0.57) and high-dose ICS groups (adjusted OR=0.32, 95% CI: 0.22-0.45). Otherwise healthy ICS users had significantly lower HOMA-IR (adjusted mean difference, -0.20; P<0.001) and better glucose tolerance than healthy controls.

Conclusions: Adults asthmatics on long-term ICS show better glucose metabolic profile than controls from the general population.

76 Association of inhaled corticosteroids with insulin resistance, adiponectin and high-sensitivity C reactive protein in adults with asthma

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Introduction: Adiponectin and high-sensitivity C reactive protein (hs-CRP) are inflammatory biomarkers that have been shown to predict the development of diabetes mellitus (DM) and prediabetes, with high levels of adiponectin associated with reduced risk. In our study on the effect of inhaled corticosteroids (ICS) on glucose metabolism (ICSD Study), we showed that long-term ICS use is associated with reduced risk of DM and prediabetes in adults with stable asthma. In this study, we investigated the association of ICS on these predictive biomarkers in otherwise healthy asthmatics (without physician-diagnosed cardiometabolic diseases) who were long-term ICS users.

Methods: A total of 285 otherwise healthy asthmatics, aged ≥35-74 years, who used ICS regularly for ≥6 months were recruited from four asthma clinics from the territory of Hong Kong. We excluded those with exacerbation in recent 4 weeks or had taken systemic steroid (SS) in recent 6 months. Each asthmatic was matched 1:1 on age, gender and body mass index (BMI) with healthy controls from the Hong Kong Cardiovascular Risk Factors Prevalence Study II. All subjects had normal glucose tolerance on a 75-g oral glucose tolerance test. The lifetime cumulative budesonide dipropionate equivalent dose of ICS and numbers of SS prescriptions were ascertained. Cumulative median ICS dose was used as the cut-off for high-dose (>2000 mg) and low-dose (1-2000 mg) ICS. The dose associations of ICS with insulin resistance (HOMA-IR), adiponectin, and hs-CRP were evaluated with multivariate regressions adjusting for potential confounders including lifetime SS prescriptions and asthma severity variables.

Results: The mean age was 48±9.4 years, and mean BMI was 22.7±3.3 with 36% males. The median (interquartile range) dose of ICS, duration of ICS use, and the total dose of SS prescriptions were 2192 (848-3894) mg, 9 (5-13) years, and 4 (1-11.5) respectively. Long-term ICS use was significantly associated with a lower HOMA-IR for both low-dose (adjusted mean difference -0.32, P<0.001) and high-dose ICS groups (adjusted mean difference -0.40, P<0.001), compared with healthy controls. Besides, a significantly higher adiponectin level was found for both low-dose (adjusted mean difference 0.32, P<0.001) and high-dose ICS groups (adjusted mean difference 0.33, P=0.001). No significant association was found for hs-CRP.

Conclusions: Long-term ICS use is associated with significantly higher levels of adiponectin and lower insulin resistance, but not hs-CRP, in otherwise healthy asthmatics, compared with healthy controls.

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Epidermal growth factor stimulates cell proliferation by activating voltage-gated potassium channels in rat bone marrow–derived mesenchymal stem cells

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Introduction: We have previously found that voltage-gated delayed rectifier potassium current (IKDR, encoded by Kv1.2 and Kv2.1) participated in regulation of cell cycling progression in rat mesenchymal stem cells (MSCs) from bone marrow. The present study was designed to investigate whether epidermal growth factor (EGF) regulates cell growth is mediated by activating IKDR.

Methods: Whole-cell patch voltage-clamp, RT-PCR, Western blots, siRNA, cell proliferation assay were employed in the present study

Results: EGF increased cell proliferation in a concentration-dependent manner, and the effect was countered by the broad-spectrum protein tyrosine (PTK) inhibitor genistein and the EGFR kinase inhibitor AG556. We found that genistein and AG556 inhibited IKDR in a concentration-dependent manner. The protein tyrosine phosphatase (PTP) inhibitor orthovanadate enhanced IKDR, and counted the inhibitory effect of IKDR by genistein or AG556, suggesting the PTK-mediating modulation of IKDR. Interestingly EGF also increased IKDR. Downregulation of IKDR with siRNA targeting to Kv1.2 or Kv2.1 channels inhibited basal proliferation, and prevented EGF-stimulated proliferation in rat MSCs.

Conclusion: These results demonstrate for the first time that EGF stimulates cell proliferation activating IKDR, and silencing Kv1.2 or Kv2.1 channels prevents the augmentation of proliferation by EFG, indicating that Kv1.2 and Kv2.1 channels mediate EGF effect in regulating cell growth in rat MSCs.

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Effects of hepatitis B virus quasispecies and reverse transcriptase variants on treatment responsiveness to entecavir

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Introduction: Entecavir is an effective antiviral agent against hepatitis B virus (HBV). However, some patients may have slower response to entecavir. We aimed to determine whether there are HBV reverse transcriptase (rt) sequence polymorphism, as well as quasispecies complexity and diversity, which are associated with slower treatment response.

