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25th Medical Research Conference, 18 January 2020

Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

ABSTRACT	TITLE	PAGE
1	**Angiotensin-converting enzyme inhibitors/angiotensin receptor blockers and risk of colorectal cancer after negative baseline colonoscopy: a territory-wide study with propensity score analysis** *KS Cheung, L Chen, EW Chan, WK Seto, ICK Wong, WK Leung*	6
2	**Natural clinical course of progressive supranuclear palsy in Chinese patients in Hong Kong** *YF Shea, ACK Shum, SC Lee, PCK Chiu, KS Leung, YK Kwan, CKF Mok, HWF Chan*	6
3	**Prevalence of cognitive impairment among peritoneal dialysis patients: a systematic review and meta-analysis** *YF Shea, SC Lee, MYM Mok, FHW Chan, TM Chan*	7
4	**Mouse model of neuromyelitis optica spectrum disorders with aquaporin-4 autoimmunity and spinal cord pathologies** *LW Yick, KH Chan*	7
5	**Controlled attenuation parameter measurements predict fibrosis progression and hepatitis B surface antigen seroclearance in virologically quiescent chronic hepatitis B: a prospective study with paired transient elastographies** *LY Mak, RWH Hui, J Fung, MF Yuen, WK Seto*	8
6	**Targeting polyamines for treatment of malignant pleural mesothelioma in xenograft models** *SK Lam, S Yan, JCM Ho*	8
7	**Protease-activated receptor-1 antagonist vorapaxar ameliorates kidney injury and tubulointerstitial fibrosis in experimental obstructive nephropathy** *SWY Lok, WH Yiu, WH Liu, H Li, R Xue, LYY Chan, JCK Leung, KN Lai, SCW Tang*	9
8	**Long intergenic non-coding RNA p21 mediates lipotoxicity-induced kidney injury** *B Li, JCK Leung, LYY Chan, WH Yiu, KN Lai, SCW Tang*	9
9	**Prevalence of beta-lactam allergies and factors predicting genuine allergies in Hong Kong** *PH Li, LQC Siew, I Thomas, TJ Watts, KL Ue, K Rutkowski, CS Lau*	10
10	**Aetiologies of idiopathic anaphylaxis and importance of allergological evaluation in Hong Kong** *PH Li, CY Wong, CS Lau*	10

ABSTRACT	TITLE	PAGE
11	**Altered expression of immune regulatory receptors upon combination of chemotherapy and checkpoint blockade immunotherapy in a lung cancer mouse model** *S Yan, SK Lam, JCM Ho*	11
12	**Perturbed ERK-DRP1 signalling contributes to impaired mitophagy in parkinsonian leucine-rich repeat kinase 2^{R1441G} mutant mice** *PWL Ho, HF Liu, CT Leung, EES Chang, YK Choi, SYY Pang, SL Ho*	11
13	**Role of brain-derived neurotrophic factor in cigarette smoke-exposed rats** *AKC Chan, JCW Mak*	12
14	***Dendrobium officinale* polysaccharides attenuate cigarette smoke–induced airway inflammation in vivo** *YM Liang, RX Dui, R Chen, PH Chu, MSM Ip, Y Zhang, JCW Mak*	12
15	**Bidirectional role of peptidase M20 domain containing 1 in the regulation of body weight** *R Yang, L Xu, X Yan, Z Huang, A Xu*	13
16	**Leisure time, occupational aerobic physical activity, and mortality risk in adults of United States: National Health Nutrition and Examination Survey 2007-2016** *B Or, MF Tsoi, TT Cheung, BMY Cheung*	13
17	**Decreased survival in young adults with stage 1 and 2 hypertension in the National Health and Nutrition Examination Survey III** *B Or, MF Tsoi, TT Cheung, BMY Cheung*	14
18	**Leucine-rich repeat kinase 2 kinase inhibitor affects phosphorylation of alpha-synuclein at serine-129 in vitro and in vivo** *EES Chang, PWL Ho, HF Liu, CT Leung, YK Choi, SYY Pang, SL Ho*	14
19	**Biological effects of a highly focused, scanned, near-infrared laser on dermal pigment** *D Manstein, H Chan, L Bowes, J Ting, V Zuo, I Erenburg, J Bhawalkar, R Anderson*	15
20	**Upregulation of peptidyl arginine deiminase 4 in neutrophil in systemic lupus erythematosus** *J Ma, CF Ko, JX Chow, CS Lau, VSF Chan*	15
21	**Interferon-alpha induces AIM2 inflammasome response in systemic lupus erythematosus patients** *JX Chow, IKY Lam, SYY Wong, CS Lau, VSF Chan*	16
22	**Toll-like 4 receptor signalling modulates long non-coding RNA LINC02207 isoforms expressions in systemic lupus erythematosus** *CF Ko, J Ma, S Wang, CS Lau, VSF Chan*	16
23	**Human leukocyte antigen–B*5801 screening to avoid allopurinol-induced severe cutaneous adverse reactions in Chinese patients with chronic kidney disease: a prospective study** *SM Wong, JCY Chan, SCW Tang, BCY Cheung, J Kwok, MWM Chan, HHL Chan, CK Yeung*	17