Methods: Pre-treatment HBV rt amino acid sequence from 370 patients who had entecavir therapy were determined by DNA sequencing. Sequencing data were associated with their virological outcome, as defined by optimal response (undetectable HBV DNA at year 1) or partial response (HBV DNA >60 copies/mL). Clonal sequencing was performed on the partial responders and optimal responders in a 2:1 ratio. Quasispecies diversity and complexity were determined.

Results: Twenty rt variants were detected exclusively in the partial responders. However, due to their rare occurrence, their association with entecavir response was not statistically significant. There were another 17 rt variants which existed in a significantly higher frequency in the partial responders than in the optimal responders. Multivariate analysis revealed that high baseline HBV DNA level, hepatitis B e antigen (HBeAg)–positivity and the rt variant rt124N were associated with partial entecavir response. HBV quasispecies complexity and diversity were higher in the optimal responders than in the partial responder.

Conclusions: We have identified 20 rt variants exclusively in the partial responders and 17 rt variant which had a significantly different distribution among the optimal and partial responders. High baseline HBV DNA, HBeAg-positivity and rt124N were associated with partial entecavir response at year 1.

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Role of HLA-DP polymorphisms on chronicity and disease activity of hepatitis B infection in the Chinese

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Introduction: The association between HLA-DP single nucleotide polymorphisms (SNPs) and chronic hepatitis B virus (HBV) infection varies between different populations. We aimed to study the association between HLA-DP SNPs and HBV infection and disease activity in the Chinese population of Hong Kong.

Methods: We genotyped SNPs rs3077 (near HLA-DPA1) and rs9277378 and rs3128917 (both near HLA-DPB1) in 500 HBV carriers (hepatitis B surface antigen [HBsAg]–positive), 245 non-HBV infected controls (HBsAg- and antibody to hepatitis B core protein [anti-HBc]–negative), and 259 subjects with natural HBV clearance (HBsAg-negative, anti-HBc-positive). Inactive HBV carriers state was defined by HBV DNA levels <2000 IU/mL and persistently normal alanine aminotransferase level for least 12 months.

Results: Compared to the non-HBV infected subjects, the HBV carriers had a significantly lower frequency of the rs3077 T allele (P=0.0040), rs9277378 A allele (P=0.0068) and a trend for lower frequency of rs3128917 T allele (P=0.054). Comparison between the HBV clearance subjects and HBV carriers showed that these alleles were associated with an increased chance of HBV clearance (rs3077: odds ratio [OR]=1.41, P=0.0083; rs9277378: OR=1.61, P=0.00011; rs3128917: OR=1.54, P=0.00017). Significant associations between HLA-DP genotypes and HBV clearance were also found under different genetic models. Haplotype TAT was associated with an increased chance of HBV clearance (OR=1.64, P=0.0013). No association was found between these SNPs and HBV disease activity.

Conclusion: Specific alleles and haplotypes of HLA-DP SNPs rs3077, rs9277378 and rs3128917 were associated with chronicity of HBV disease in the Chinese. Further studies are required to determine whether these SNPs influence the disease endemicity in different ethnic populations.

Application of COLD-PCR sequencing for the early detection of HBV antiviral drug resistant mutations

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Introduction: Polymerase chain reaction (PCR)–sequencing is often used to detect mutations in a DNA sequence. However, it is often limited by a lack of sensitivity when detecting minor mutations within a mixed pool of variant sequences. We aimed to adopt a modified CO-amplification at lower denaturation temperature–PCR (COLD-PCR) method, with a lower-than-normal denaturation temperature during PCR thermal cycle, for the early detection of hepatitis B virus (HBV) drug resistance mutations in patients treated with antiviral agents.

Methods: Detection sensitivity of COLD-PCR sequencing was determined using plasmids containing mixed proportions of HBV wild-type and mutant sequences. The performance of COLD-PCR sequencing in detecting drug resistance mutations was compared to that of normal PCR-sequencing and a line probe (LiPA) assay, using clinical samples obtained from 106 lamivudine-treated and 29 telbivudine-treated patients.

Results: Normal PCR-sequencing could detect mutations only if they exist in ≥25% in a mixed population of HBV plasmids, while COLD-PCR could detect mutations in as low as 5 to 10% of the population. All 135 (106 lamivudine and 29 telbivudine) patients had viral breakthrough at various yearly time-points and had drug resistance mutations rt204V/I detected by the LiPA assay. Among these patients, COLD-PCR sequencing could detect mutations in 95 (70%) patients and normal PCR could only detect mutations in 78 (58%) patients (P=0.031).

Conclusion: COLD-PCR sequencing is superior to normal PCR sequencing in detecting minor mutations in a mixed population, although both methods are less sensitive than the LiPA assay. Nevertheless, COLD-PCR sequencing can provide a simple and less expensive method to detect minor mutations.

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Ki67 proliferation index in invasive lobular carcinoma of the breast: clinicopathologic correlation and prognostic significance

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Introduction: Invasive lobular carcinoma (ILC) of the breast has distinct clinical and biological characteristics, including lower Ki67, when compared with invasive ductal carcinoma (IDC). Ki67 is an established independent negative prognosticator in IDC for disease-free survival and overall survival, although its role in ILC remains undefined due to lack of dedicated studies.