ABSTRACT	TITLE	PAGE
24	**Long non-coding RNA LINC02207 in systemic lupus erythematosus** *S Wang, CF Ko, J Ma, CS Lau, VSF Chan*	17
25	**Role of orosomucoid 1–like protein 3 on cigarette smoke–induced airway inflammation, mucus hypersecretion, and activation of the unfolded protein response in human airway epithelial cells** *R Chen, MSM Ip, JCW Mak*	18
26	**Serum growth differentiation factor 15 levels are closely associated with the progression of non-alcoholic fatty liver disease** *X Yan, Z Huang, R Yang, L Xu, A Xu*	18
27	**Potential therapeutic role of interleukin 9 in a mouse lung cancer model** *Y Feng, S Yan, SK Lam, JCM Ho*	19
28	**Targeting PIN1 as a potential novel therapeutic strategy against activated B cell–like subtype of diffuse large B cell lymphoma** *CW Cheng, D Chau, LM Yue, E Tse*	20
31	**Safety and efficacy of lithium carbonate as the second-line antithyroid drug in the treatment of Graves' disease: a retrospective case series in Hong Kong Chinese patients** *ACH Lee, CH Lee, JKY Lam, YC Woo*	21
32	**Systemic therapy treatment outcomes for recurrent hepatocellular carcinomas following liver transplants in Hong Kong** *BCW Li, GW Kwok, JWY Chiu, RCY Leung, GKB Shing, V Tang, TCC Yau*	21
33	**Exploring the utility of auto-antibodies in immune checkpoint blockade for advanced cancers** *GW Kwok, BCW Li, RCY Leung, JWY Chiu, TCC Yau*	22
34	**Exercise improves cardiac dysfunction via fibroblast growth factor 21–sirtuin3 signalling in type 2 diabetic mice** *LG Jin, LL Geng, L Ying, Y Pan, R Yang, WH Woo, A Xu*	22
35	**Interaction between genetic variants and haemoglobin A1c on the risk of sight-threatening diabetic retinopathy** *KKK Ng, CH Lee, YC Woo, WS Chow, RLC Wong, CHY Fong, A Xu, PC Sham, KSL Lam, CYY Cheung*	23
37	**Role of G protein-coupled receptor 110 in the pathogenesis of obesity** *Z Huang, L Xu, R Yang, X Yan, S He, A Xu*	24
38	**Metabolic factors and adipokine levels are associated with fibrosis evolution in chronic hepatitis B patients on nucleoside analogue therapy** *RWH Hui, LY Mak, CH Lee, J Fung, DKH Wong, A Xu, KSL Lam, MF Yuen, WK Seto*	25
39	**Mechanosensitive ion channel Piezo1 controls cell fate determination of bone marrow mesenchymal stem cells** *B Wang, LY Cheong, Y Ma, XY Hui, E Honore, A Xu*	26
40	**Detection of hepatitis B virus covalently closed circular DNA and integrated hepatitis B virus DNA in hepatitis B surface antigen–negative hepatocellular carcinoma patients** *DKH Wong, S Cheng, LY Mak, E To, RCL Lo, TT Cheung, WK Seto, J Fung, K Man, CL Lai, MF Yuen*	26
41	**Inducing beige adipocyte from human-induced pluripotent stem cells without gene transfer** *Y Cheng, XF Gao, PT Liu, XY Hui, A Xu*	27
42	**Risk factors of community-acquired pneumonia in patients with spondylarthritis** *HY Chung, PY Fong, CL Lau, HY Nip; Hong Kong Society of Rheumatology*	27
43	**Adipocytes regulate osteogenesis in a novel lipoatrophic mouse model** *LY Cheong, Z Liu, B Wang, Y Ma, A Xu*	28
44	**Incidence of cancer in patients taking valsartan or amlodipine for ≥1 year** *TH Chan, MF Tsoi, BMY Cheung*	28

ABSTRACT	TITLE	PAGE
45	**Adipose tissue–targeted liposomal drug delivery system for treatment of obesity** *K Chen, W Wang, XY Hui, A Xu*	29
46	**Cardiovascular outcomes in trials of new antidiabetic drug classes** *Y Fei, MF Tsoi, BMY Cheung*	29
47	**Efficacy and safety of $P2Y_{12}$ inhibitors in patients with acute coronary syndrome: a network meta-analysis** *Y Fei, CK Lam, BMY Cheung*	30
48	**Optimal duration of dual antiplatelet therapy after drug-eluting stents implantation: a network meta-analysis of randomised controlled trials** *Y Fei, MF Tsoi, BMY Cheung*	30
49	**Association between gout and depression: The United States National Health Nutrition and Examination Survey 2007-2016** *MF Tsoi, BMY Cheung, CS Lau, TT Cheung*	31
50	**Efficacy of Janus kinase inhibitors or biologic disease-modifying antirheumatic drugs in psoriatic arthritis patients: a network meta-analysis** *MF Tsoi, Y Fei, BMY Cheung, CS Lau, TT Cheung*	31
51	**Comparative effectiveness of Janus kinase inhibitors and biologic disease-modifying antirheumatic drugs in rheumatoid arthritis patients with an inadequate response to tumour necrosis factor alpha inhibitors: a network meta-analysis** *MF Tsoi, Y Fei, BMY Cheung, CS Lau, TT Cheung*	32
52	**Cardiovascular safety of Janus kinase inhibitors in patients with rheumatoid arthritis: a meta-analysis** *MF Tsoi, Y Fei, B Or, TT Cheung, BMY Cheung*	32
53	**Blood and urine inorganic and organic mercury levels in the United States: The United States National Health and Nutrition Examination Survey 1999-2016** *ASC So, MF Tsoi, TT Cheung, BMY Cheung*	33
54	**Ankylosing Spondylitis Disease Activity Score is associated with both the extent and intensity of diffusion-weighted magnetic resonance imaging spinal inflammation in active axial spondylarthritis** *THB Yan, CY Wong, YCC Lau, HY Chung, ETF Chui, KH Lee, HHL Tsang, SCW Chan, CS Lau*	33
55	**Efficacy and safety of ticagrelor versus clopidogrel in patients with stable coronary artery diseases: a systematic review and meta-analysis** *Q Feng, Y Fei, MF Tsoi, BMY Cheung*	34
56	**Ticagrelor reduced risk of infection compared with clopidogrel: a meta-analysis** *HL Li, MF Tsoi, Q Feng, T Fei, BMY Cheung*	34
57	**Magnetic resonance imaging inflammation of facet and costovertebral joints is associated with restricted spinal mobility and worsened functional status** *MHB Kwong, SC Chow, HTA Lee, HY Chung*	35
58	**Identification of biomarkers by immunoprofiling for immunotherapy in advanced hepatocellular carcinoma** *V Ting, CC Wong, YL Kwong, TCC Yau*	35
59	**Neutrophil serine proteinases exacerbate atherosclerosis by increasing intestinal permeability and endotoxinaemia in mice** *Y Pan, HT Chau, Q Wang, LG Jin, KSL Lam, A Xu*	36
60	**Incidence of hospitalised hypokalaemia in patients with indapamide prescription: a population-based study** *V Tang, MF Tsoi, TT Cheung, BMY Cheung*	36