Methods: We analysed a consecutive cohort of ILC patients undergoing upfront surgery in a tertiary referral centre in Hong Kong between October 2000 and September 2008. Tumour Ki67 levels were correlated with various clinicopathological parameters and recurrence outcomes. Univariate and multivariate regression analyses were also performed.

Results: A total of 144 patients were included in the analysis. All were female, with a median age of 50 (range, 34 - 82) years. Higher Ki67 was significantly associated with higher tumour grade (Spearman correlation coefficient, r=0.2, P=0.028), tumour size (r=0.181, P=0.047), lymphovascular infiltration (r=0.218, P=0.031), and lymph node involvement (r=0.242, P=0.005). However, there was no significant correlation between Ki67 level and ER, PR or HER2 status. Moreover, Ki67 failed to emerge as an independent predictor of recurrence on univariate and multivariate regression analyses.

Conclusion: Although high levels of Ki67 correlated with poor-risk pathological features, it did not independently affect recurrence and had little prognostic role in ILC. Recurrence risk in ILC was not indicated by aggressiveness of individual primary tumours, but possibly by the lobular histology, which has a predilection for bilaterality, multicentricity and late recurrence thus reflects generalised predisposing changes in the breast epithelium.

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A single nucleotide polymorphism of interleukin-6 gene is related to plasma adrenomedullin levels

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Introduction: Plasma adrenomedullin (ADM) level is elevated in inflammation and is associated with cardiovascular diseases. Single nucleotide polymorphisms (SNPs) in the ADM gene have been found to be associated with ADM level. The 5′-flanking region of ADM is known to contain a consensus sequence of the binding site for the nuclear factor for interleukin-6 (IL-6). Therefore, we investigated if plasma ADM level is related to SNPs in the IL6 gene.

Methods: Plasma ADM level was measured in 476 subjects (236 men, 240 women; mean age, 50.9±10.8 years) in the Hong Kong Cardiovascular Risk Factor Prevalence Study-2 (CRISPS2). The subjects were genotyped for three tagging SNPs in the IL-6 gene.

Results: The minor allele frequencies of the IL6 SNPs rs17147230, rs1800796 and rs2069837 were 41.8%, 20.0% and 15.4%, respectively. The SNP rs17147230, was associated with plasma ADM level after adjusting for age and sex (β=-0.096, P=0.034). The association was significant in women (β= −0.115, P=0.021) but not in men. Amongst all subjects, plasma ADM level decreased with an increasing number of minor alleles of rs17147230 in multivariate analysis (P=0.034). Compared to subjects with AA genotype, subjects with TT genotype had a plasma ADM level 12.8% lower (95% confidence interval: 0.6%-23.5%, P=0.041).

Conclusion: Plasma ADM level is related to the SNP rs17147230 in IL6 gene. The effect of the polymorphism on inflammation and cardiovascular disease remains to be determined.

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Plasma level of adrenomedullin is influenced by a single nucleotide polymorphism in the adiponectin gene

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Objective: Adrenomedullin (ADM) and adiponectin are both adipokines associated with inflammation. The plasma level of these peptides is influenced by single nucleotide polymorphisms (SNPs) in the *ADM* and *ADIPOQ* genes, respectively. There is some evidence that ADM may regulate adiponectin gene expression, but whether adiponectin can regulate ADM expression is unclear. We investigated if *ADIPOQ* SNPs influence plasma ADM level.

Methods: Plasma ADM level was measured in 476 subjects in the Hong Kong Cardiovascular Risk Factor Prevalence Study-2 (CRISPS2). We genotyped them for two *ADIPOQ* SNPs that are known to be associated with plasma adiponectin level.

Results: The minor allele frequencies of *ADIPOQ* SNPs rs182052 and rs12495941 were 40.6% and 42.2%, respectively. Plasma ADM level was associated with rs182052 after adjusting for age and sex (β=0.104, P=0.023). In multivariate analysis, plasma ADM level increased with the number of minor alleles of rs182052 carried (P=0.013). Compared to subjects with GG genotype, subjects with AA genotype had 17.7% higher plasma ADM level (95% confidence interval: 3.6%-33.7%, P=0.013). In subgroup analysis, the association remains significant in diabetic patients (β=0.344, P=0.001) but not in normal subjects.

Conclusion: Plasma ADM level is related to SNP rs182052 in the *ADIPOQ* gene. The interaction between these two important peptides involved in obesity and inflammation warrants further study.

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EBUS-TBNA gives adequate tissue information on cell type in lung cancer

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Introduction: In formulating systemic treatment in patients with advanced stage lung cancer, it is now considered imperative to know the cell type such as squamous carcinoma, adenocarcinoma and large cell carcinoma as chemotherapeutic agents would be tailored to treat different cell types. In the authors' centre, the adoption of using epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKI) as the first-line treatment has been the treatment of choice in patients shown to have activated EGFR mutation. Adequate tissue obtained during diagnostic procedures for cell typing and molecular profiling is therefore important in formulating personalised treatment in lung cancer patients nowadays.

Methods: Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) was performed under local anaesthesia in patients presented with mediastinal abnormality suspected of or confirmed lung cancer for diagnosis and staging purpose. Once malignancy was confirmed from the pathological materials, exact cell type, origin of tumour, differentiation were recorded. In the later phase of the study period since October 2008, molecular profiling was also deployed to confirm the EGFR mutation, ALK translocation status and Kras.

Results: Over the 4 years' study period started from August 2006, there were 269 EBUS-TBNA performed in 258 patients (median age 62 years, male 63.2%). Of the 209 patients (81%) confirmed having malignancy as their final diagnosis, EBUS-TBNA was able to detect malignancy in 162 patients, 25 (15.4%) of whom were having extrathoracic malignancy (breast, head and neck, renal, gastrointestinal, hepatobiliary, uterus and leiomyosarcoma) and primary lung cancer in 133 (63.6%). Among those 133 patients confirmed having primary lung cancer, 117 (88.0%) had exact cell type delineated: adenocarcinoma 50.4%, squamous cell carcinoma 13.5%, small cell carcinoma 10.5%, large cell carcinoma 6.8%, poorly differentiated carcinoma 6.0%, mucoepidermoid carcinoma 0.8%. For those 40 patients who had molecular profiling performed, patients with adequate tissue for EGFR mutation and/or ALK translocation and Kras mutation were obtained in 38 (95.0%). Of the 162 patients confirmed to have malignancy by EBUS-TBNA, only 20 (12.3%) had revealed non–small-cell lung cancer without knowing the exact cell type, differentiation of the tumour, EGFR status or primary origin of the tumour. In the 209 patients with final diagnosis of malignancy, the sensitivity was 87.4.0% and negative predictive value was 74.0%.

Conclusion: EBUS-TBNA is effective in subtyping of tumour cells and molecular profiling in patients with lung cancer.

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Simple clinical risk score model in prediction of incident diabetes mellitus: a population-based 13-year prospective study in Hong Kong

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Objective: To develop a simple risk score model for estimating 13-year risk of type 2 diabetes (T2DM) for Southern Chinese.

Methods: A total of 2499 subjects who had oral glucose tolerance tests (OGTT) done at baseline and reassessment visits of the Hong Kong Cardiovascular Risk Factor Prevalence Study (CRISPS, 1995-6 and CRISPS-3, 2005-2008) were included for analysis. There were 205 new T2DM cases after a median interval of 13.0 years. Logistic regression models were used to predict incident T2DM, starting with an easy-to-measure clinical characteristics model (ECC), followed by more complex ones which included fasting glucose (ECC+IFG) and OGTT (ECC+IFG/IGT), based on a mathematically derived point-scoring system. Receiver operating characteristic (ROC) curve was used to assess the performance of different methods for diabetes risk prediction.

Results: Clinical characteristics with statistically significant difference between the incident diabetes and the non-diabetic group (age, family history of T2DM, body mass index, and hypertension) and those of clinical relevance (smoking and physical activity) were included in the ECC model. This model showed good predictive performance for development of T2DM with an area under ROC curve (AUC) of 0.74 (95% confidence interval: 0.71-0.78). More complex model did not (ECC+IFG, AUC=0.76, P=0.07) or only modestly improved (ECC+IFG/IGT, AUC=0.79, P<0.05) the predictive performance of the ECC model. Based on ROC curve analysis, a risk score of 6 should be the optimal cut-off for the ECC model. The 13-year T2DM risk in subjects with a risk score of more than 6 is ≥8% (sensitivity 74.1%, specificity 62.0%).

Conclusions: Simple and easy-to-measure clinical characteristics, which can be self-checked by the general public, effectively predict diabetes. They could be used to construct a point scoring T2DM prediction algorithm to estimate the risk of new T2DM in Southern Chinese.

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Lupus monocyte-derived dendritic cells treated with 1α,25-dihydroxyvitamin D3 and dexamethasone are tolerogenic and induce IL-10 producing regulatory T cells

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Background: The pathogenesis of systemic lupus erythematosus (SLE) is characterised by dysregulated dendritic cells (DCs) and autoreactive T and B cells.

Objective: To examine if alternatively activated DCs (aaDCs) derived from SLE patients possess tolerogenic properties and to delineate the underlying mechanisms.

Methods: CD14+ monocytes from SLE patients and healthy subjects were cultured under IL-4 and GM-CSF to generate DCs. aaDCs generated by combination of 1α,25-dihydroxyvitamin D3 (vitD3) and dexamethasone were examined for phenotype and tolerogenic functions compared with lipopolysaccharide (LPS)–matured DCs (matDCs). Activation markers and intracellular cytokine expression of T cells and/or DCs were measured by flow cytometry. ELISA was used to measure cytokine levels in supernatant. Western blot was performed for detection of RelB protein expression.