ABSTRACT	TITLE	PAGE
61	**Gut microbiome-derived lipopolysaccharide contributes to pathogenesis of murine lupus nephritis** *J Yu, C Chan, ACP Tai, S Yung, TM Chan*	37
62	**Artificial intelligence–assisted real-time detection reduces missed lesions during colonoscopy: a retrospective and prospective study** *TKL Lui, C Hui, VWM Tsui, KS Cheung, KL Ko, CC Fu, SY Wong, WK Leung*	37
63	**Cholinergic receptor nicotinic alpha 7 subunit mediates cigarette smoke–induced PD-L1 expression** *HH Kwok, NCM Lee, J Deng, DCL Lam*	38
64	**Role of oncofetal protein *HMGA2* in the development of hepatitis B virus–associated hepatocellular carcinoma** *FY Huang, DKH Wong, WK Seto, KY Lai, CL Lai, MF Yuen*	38
65	**Cardiac cell patches with decellularised placenta and human-induced pluripotent stem cell–derived cardiomyocytes for myocardial repair** *Y Jiang, S Sun, Z Zhen, SY Liao, HF Tse*	39
66	**Mesenchymal stem cell preconditioning facilitates therapeutic efficacy of transplanted cells in myocardial infarcted heart** *S Sun, WH Lai, SY Liao, HF Tse*	39
67	**Point-of-care ultrasound augments physical examination learning in undergraduate medical students** *CK Wong, JJ Hai, KY Chan, KC Un, M Zhou, D Huang, YY Cheng, HF Tse, P Yeung, PS Yip, VKS Li, A Chan, M Cheung, S Cheung, CP Lau, CW Siu*	40
68	**Bone density and quality in prediabetes among postmenopausal Chinese women: the role of fibroblast growth factor 21** *DTW Lui, CH Lee, VWK Chau, CHY Fong, KMY Yeung, JKY Lam, ACH Lee, WS Chow, KCB Tan, YC Woo, KSL Lam*	40
69	**Statin use reduces incident hip fractures among older Chinese people with type 2 diabetes, independent of mean haemoglobin A1c and duration of diabetes** *DTW Lui, CH Lee, WS Chow, CHY Fong, CW Siu, YC Woo, KSL Lam*	41
70	**Liver-specific adeno-associated virus 2/8 trimeric adiponectin gene transfer reduces β-amyloidosis and improves learning and memory in a mouse model of Alzheimer's disease** *RCL Ng, M Jian, M Bunting, OKF Ma, JSC Kwan, KH Chan*	41
71	**Adiponectin paradox in the association between circulating adiponectin levels and incident cancer in patients with type 2 diabetes** *CH Lee, DTW Lui, CYY Cheung, CHY Fong, MAA Yuen, WS Chow, YC Woo, A Xu, KSL Lam*	42
72	**Artificial haemoglobin YQ23 increases circulating mesenchymal stem cells and promotes angiogenesis in a mouse model of hind limb ischaemia** *HC Han, FC Tzang, SH Lau, WH Lai, YM Lau, KM Ng, YK Lee, BP Yan, CK Wong, Y Feng, N Tan, JY Chen, JJ Hai, CW Siu*	42
	Author Index	43

1

Angiotensin-converting enzyme inhibitors/angiotensin receptor blockers and risk of colorectal cancer after negative baseline colonoscopy: a territory-wide study with propensity score analysis

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Introduction: Whether angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) modify colorectal cancer (CRC) risk remains controversial. We aimed to determine the association between ACEI/ARB use and subsequent CRC risk after a baseline colonoscopy negative for CRC.

Methods: This is a retrospective cohort study using a territory-wide electronic healthcare database in Hong Kong recruiting patients ≥40 years undergoing colonoscopies between 2005 and 2013. Exclusion criteria included prior CRC, inflammatory bowel disease, prior colectomy and CRC detected within 6 months of index colonoscopy. Medication use was traced up to 5 years before index colonoscopy, and ACEI/ARB use was defined as at least 90-day use within 5 years before the index colonoscopy. Post-colonoscopy colorectal cancer (PCCRC) was defined as cancer diagnosed between 6 and 36 months after the index colonoscopy. Sites of CRC were categorised as proximal (proximal to splenic flexure) and distal cancer. The adjusted hazard ratio (aHR) of PCCRC with ACEI/ARB use was derived by propensity score adjustment based on covariates (including patient factors, concurrent medication use and endoscopy centre's performance).

Results: Among 187 897 eligible patients (48.9% male), 854 (0.45%) were diagnosed with PCCRC (proximal cancer: 147 [17.2%]). The median age at PCCRC diagnosis was 75.9 years (interquartile range [IQR]=65.5-83.8 years), while the median time from index colonoscopy to PCCRC diagnosis was 1.2 years (IQR=0.8-1.9 years). There were 30 856 (16.4%) ACEI/ARB users. Angiotensin-converting enzyme inhibitors/angiotensin receptor blockers were associated with a lower risk of CRC that developed <3 years after index colonoscopy (aHR=0.78, 95% confidence interval [CI]=0.64-0.96), but not CRC that developed >3 years (aHR=1.18, 95% CI=0.88-1.57). Subgroup analysis shows that aHRs were 0.83 (95% CI=0.51-1.35) for proximal and 0.77 (95% CI=0.61-0.97) for distal cancer. Every single year increase in ACEI/ARB use was associated with a 5% reduction in CRC risk. Subgroup analysis shows that ACEI/ARB use was associated with lower risk of PCCRC in patients aged ≥55 years (aHR=0.79, 95% CI=0.65-0.98) and those with history of colonic polyps (aHR=0.71, 95% CI=0.52-0.97).

Conclusions: Angiotensin-converting enzyme inhibitors/angiotensin receptor blockers were associated with a lower CRC risk in a duration-response manner after a baseline colonoscopy negative for CRC.

2

Natural clinical course of progressive supranuclear palsy in Chinese patients in Hong Kong

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Introduction: Progressive supranuclear palsy (PSP) is one of the most frequent types of atypical parkinsonism. There is no study of its natural clinical course among Chinese patients.

Methods: Twenty one PSP patients with magnetic resonance imaging brain confirmation were retrospectively recruited from geriatrics clinic of Queen Mary Hospital and Tuen Mun Hospital. Clinical information was retrospectively retrieved from clinical records, including age of onset, age of presentation, age of death, symptoms duration, education level, sex, presenting scores of the Cantonese version of the Mini-Mental State Examination, clinical symptoms, history of levodopa or dopamine agonists intake and response. The clinical symptoms were clustered into different categories: motor symptoms, bulbar symptoms, cognitive symptoms, and others. The date of development of the above symptoms clusters was retrospectively traced.

Results: Motor symptoms developed early along the clinical course. Cox proportional hazard model showed that the number of episodes of pneumonia and the shorter the time to vertical gaze and pneumonia predicted mortality. Apathy, dysphagia, pneumonia, caregiver's stress and pressure injuries when analysed as time-dependent covariates predicted mortality. There was a significant negative correlation between the age of presentation and time to mortality from presentation (Pearson correlation=-0.54, P=0.04). Up to 40% of caregivers complained of stress along the clinical course.

Conclusion: Important clinical milestones, including the development of dysphagia, vertical gaze palsy, significant caregiver stress, pressure injuries and pneumonia, may be the ideal timing for advanced care planning for PSP patients.