Results: Compared with matDCs, lupus aaDCs displayed semi-mature phenotype with low level of expression of co-stimulatory molecules and maturation markers (CD83, CD40) that remained stable despite challenge by CD40L, CpG-DNA, and SLE serum. Lupus aaDCs also possessed tolerogenic phenotype with suppressive effect on allogeneic T cell activation (CD25 and CD69 expression) and proliferation. These aaDCs polarised naïve CD45RA+T cells into T effector cells with IL-10+FoxP3+ phenotype and skewed memory CD45RO+ T cells to less inflammatory phenotype with reduced expression of IFN-γ and IL-17. Compared with matDCs, aaDCs were found to express reduced level of RelB, a transcription factor regulating DC differentiation and maturation.

Conclusion: Lupus aaDCs demonstrated tolerogenic properties with induction of IL-10 producing T cells that have regulatory functions. Reduced expression of RelB in aaDCs may be the underlying mechanism for their tolerogenicity.

87 Plasmacytoid dendritic cells exhibit elevated TLR7 responses in diseased New Zealand black/white F1 mice

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Introduction: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease complicated by the involvement of multiple organ systems and its diverse clinical manifestations. Typically, SLE patients have been characterised by the presence of high titres of type I interferon (IFN) and anti-nuclear antibodies (ANA) in their sera. Plasmacytoid dendritic cells (pDCs), also known as the most potent natural type I IFN-producing cells, are considered as the key pathogenic factor in SLE. The New Zealand black/white (NZB/W) F1 mouse is a well-established lupus model as it spontaneously develops serum ANA followed by lupus nephritis and immune complex depositions in the kidneys. In this study, the NZWB/W F1 mice have been employed for investigations on the pathogenic involvement of pDCs in SLE, hypothesising that pDCs from an SLE origin are more responsive to TLR stimulations.

Methods: The mice were divided into two groups based on their physiological status. For mice older than 30 weeks with high ANA and positive proteinuria, they were classified as diseased mice. Those aged 10-to-15 weeks old and negative for both parameters were defined as pre-diseased and used as controls. Age-matched maternal strains, NZW mice, for each group were sacrificed as controls for the age-difference factor. Bone marrow (BM)–derived pDCs were generated with mouse Flt-3 ligand and their responses to toll-like receptor (TLR)-7 and TLR-9 stimulations were compared between groups.

Results: Pre-diseased and diseased mice have similar percentages of haematopoietic precursor cells in the BM, which gave rise to comparable number of pDCs after 8 days of culture. However, elevated TLR7 responses were observed in pDCs derived from BM of diseased mice. There were significantly increased up-regulations of MHC class II and CD40 expressions in TLR7 stimulated pDCs from diseased F1 mice comparing to those from pre-diseased mice ($P<0.01$, $n=8$). Interferon alpha and interleukin (IL)-6 secreted by TLR-7 and TLR-9 stimulated pDCs were evaluated. No significant differences were found between the diseased and pre-diseased mice. BM-derived pDCs from the NZW mice had no differences in response to TLR7 and TLR9 despite their age difference.

Conclusion: Results in this study demonstrated that pDCs in SLE exhibit heightened responses to TLR7 activation. This suggests that the pathogenic role of pDCs in SLE may be relevant to a potentially defective TLR7 pathway.

88 Long-term outcomes of lupus nephritis patients treated with corticosteroids and mycophenolate mofetil

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Introduction: Corticosteroids and mycophenolate mofetil (MMF) have proven efficacy when used as induction and maintenance treatment for proliferative lupus nephritis (LN), but the long-term data of this immunosuppressive regimen are awaited.

Methods: We conducted a single-centre retrospective study on patients with Class III/IV±V LN who have received prednisolone and MMF continuously for induction and maintenance immunosuppression.

Results: In this study, 65 patients were included; 31 patients were treated with prednisolone and MMF throughout (Group I). 23 patients had their MMF replaced with azathioprine (AZA) [Group II] and 11 patients with calcineurin inhibitors (CNI) [Group III] at some point during the maintenance phase. The follow-up was 91.9±47.7 months. The 10-year patient and renal survival rates were 91% and 86%, respectively, with no difference between the three groups. Group I patients had higher relapse-free survival than Group II and Group III patients (76% vs 56% vs 43% respectively at 5 years; 69% vs 32% vs 0% respectively at 10 years; I vs II, $P=0.049$; I vs III, $P=0.019$; II vs III, $P=0.490$). Patients who received MMF for >24 months showed superior relapse-free survival than those treated for shorter durations (88% vs 48% respectively at 5 years; 81% vs 28% respectively at 10 years; $P<0.001$). Group I patients had better renal function at 10 years. The side-effects profile was similar between the three groups except more anaemia observed in the Group I.

Conclusion: Continuous treatment with corticosteroids and MMF as both induction and long-term maintenance immunosuppression confers favourable long-term outcome. Treatment with corticosteroids and MMF for >24 months is advisable in view of the lower risk of relapse.

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(This abstract was withdrawn.)

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Biomarker analysis from a phase I/II study of foretinib, an oral multikinase inhibitor targeting MET, RON, AXL, TIE-2 and VEGFR in advanced hepatocellular carcinoma

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Introduction: A phase I/II dose-escalation and expanded-cohort study of the safety, tolerability, pharmacokinetics, and efficacy of foretinib (GSK163089) administered daily to Asian patients with advanced hepatocellular carcinoma (HCC) was conducted. A cytokine and angiogenic factor (CAF) biomarker panel was evaluated before and during foretinib treatment.

Methods: Efficacy was evaluated by modified RECIST criteria for HCC. Patients treated at the maximum tolerated dose of 30 mg QD with samples available for CAF evaluation were analysed (n=38). Plasma samples pre-dosing at days 1, 8, 15, and 22 were obtained. 26 CAF markers were assessed: Ang2, BMP9, Clusterin, ESelectin, EGF, FasL, HGF, FGF, IGFBP1, IGFBP3, IL6, IL8, MMP9, OPN, PLGF, SCF, TIMP2, TRAIL, TSP2, VCAM1, VEGF, VEGFR2, ECadherin, fibronectin, TGF-beta 1, thrombomodulin. CAF assays were conducted using SearchLight (Aushon Biosystems). Baseline covariates were evaluated. Statistical analysis was conducted using Cox regression, ANOVA, and spearman correlation; $P<0.01$ was considered statistically significant.

Results: Median time-to-progression and overall survival (OS) were 4.2 months (95% confidence interval, 2.8-8.2) and 15.7 months (7.0-not reached), respectively. In univariate OS models, higher baseline levels of IL8 ($P<0.0001$), IL6 ($P=0.0003$), TSP2 ($P=0.0015$), MMP9 ($P=0.0072$), and IGFBP1 ($P=0.0075$) were associated with shorter OS. When divided by quartiles (Q), there were no deaths (all patients censored) among patients in the lowest Q for IL6 or IL8 (n=9); in the highest Q there were 10/10 and 9/10 deaths, respectively. In multivariate models, baseline IL8 and IL6 were independent predictors of OS. In a combined model of IL8 and IL6 a group of patients with no deaths (n=16 patients all censored) versus a group with 17 deaths (and 5 censored) was identified.

Conclusion: In Asian advanced HCC patients treated with foretinib, IL8 and IL6 are independent predictors of OS, individually, and in a combined model. Additional studies are needed to confirm this finding and determine if predictive or prognostic.

91 Phase 1 study investigating everolimus combined with sorafenib in patients with advanced hepatocellular carcinoma

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Introduction: Sorafenib is the only therapy shown to improve overall survival in patients with advanced hepatocellular carcinoma (HCC). Combination therapy targeting multiple signalling pathways may improve outcomes in advanced HCC.

Methods: A phase 1 study of the mTOR inhibitor everolimus plus sorafenib 400 mg twice daily was performed in patients with measurable, advanced HCC naive to systemic therapy. Everolimus was initiated at 2.5 mg once daily and increased per a Bayesian sequential dose-escalation scheme based on the dose-limiting toxicities (DLTs) experienced within the first 28 days of treatment. Primary endpoint was determination of the maximum tolerated dose (MTD) of everolimus in combination with sorafenib. Secondary endpoints included tolerability and efficacy.

Results: Thirty patients were enrolled; 25 were evaluable for MTD determination. One of 12 patients treated with everolimus 2.5 mg once daily and 6 of 13 patients treated with everolimus 5.0 mg once daily experienced a DLT, most commonly thrombocytopenia (n=5). All patients experienced ≥1 adverse event, most commonly diarrhoea (66.7%), hand-foot skin reaction (66.7%), and thrombocytopenia (50.0%). Best overall response was stable disease (62.5% and 42.9% in the 2.5-mg and 5.0-mg cohorts, respectively). Median time to progression and overall survival in the 2.5-mg cohort were 4.5 months and 7.4 months, respectively, and 1.8 months and 11.7 months, respectively in the 5.0-mg cohort.

Conclusion: The everolimus MTD in combination with sorafenib 400 mg twice daily was 2.5 mg once daily. The inability to achieve a biologically effective everolimus concentration at the MTD precluded phase 2 study of this combination.

92 A multi-centre phase II trial of bevacizumab (B) pre- and post-transarterial chemoembolisation treatment for patients with localised unresectable hepatocellular carcinoma: interim safety report

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Introduction: Transarterial chemoembolisation (TACE) prolongs survival for selected patients with unresectable hepatocellular carcinoma (HCC). However, a significantly elevated circulating VEGF level, which contributed to neovascularisation and tumour re-growth, was reported following TACE. VEGF has a fundamental role in tumour angiogenesis and is targeted by the humanised mAb bevacizumab (B). Given the short half-life of B (1.4 days) and a peak circulating VEGF level days after TACE, this trial investigated the feasibility and tolerability of adjunctive B pre- (within 24-48 hours) and post-TACE (2-weekly) for optimised inhibition of tumour angiogenesis.