3

Prevalence of cognitive impairment among peritoneal dialysis patients: a systematic review and meta-analysis

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Introduction: Cognitive impairment (CI) is common among patients on peritoneal dialysis (PD), but it is under-recognised and systematic review on its prevalence and impact across different geographical locations or patient characteristics is lacking.

Methods: A search of the literature on CI in PD patients published between 1 January 1980 and 25 April 2019 was conducted. Meta-analysis using a random-effects model was performed to determine the pooled estimate of the prevalence of CI. Meta regression was performed to identify factors contributing to the variance of prevalence rate. A systematic review was also performed to study risk factors of CI and its impact on clinical outcomes.

Results: Eight studies were included and the relevant data from 1736 patients were extracted for analysis. Meta-analysis revealed a pooled prevalence of CI at 28.7% (95% confidence interval=15.9%-46%). Meta regression analyses showed that the prevalence of CI was unrelated to patient's age, sex, duration of PD, healthcare policy of dialysis modality, the prospective or retrospective nature of studies, or year of publication. Systematic review of 20 studies showed that older age, female sex, and lower education were risk factors for CI. Potential reversible factors for CI include electrolytes disturbances, depression, and vitamin D deficiency. Also, CI was associated with a higher risk of hospitalisation, mostly due to PD-related peritonitis.

Conclusions: Cognitive impairment is common in patients on long-term PD. Screening for CI should be considered in PD patients with increased risk.

4

Mouse model of neuromyelitis optica spectrum disorders with aquaporin-4 autoimmunity and spinal cord pathologies

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Objective: To establish a mouse model of neuromyelitis optica spectrum disorders (NMOSD) with aquaporin-4 (AQP4) autoimmunity seropositive for immunoglobulin G (IgG) AQP4 autoantibodies.

Methods: We performed DNA immunisation in wild-type mice with plasmid encoding mouse AQP4 M23 isoform via in vivo electroporation of skeletal muscle.

Results: DNA immunisation–induced inflammation and increased expression of AQP4 in muscle membrane in a punctuate distribution were suggestive of high-order orthogonal array of particles. Immunoglobulin G AQP4 autoantibodies were detected by cell-based assay in peripheral blood of mice immunised with AQP4 gene and pretreatment with CFA and PTx, but not in control mice. Immunoglobulin G AQP4 autoantibodies infiltrated into the spinal cord through a disrupted blood-brain barrier. Mice seropositive for IgG AQP4 autoantibodies had spinal cord pathologies including loss of AQP4 and glial fibrillary acidic protein, deposition of complement activation products (C5b-9), infiltration of macrophages, neutrophils and eosinophils, activation of microglia/macrophages, increased proinflammatory cytokines (tumour necrosis factor α, interleukin 1β and interleukin 6) levels, demyelination and loss of oligodendrocytes and axons. Importantly, these spinal cord pathologies in mice with IgG AQP4 autoantibodies were associated with motor impairment.

Conclusions: We developed a mouse model of NMOSD with circulating IgG AQP4 autoantibodies and NMOSD-like spinal cord pathologies. This model is useful for studying pathogenesis and novel therapeutics in NMOSD.

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5

Controlled attenuation parameter measurements predict fibrosis progression and hepatitis B surface antigen seroclearance in virologically quiescent chronic hepatitis B: a prospective study with paired transient elastographies

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Introduction: Concomitant hepatic steatosis is common in patients with chronic hepatitis B (CHB) infection. The impact of such on liver outcomes are controversial. We aimed to study the effect of hepatic steatosis on risk of fibrosis progression and hepatitis B surface antigen (HBsAg) seroclearance.

Methods: Untreated patients with CHB with normal alanine aminotransferase and low viraemia (defined as serum hepatitis B virus [HBV] DNA <2000 IU/mL) were prospectively recruited for baseline and 3-year transient elastography assessment. Fibrosis staging is defined according to the EASL-ALEH guidelines: no significant fibrosis (F0/F1): liver stiffness (LS) <6 kPa; advanced fibrosis (F3): LS ≥9 kPa; and cirrhosis (F4): LS ≥12 kPa. Fibrosis progression is defined as ≥1 stage increment of fibrosis at reassessment. Hepatic steatosis is defined as controlled attenuation parameter (CAP) ≥ 248 dB/m, while severe hepatic steatosis is defined as CAP ≥280 dB/m.

Results: A total of 330 patients (median age 50.5 years, 41.2% male) had paired transient elastography assessment. The median HBV DNA was 189 (interquartile range [IQR]=52-624) IU/mL. The median follow-up duration was 36.4 (IQR=34.1-39.0) months. At baseline, 51.2% and 28.8% had steatosis and severe steatosis, respectively. Only 4.7% had F3/F4 at baseline, while the proportion of patients with F3/F4 increased to 8.7% at 3 years. The rate of liver fibrosis progression in patients with persistent severe steatosis was significantly higher than that of persistently non-steatotic patients (41.3% vs 23%, P=0.017). For those who had new-onset severe steatosis, the risk of fibrosis progression was 34.6%. Twenty four (7.3%) patients were started on antiviral agent for virological breakthrough at a median interval of 21.8 (IQR=15.2-26.0) months. Twenty two (6.7%) patients developed HBsAg seroclearance during the follow-up period. The cumulative probability of HBsAg seroclearance in patients with baseline steatosis was 9.9% compared with 3.7% in patients without baseline steatosis (P=0.008). Presence of hepatic steatosis was associated with significantly higher chance of HBsAg seroclearance (hazard ratio=2.998; 95% confidence interval=1.143-7.865; P=0.026).

Conclusion: Measurements of CAP via transient elastography can prognosticate virologically quiescent CHB. Hepatic steatosis was associated with higher risk of fibrosis progression and 3-fold increase in HBsAg seroclearance rate than those without hepatic steatosis.

6

Targeting polyamines for treatment of malignant pleural mesothelioma in xenograft models

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Introduction: Inhaling asbestos fibres is the commonest cause of malignant pleural mesothelioma (MPM). Although the use of asbestos has been restricted, the incidence of MPM is still rising due to a long lag time in malignant transformation. In 2004, the United States Food and Drug Administration approved a combination of pemetrexed with cisplatin for treatment of unresectable MPM. However, overall prognosis is still extremely poor. As such, development of novel therapeutic options is urgently needed. Ornithine decarboxylase (ODC) is highly expressed in 211H and H226 MPM xenografts and clinical tumour samples. Upregulation of ODC increases polyamine production and enhances tumour growth. Alpha-difluoromethylornithine (DFMO) is a specific ODC inhibitor which can suppress polyamines production. This study aimed to disclose the therapeutic effect of DFMO in MPM xenograft models.

Methods: Nude mice were subcutaneously inoculated with human MPM cells (211H or H226). Mice were treated with DFMO in drinking water when tumour size reached 50 to 100 mm^3. Mice with tumour size >600 mm^3 were considered reaching humane endpoint. Spermidine levels, protein expression, cytokines concentrations and apoptosis were investigated by Dot plot, western blot, enzyme-linked immunosorbent assay and terminal deoxynucleotidyl transferase dUTP nick end labelling assay, respectively. Nitrated protein was identified using Dionex UltiMate 3000 RSLCnano system coupled to Thermo Fisher Orbitrap Fusion Lumos Tribrid mass spectrometer (Centre for PanorOmic Sciences, The University of Hong Kong).

Results: Alpha-difluoromethylornithine–suppressed tumour growth in both xenografts. DFMO increased median survival from 29 days in control arm to 41 days in treatment arm in mice with 211H xenografts (P=0.0234), while from 30 days to 43.5 days in those with H226 xenografts (P=0.0050). There was no synergism when combining DFMO with either cisplatin or pemetrexed. The tumour suppressive effect of DFMO was more effective when compared with cisplatin or pemetrexed alone. Upon DFMO treatment, decrease in spermidine level, increase in nitrotyrosine content (nitration), and activation of apoptosis were observed in both xenografts. In addition, increase in nitrosocysteine level, increase in intratumoural interleukin 6, keratinocyte chemoattractant and tumour necrosis factor alpha as well as elevation of DNA lesion and downregulation of pAkt were induced by DFMO in H226 xenografts only. Moreover, anti-cancer effect of DFMO was partially reversed by supplement of spermidine during DFMO treatment in both xenografts. Nitration was found at the Tyr53 of the human actin sequence in both xenografts which might affect actin polymerisation.

Conclusion: Alpha-difluoromethylornithine may have a potential role in treating MPM.

Acknowledgement
This research was supported by Hong Kong Pneumoconiosis Compensation Fund Board.

Protease-activated receptor-1 antagonist vorapaxar ameliorates kidney injury and tubulointerstitial fibrosis in experimental obstructive nephropathy

7

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Background: In addition to its role in tumour invasiveness and metastases, the protease-activated receptor-1 (PAR-1) has emerged as an inducer of kidney fibrosis. Whether it can be exploited as a therapeutic target remains unknown.

Methods: We assessed the effect of direct inhibition of PAR-1 on renal fibrosis by vorapaxar (a PAR-1 antagonist), a drug currently undergoing clinical trials for cardiovascular disease, in murine unilateral ureteral obstruction (UUO) model, and in cultured rat renal proximal tubular epithelial cells (NRK-52E). Protease-activated receptor-1 signalling was studied by real-time quantitative polymerase chain reaction, western blotting, and immunohistochemical staining.

Results: In UUO kidneys, PAR-1 and its activator, thrombin, were highly expressed in tubular cells. Mice treated with vorapaxar showed diminished renal fibrotic changes with attenuated fibronectin, alpha-smooth muscle actin and collagen expression versus control. Macrophage infiltration and ERK1/2 activation were also reduced in vorapaxar-treated UUO kidneys. In NRK-52E cells, vorapaxar inhibited PAR-1 signalling, ameliorated thrombin-induced ERK1/2 activation, and suppressed the downstream transforming growth factor-beta signalling via both Smad-dependent and non-Smad-dependent MAPK signalling pathways.

Conclusion: Vorapaxar protects against kidney fibrosis in UUO model, partly via inhibition of thrombin/transforming growth factor-beta/Smad signalling. This PAR-1 targeted therapeutic strategy may provide a novel treatment approach for chronic renal fibrotic diseases.

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Long intergenic non-coding RNA p21 mediates lipotoxicity-induced kidney injury

8

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Introduction: Ectopic lipid accumulation in kidney is a key factor in the aetiology of lipotoxicity-induced kidney lesion. Emerging evidence unravels that long intergenic non-coding RNA p21 (lincRNA-p21) plays a pivotal role in diverse biological processes and diseases. However, little is known about the role of lincRNA-p21 in kidney diseases. We aimed to identify the functional role of lincRNA-p21 in lipotoxicity-induced kidney injury.

Methods: Expression of lincRNA-p21 in kidney from mice (C57BL/6J) fed with normal diet (ND; 10 kcal%) or high-fat diet (60 kcal%), as well as in palmitic acid (PA)–treated human proximal tubular epithelial cells line (HK-2 cells) was determined by quantitative reverse transcription polymerase chain reaction (qRT-PCR). Antisense locked nucleic acids GapmeR technique was utilised to silence lincRNA-p21 expression in HK-2 cells, followed by evaluation of cellular inflammation, endoplasmic reticulum (ER) stress and apoptosis by qRT-PCR after PA exposure. Western blot and enzyme-linked immunosorbent assay were performed to investigate the associated signalling cascades.

Results: Compared with ND-fed mice, a significantly increase in the expression of lincRNA-p21 was found in kidney biopsy from high-fat diet–fed mice. Consistently, markedly upregulated expression of lincRNA-p21 was observed in PA-treated HK-2 cells. By contrast, silencing lincRNA-p21 significantly counteracted PA-induced gene expression associated with inflammation (interleukin 6), ER stress (BiP, sXBP1 and CHOP), and apoptosis (BCL2). Additionally, PA suppressed PI3K/Akt/mTOR/Mdm2 signalling cascades and subsequently led to enhanced p53 activity, which consequently drove lincRNA-p21 expression in HK-2 cells.

Conclusion: Palmitic acid acts through PI3K/Akt/mTOR/Mdm2 signalling pathway to upregulate lincRNA-p21 expression in a p53-dependent manner, thereby contributing to lipotoxicity-induced pathological process in HK-2 cells.

Acknowledgement
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Prevalence of beta-lactam allergies and factors predicting genuine allergies in Hong Kong

9

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Introduction: Beta-lactams (BL) are the most widely used class of antibiotics and most frequently reported cause of drug allergy. However, the vast majority of patients are found not to be genuinely allergic after allergy evaluation. Despite devastating consequences of incorrect allergy labelling, few studies have investigated the predictors of genuine BL allergies and the local epidemiology in Hong Kong is unknown.

Methods: Demographic and allergic data from patients admitted to the acute medical wards of Queen Mary Hospital over a period of 6 months were analysed to identify the prevalence and factors associated with the presence of BL allergy labels in Hong Kong. A combined cohort of patients having completed allergy investigation for suspected BL allergies in the United Kingdom and Hong Kong were analysed. Association analysis comparing the clinical characteristics of confirmed BL allergic and non-allergic patients was performed to identify predictors of genuine allergy.