Methods: Untreated patients with localised unresectable HCC received standard TACE (8-10 weekly) and B (5 mg/kg 2-weekly intravenously). B was given 24 to 48 hours prior to each TACE. Both TACE and B were continued until PD or unacceptable toxicity. The primary endpoint was progression-free survival by RECIST. Other endpoints were OS, ORR, tumour necrosis rate and safety. Blood samples were collected to monitor circulating EPC and VEGF.

Results: A total of 29 Asian patients were enrolled between February 2008 and February 2010. Up to the time of this report, patients have received a median of three TACE sessions (range, 1-10) and a median of 12 B cycles (range, 1-41).Twelve patients remained on the study. Most treatment-related toxicities (TRTs) were grade 1 or 2; grade 3/4 TRTs were hypertension (n=4), tumour rupture (n=2), and chest discomfort, right groin pseudoaneurysm, AST elevation, malaise, hyperbilirubinaemia, thrombocytopenia and abdominal pain (all n=1). Neither tumour rupture was major; both tumours were subcapsular; one was an internal rupture and one responded well to TACE. No variceal bleeding was observed. Six deaths occurred mostly due to advanced disease, but none due to TRT.

Conclusion: The current data suggest that it is safe to use B as an adjunctive agent pre- and post-TACE. Further trials should be considered to compare the survival of inoperable HCC patients undergoing TACE with and without adjunctive B.

Serum FGF21 is a sensitive prognostic biomarker of acute liver injury in mice and men

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Background and Aims: Fibroblast growth factor 21 (FGF21) is a liver-secreted hormone with pleiotropic effects on energy metabolism and insulin sensitivity. We aimed to investigate the potential role of serum FGF21 as a biomarker for acute liver injury (ALI) in both rodents and humans.

Methods: Mouse models of ALI were induced by peritoneal injection of carbon tetrachloride (CCl4, 1 mL/kg) and acetaminophen (APAP, 300 mg/kg). Two independent cohorts of liver transplantation patients were recruited for the collection of blood samples at various time points.

Results: In both CCl4- and APAP-treated mice, serum FGF21 levels were markedly elevated by over 60-fold, which occurred before the detectable increase of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST). The marked elevation of serum FGF21 was due to the dramatic increase in hepatic FGF21 expression, but not due to hepatocyte damage. In patients receiving liver transplantation, a dramatic increase in serum FGF21 levels (approximately 25-fold) was observed as early as 2 hours after surgery, whereas the peak levels of serum ALT and AST caused by ischaemia/reperfusion injury were detected only after 24 hours. Temporal correlation analysis demonstrated a significant association of peak serum levels of FGF21 at 2 hours with the magnitude of the increase in both serum ALT and AST levels at 24 hours after liver transplantation, indicating a predictive value of serum FGF21 for liver ischaemia/reperfusion injury.

Conclusion: Serum FGF21 may represent a sensitive and specific prognostic biomarker for early detection of ALI in rodents and humans.

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Clinical features, management, and prognostic factors of status epilepticus in Chinese

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Introduction: Status epilepticus (SE) is a neurological emergency with significant mortality and morbidity. There are currently limited data regarding the causes and outcomes of SE in our locality, and identification of prognostic factors, especially those available at presentation, could lower risk of under- or over-treatment in SE.

Methods: We retrospectively studied the clinical characteristics, management, and clinical outcome of adults diagnosed with incident SE, excluding episodes due to cerebral anoxia, at a regional hospital in Hong Kong during 1 January 2007 to 31 December 2011.

Results: A total of 38 patients with incident SE were identified during the study period. The mean age was 58±22 years and 61% were males. Underlying cerebrovascular disease (34%), poor compliance to anti-convulsants in patients with known epilepsy (16%), and infection of the central nervous system (11%) were the main causes of SE. SE was associated with 21% mortality during hospitalisation period and 32% mortality within 6 months of admission. Age≥65 years, absence of prior history of seizures, a higher blood glucose level during SE and a Status Epilepticus Severity Score (STESS)≥4 were associated with 6-month mortality ($P<0.05$). Multivariate analysis subsequently identified a STESS≥4 as an independent predictor of poor prognosis (odds ratio=8.6, 95% confidence interval: 1.2-61.2, $P=0.03$).

Conclusions: SE in adults is most commonly due to underlying cerebrovascular disease and is associated with a high mortality. The STESS is a useful tool in predicting 6-month mortality.

Obesity-related biomarkers in predicting cancer development

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Introduction: In obese subjects, cytokines released from adipocytes induce chronic low-grade systemic inflammation, which may enhance the development of malignancies. We examined the relationship between baseline adipokines level and incident cancer risk in a community-based cohort of Chinese subjects.

Methods: Subjects were recruited from the Hong Kong Cardiovascular Risk Factors Prevalence Study 2 (CRISPS 2) cohort. Adipokines including interleukin-6 (IL-6), soluble tumour necrosis factor receptor 2 (sTNFR2, as a surrogate marker of tumour necrosis factor-alpha), leptin, lipocalin 2, adiponectin and adipocyte–fatty acid binding protein (A-FABP) levels were measured at baseline. Incident cancer cases were identified after a median follow-up of 9.6 years.