Results: The local prevalence of BL allergy labels was 5% in Hong Kong, which was associated with female sex and concomitant non-BL antibiotic allergy labels. After completing allergy testing, the rate of genuine BL allergy was only 14%. History of anaphylaxis and duration of <1 year since the index reaction were independent clinical predictors of genuine BL allergy. The negative predictive value of penicillin skin testing was 90%. There was an alarmingly high rate of confirmed piperacillin-tazobactam allergy.

Discussion: The estimated true prevalence of genuine BL allergy in Hong Kong is around 0.5%. This high rate of BL mislabelling highlights the need for comprehensive allergy evaluation and screening. Physicians should be reminded to ascertain history of anaphylaxis and duration since the index reaction as clinical predictors of genuine BL allergy. Piperacillin-tazobactam allergy may pose a unique challenge to our locality with high prevalence of suspected allergies, surging antibiotic resistance and lack of testing available.

Aetiologies of idiopathic anaphylaxis and importance of allergological evaluation in Hong Kong

10

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Introduction: The term idiopathic anaphylaxis (IA) refers to anaphylaxis of uncertain aetiology and is supposedly a diagnosis of exclusion. Patients are at especially high risk of recurrent episodes of life-threatening anaphylaxis due to uncertain avoidance measures. As the first and only public hospital in Hong Kong for Immunology & Allergy, Queen Mary Hospital (QMH) receives referrals from the entire territory with patients labelled with IA. However, aetiologies for previously labelled IA are often found after allergy testing. We studied all patients with previously labelled IA to identify its genuine prevalence and aetiologies.

Methods: We analysed all patients attending Immunology Clinic at QMH since its establishment from May 2018 to October 2019. Data were extracted for patient demographics, referring diagnosis, clinical manifestations, allergological investigation and results, pre-consultation prescriptions and final diagnosis.

Results: Over 230 referrals, IA made up more than 10% of all referrals to QMH. A total of 32 patients were referred for IA. The median age and delay in diagnosis was 37 (range, 16-69) years and 3 (0-20) years, respectively. Twenty (62.5%) patients were diagnosed with food-dependent exercise–induced anaphylaxis (FDEIA), 18/20 to wheat and 2/20 to shellfish. Remaining aetiologies were mastocytosis (3/32, 9.4%), buckwheat allergy (2/32, 6.3%), alpha-gal allergy (1/32, 3.1%), exercise-induced anaphylaxis (1/32, 3.1%) and lipid transfer protein allergy (1/32, 3.1%). Aetiologies were not yet ascertained in four (12.5%) patients. Only five (15.6%) patients were prescribed adrenaline auto-injectors.

Discussion: Despite being previously labelled as "idiopathic" and at risk of recurrent life-threatening anaphylaxis, aetiologies were identified in over 80% of patients and less than one fifth of cases were prescribed adrenaline auto-injectors. This highlights the importance of comprehensive allergological evaluation and consultation. Most patients were diagnosed with food-dependent exercise–induced anaphylaxis; this would be an interesting field of further research in Hong Kong.

11

Altered expression of immune regulatory receptors upon combination of chemotherapy and checkpoint blockade immunotherapy in a lung cancer mouse model

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Introduction: Lung cancer remains as the most lethal cancer disease in Hong Kong. Although blockade of inhibitory immune checkpoints achieved remarkable clinical outcomes, acquired immune resistance arises as dynamic interactions between the cancer cells and the tumour-associated microenvironment evolve. Exploration on alternative strategies to battle against lung cancer is therefore necessary for long-term tumour suppression.

Methods: Murine lung cancer model was established by inoculation of Lewis lung carcinoma cells subcutaneously on the flank of C57BL/6J mice. Chemotherapy (cisplatin or pemetrexed) or antibody to programmed cell death protein 1 (anti-PD-1) alone, or in combination started at 7 days post-inoculation for 2 weeks. Tumour growth was measured by digital calliper and survival rate was recorded. Mice were sacrificed when they reached the humane endpoint. Splenocytes and tumour cells were harvested and expression of immune regulatory markers (including PD-L1, PD-1, Tim-3, OX-40, GITR, LAG-3 and CTLA-4) was analysed by flow cytometry.

Results: While PD-1 blockade delayed tumour growth ($P<0.001$), no significant difference in overall survival was observed. Systemically, anti-PD-1 immunotherapy induced upregulation of Tim-3, OX-40 and GITR; cisplatin ± anti-PD-1 induced upregulation of GITR; whilst OX-40 is induced in both combinations of chemotherapy and anti-PD-1. Tumour-infiltrating T cells were increased in anti-PD-1–treated mice, while combination of pemetrexed and anti-PD-1 induced upregulation of co-stimulatory receptors including GITR and OX-40 within tumour.

Conclusion: Cisplatin and pemetrexed induced different systematic and intratumoural immune responses when used alone or in combination with anti-PD-1 treatment. Increased expression of co-stimulatory receptors in the tumour in response to combination therapy provide insights for investigation of synergism on anti-PD-1 and alternative therapeutic targets.

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12

Perturbed ERK-DRP1 signalling contributes to impaired mitophagy in parkinsonian leucine-rich repeat kinase 2^{R1441G} mutant mice

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Background: Leucine-rich repeat kinase 2 (LRRK2) mutations represent the most common genetic risk in Parkinson's disease. Damaged mitochondria undergo initial fission followed by ubiquitination and mitophagy. Extracellular signal-regulated kinase (ERK) activation promotes DRP1 phosphorylation for mitochondrial fission. We aimed to explore whether LRRK2 mutation affects ERK phosphorylation and DRP1-mediated mitophagy.

Methods: Level of ERK1/2 and DRP1 phosphorylation was determined by immunoblotting in littermate-matched wild-type (WT) and LRRK2^{R1441G} knock-in mouse embryonic fibroblasts (MEFs) after stressed by mitochondrial uncoupler, FCCP (10 uM; 30 min). Stable cultures of WT and mutant MEFs overexpressing mitochondria-specific photoactivatable PAmCherry were developed for mitochondrial degradation flow cytometry assays. Contribution of DRP1 in mitophagy was determined after siRNA-mediated knockdown.

Results: The LRRK2 mutant MEFs exhibited lower rate of mitochondrial degradation than WT. Knockdown of DRP1 expression reduced mitochondrial degradation. FCCP treatment caused ERK1/2 phosphorylation in WT but not in LRRK2 mutant MEFs. Total DRP1 levels were similar between WT and mutant MEFs. However, DRP1-pSer616 phosphorylation was lower in mutant MEFs under FCCP toxicity.

Conclusions: Mitochondrial stress-induced ERK1/2 and DRP1 phosphorylation were impaired in LRRK2 mutant MEFs suggesting that LRRK2^{R1441G} mutation perturbs ERK-DRP1 signalling. Impaired ERK-DRP1 signalling in LRRK2 mutant cells may contribute to slower mitophagy in the LRRK2 mutant cells.

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Role of brain-derived neurotrophic factor in cigarette smoke-exposed rats

13

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Introduction: Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family of growth factors, is synthesised in the endoplasmic reticulum. Chronic obstructive pulmonary disease (COPD) is characterised by persistent respiratory symptoms and airflow limitation, in which cigarette smoking is a major risk factor. One previous report demonstrated that lung function of stable COPD patients is associated with the serum levels of BDNF. Cigarette smoke (CS)–induced oxidative stress was also found to increase the expression of BDNF in airway smooth muscle cells. Therefore, this study aimed to investigate the relationship between duration of CS exposure and serum levels of BDNF in CS-exposed passive smoking rat model.

Methods: Male Sprague-Dawley (SD) rats, aged 1.5 months, were subjected to 7-day and 56-day CS exposure (SCIREQ inExpose System) to simulate acute and subchronic conditions in COPD. During the subchronic exposure, dosage of *Dendrobium officinale* polysaccharides (50 mg/kg and 200 mg/kg) was administered daily by oral gavage after CS exposure for 28 days to determine its therapeutic effect in this model. In addition, rat serum from SD rats aged 4 months, 8 months and 15 months were also sacrificed to determine the effects of age on baseline serum levels of BDNF.

Results: Duration of CS exposure was found to be positively correlated with serum levels of BDNF. *Dendrobium officinale* polysaccharides showed its dose-dependent effects on reducing CS-induced serum BDNF levels. The baseline serum levels were also found to increase with age.

Conclusion: These findings suggest that BDNF may be used as a biomarker in smokers and *Dendrobium officinale* polysaccharides may be used as a novel therapy in the treatment of CS-mediated COPD.

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Dendrobium officinale polysaccharides attenuate cigarette smoke–induced airway inflammation in vivo

14

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Introduction: Polysaccharides extracted from *Dendrobium officinale* (DOP), a valuable traditional Chinese Medicine herb, have antioxidant and anti-inflammatory effects. This study aimed to determine whether DOP treatment has any therapeutic effect on cigarette smoke (CS)–induced airway inflammation in a rat passive smoking model.

Methods: Sprague-Dawley rats were exposed to either sham air or CS generated by SCIREQ inExpose inhalation exposure system for 1 hour per day for 56 days. From day 29, rats were given DOP or N-acetylcysteine (NAC, as a positive control) by oral gavage. After the last exposure, rats were sacrificed and bronchoalveolar lavage fluid (BAL), blood and lung tissues were collected for further assays.

Results: Complete blood count test showed that CS exposure significantly elevated lymphocytes and monocytes, which was reversed by NAC or DOP treatment. Treatment with NAC or DOP also inhibited CS-induced elevation of macrophages and neutrophils in BAL. In support, DOP treatment significantly inhibited CS-induced elevation of serum and lung CINC-1 levels. However, DOP and NAC significantly attenuated CS-induced elevation of malondialdehyde level in lung. Furthermore, DOP showed inhibition of CS-induced NF-kappaB and MAPK (ERK and p38) activation in lung.

Conclusion: *Dendrobium officinale* attenuated smoking-related airway inflammation via the inhibitory effect on NF-kappaB and MAPK signalling pathway in a rat model of passive smoking, which may have beneficial effect for patients with chronic obstructive pulmonary disease.

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Bidirectional role of peptidase M20 domain containing 1 in the regulation of body weight

15

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Introduction: Peptidase M20 domain containing 1 (PM20D1) is a bidirectional enzyme catalysing the condensation of monounsaturated fatty acid (C18:1) and amino acid (phenylalanine) to generate N-acyl amino acid (C18:1-Phe) and also the reverse hydrolytic reaction. Although we have demonstrated a significant increase of circulating PM20D1 concentration in diet-induced obese mice, the role of PM20D1 in the pathogenesis of obesity in vivo is unclear.

Methods: Adeno-associated virus serotype 8–mediated gene delivery system was developed to overexpress PM20D1 in C57BL6N mice. Mice were fed with standard chow or high-fat diet (HFD) and housed at either thermoneutral (30°C) or cold (6°C) temperatures for 15 days. Body weight and food intake was measured on a weekly basis. Body composition was determined using nuclear magnetic resonance. Serum levels of C18:1 and C18:1-Phe were measured by liquid chromatography–mass spectrometry. The effects of Zn^{2+} on catalytic activities of PM20D1 were explored by in vitro studies.

Results: Under the thermoneutral condition, mice with overexpression of PM20D1 gained more weight upon HFD feeding due to increased food intake. Whereas under the cold condition, overexpression of PM20D1 in mice significantly reduced body weight as a result of reduced food intake. Consistently, diet-induced glucose intolerance in mice with PM20D1 overexpression was exacerbated when they were housed at thermoneutrality, but ameliorated when they are exposed to cold environment. Liquid chromatography–mass spectrometry analysis showed that serum levels of C18:1 were lower in mice with PM20D1 overexpression at thermoneutrality, but were higher in these mice after cold exposure. Notably, serum levels of C18:1-Phe exhibited opposite changes. In addition, further study showed administration of C18:1, but not C18:1-Phe, to mice reduced body weight by decreasing food intake. Lastly, Zn^{2+} inhibited the synthetic activity of PM20D1 with no effect on its hydrolytic activity as shown by in-vitro studies.

Conclusion: This study shows the different roles of PM20D1 in the regulation of body weight under different temperatures, which was attributed to bidirectional enzymatic effects of PM20D1 on its metabolite C18:1. This may be achieved by the change in the serum level of Zn^{2+}, which is a cofactor of PM20D1 in a temperature dependent manner.

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Leisure time, occupational aerobic physical activity, and mortality risk in adults of United States: National Health Nutrition and Examination Survey 2007-2016

16

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Objectives: Regular aerobic physical activity (PA) is recommended in the Physical Activity Guidelines of the United States. We investigated whether both leisure time physical activity (LTPA) and occupational physical activity (OPA) reduce mortality.

Methods: We analysed physical activity data of 28 735 participants and mortality data of 18 498 participants aged 18 to 64 years in the United States National Health and Examination Survey 2007-2016 using R 3.6.1.