Results: A total of 1897 subjects were included in the final analysis. During the study period, 99 subjects developed cancers. For the baseline biomarkers measurements, subjects who developed cancers had significantly higher level of hsCRP, IL-6, sTNFR2 and lipocalin 2. After adjustment for conventional risk factors, only baseline pro-inflammatory adipokines IL-6 (hazard ratio [HR]=1.51, 95% confidence interval [CI]: 1.16-1.97) and sTNFR2 (HR=2.36, 95%CI: 1.16-4.81) predicted cancer development.

Conclusion: Our data supported the hypothesis that chronic low-grade systemic inflammation caused by obesity could increase the risk of malignancy.

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Skin autofluorescence correlates with microvascular complications in type 2 diabetes

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Introduction: Skin autofluorescence (AF) is a marker of advanced glycation endproducts (AGEs) accumulation in the body, a process which has been implicated in diabetic complications. We investigated the relationship between skin AF and microvascular complications in type 2 diabetic subjects.

Methods: Subjects were recruited from the diabetic complication assessment programme of our hospital. Skin AF was measured over the volar surface of the forearms using the AGE Reader. Anthropometric and biochemical data, including HbA1c, were collected. Statistical analysis was performed with SPSS 19.0.

Results: Skin AF was measured in 322 Chinese type 2 diabetic subjects (192 male, 130 female, 63.9±11.0 years), of which 229 had one or more microvascular complication(s). The median diabetes mellitus (DM) duration was 10 years (interquartile range, 6-15 years) and the mean HbA1c was 7.84±1.35%. Skin AF correlated positively with age (r=0.350, P<0.001), DM duration (r=0.256, P<0.001), serum creatinine (r=0.280, P<0.001) and smoking pack-years (r=0.287, P=0.006), but not HbA1c (P=0.306). Skin AF was higher in subjects with any microvascular complication (2.41±0.48 AU vs 2.17±0.37 AU, P<0.001), retinopathy (2.41±0.46 AU vs 2.26±0.45 AU, P=0.006), nephropathy (2.49±0.49 AU vs 2.20±3.98 AU, P<0.001) and neuropathy (2.56±0.51 AU vs 2.27±0.42 AU, P<0.001) compared to those without the respective complication. Skin AF was independently associated with nephropathy (OR for 1AU increase in AF=2.65 [1.42-4.95]; P=0.002) and neuropathy (OR for 1 AU increase in AF=2.28 [1.15-4.54]; P=0.019) after adjusting for gender, age, smoking status and DM duration. The optimal skin AF cut-off values for having retinopathy, nephropathy, neuropathy or any microvascular complication were 2.0475 AU (sensitivity 83.2%, specificity 33.0%), 2.263 AU (sensitivity 68.8%, specificity 62.0%), 2.307 AU (sensitivity 70.1%, specificity 57.6%), and 2.263 AU (sensitivity 59.8%, specificity 63.6%) respectively on ROC analysis.

Conclusion: Skin AF correlates with diabetic complications, in particular nephropathy and neuropathy, in Chinese type 2 diabetic subjects. The AGE Reader might serve as a simple and non-invasive method to evaluate the risk of diabetic microvascular complication.

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Adipocyte fatty acid binding protein deficiency protects mice against diabetic cardiomyopathy and myocardial ischaemia/reperfusion induced cardiac injury

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Introduction: Diabetes increases the risk of heart dysfunction by inducing cardiomyopathy. We have previously demonstrated that serum levels of adipocyte fatty acid–binding protein (A-FABP), a fat-derived adipokine, increased significantly in patients with heart failure and are independently associated with the deterioration of heart function. This study investigated the role of A-FABP in the pathogenesis of diabetic cardiomyopathy and myocardial ischaemia/reperfusion (MI/R) induced cardiac injury.

Methods: Mice genetically deficient in A-FABP (A-FABP-/-) and their wild-type (WT) littermates were randomly allocated to non-diabetic and streptozotocin (STZ) induced diabetic groups. Each group contains sham operated mice and mice with MI/R injury. Left ventricular function was evaluated by pressure-volume loops. Cardiomyocyte apoptosis and superoxide production were measured by immunohistochemistry. A-FABP mRNA and protein expression in heart tissue were determined by real time quantitative PCR and Western blot analysis.

Results: After MI/R, WT mice showed increased myocardial infarct size, apoptotic index, and superoxide production which accompanied by decreased left ventricular function compared with A-FABP-/- mice either in non-diabetic or diabetic groups. Both mRNA and protein expression levels of A-FABP in heart tissue were significantly increased after MI/R injury and diabetes induction. Immunofluorescence staining showed that A-FABP was colocalized with endothelial marker CD31. eNOS phosphorylation and NO production in the heart tissue decreased significantly in WT mice after MI/R while elevated significantly in that of A-FABP-/- mice after MI/R injury and diabetes induction.

Conclusion: A-FABP deficiency protects mice against diabetic cardiomyopathy and myocardial ischemia/reperfusion induced cardiac injury, which may through inducing eNOS phosphorylation and NO production in the heart tissue.

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