Results: The proportions of participants having ≥150 min/week of moderate intensity aerobic PA or equivalent from LTPA, OPA and total PA were 39.6% (38.0%-41.2%), 37.5% (36.2%-38.7%) and 61.7% (60.6%-62.9%), respectively. For survival analysis, the median follow-up was 4.8 (interquartile range=2.8-6.8) years. Log rank test showed that all the groups with ≥150 min/week of LTPA had higher survival compared with the group with no PA (all P<0.001), whereas the groups having ≥150 min/week of OPA but <150 min/week of LTPA did not have higher survival (P=0.166, 0.584). In stratified multivariable Cox regression, LTPA ≥150 min/week (hazard ratio [HR]=0.48, 95% confidence interval [CI]=0.33-0.69) with 50≤OPA<100 min/week (HR=0.31, 95% CI=0.14-0.71) was associated with lower all-cause mortality. In contrast, 150≤OPA<300 min/week and OPA ≥300 min/week were not (HR=0.63, 95% CI=0.27-1.47; HR=1.02, 95% CI=0.68-1.53, respectively).

Conclusions: Adults doing ≥150 min/week of moderate intensity or equivalent LTPA have half the risk of death. Occupational physical activity is not associated with lower mortality. Our findings support the differentiation between LTPA and OPA in the guidelines. Leisure time physical activity should be encouraged, especially since more than half of adult Americans do not have enough aerobic exercises from LTPA.

17

Decreased survival in young adults with stage 1 and 2 hypertension in the National Health and Nutrition Examination Survey III

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Introduction: The 2017 American College of Cardiology/American Heart Association guideline for the Prevention, Detection, Evaluation and Management of High Blood Pressure in Adults updated the classification of blood pressure (BP) and treatment recommendations for each BP category. We studied in young adults whether elevated BP, stage 1 and 2 hypertension increased all-cause mortality.

Methods: A total of 3747 participants aged 20 to 29 years with BP measurements and mortality data in the United States National Health and Examination Survey III were included. They were stratified according to the hypertension status and followed until 31 December 2015 with a median follow-up of 24.0 years. Time ratios (TRs) and 95% confidence intervals (CIs) were calculated in R 3.6.0. As Cox proportional hazards assumption was not satisfied, we used a Weibull accelerated failure time model.

Results: The adjusted TRs were 0.80 (95% CI=0.48-1.32) for elevated BP, 0.81 (95% CI=0.59-1.11) for stage 1 hypertension, and 0.62 (95% CI=0.41-0.94) for stage 2 hypertension. After adjusting for body mass index (BMI), the respective TRs became insignificant (0.84 [95% CI=0.51-1.40], 0.88 [95% CI=0.65-1.18], and 0.73 [95% CI=0.47-1.14]). In sex-specific analysis, the adjusted TRs were 0.74 (95% CI=0.44-1.26), 0.70 (95% CI=0.49-0.99), and 0.57 (95% CI=0.35-0.91) for men, and 0.91 (95% CI=0.22-3.70), 1.98 (95% CI=0.75-5.26), and 0.70 (95% CI=0.36-1.34) for women.

Conclusions: During more than two decades of follow-up, young adult men with stage 1 and stage 2 hypertension had decreased life expectancy while young adult women might not. Our findings justify the early detection and management of hypertension. The decreased life expectancy is partly explained by BMI, so lifestyle changes including weight control are important.

18

Leucine-rich repeat kinase 2 kinase inhibitor affects phosphorylation of alpha-synuclein at serine-129 in vitro and in vivo

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Background: Leucine-rich repeat kinase 2 (LRRK2) mutations, one of the most common genetic risks of Parkinson's disease (PD), are associated with hyperkinase activity. We have previously shown that aged LRRK2^{R1441G} mutant knock-in mice exhibit greater accumulation of alpha-synuclein aggregates in the brain compared with age-matched wild-type controls. Alpha-synuclein phosphorylated at serine-129 (pSer129) promotes alpha-synuclein aggregation; over 90% of alpha-synuclein in dementia with Lewy bodies and PD is phosphorylated at Ser129 in contrast to only 4% in normal brains. The potential link between pSer129 and LRRK2 mutation-induced hyperactivity remains unclear. We aimed to determine whether LRRK2 kinase inhibitor GNE-7915 reduces pSer129 levels in human neuronal SH-SY5Y cells overexpressing alpha-synuclein and in LRRK2^{R1441G} knock-in mutant mice striatum.

Methods: Three-month-old LRRK2^{R1441G} knock-in mice and age-matched wild-type controls were injected subcutaneously with either vehicle or GNE-7915 (50-75 mg/kg). Mice were sacrificed 4 hours after injection immunoblotting analysis of striatal tissues for pSer129 levels. Stable human neuronal SH-SY5Y cells overexpressing alpha-synuclein were treated with GNE-7915 (10-2500 nM) for 24 hours for immunoblotting.

Results: GNE-7915 injection at 75 mg/kg significantly reduced pSer129 levels in LRRK2^{R1441G} knock-in mouse striatum. GNE-7915 also reduced pSer129 levels in human neuronal SH-SY5Y cells.

Conclusions: GNE-7915 reduced pSer129 levels in both human neuronal SH-SY5Y cells and striatum of LRRK2^{R1441G} knock-in mice. These results highlight a potential therapeutic link between LRRK2 kinase inhibition and pSer129 level in synucleinopathies of PD.

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Biological effects of a highly focused, scanned, near-infrared laser on dermal pigment

19

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Background: Melasma and post-inflammatory hyperpigmentation (PIH) are common, difficult-to-treat examples of dermal melanosis particularly troublesome in darker skin types. Melanin is retained in dermal phagocytic cells and dermal pigmentation can take >2 years to clear. Effectively, dermal melanosis is a melanin "tattoo". No highly effective treatments are available.

Methods: For animal model of dermal pigment, melanin tattoos were used to mimic dermal melanin deposits. Device setting included 1064 nm Q-switched fibre laser, 5 kW peak power, 10 W average power, focusing lens NA=0.5, treatment area 10 × 10 mm, raster scanning in x-y plane, focal depth adjustable with mm precision, and cooled sapphire window to prevent bulk heating.

Results: Dermal melanin appearance was reduced immediately post-treatment. Microscopic images shown damaged melanophages post-treatment. Further work is underway to elucidate the mechanisms involved. The melanin tattoos were shown to be stable for over 2 months.

Conclusions: Melanin tattooing is a reasonable animal model for PIH. It is possible to target dermal melanin by rapidly scanning an intradermal-focused Q-switched fibre laser at 1060 nm. Epidermal damage can be avoided, while dermal pigment is affected. It is possible to immediately reduce the appearance of dermal melanin. Future work is warranted to further elucidate mechanisms at the target melanin, re-design with the ability to treat a large field, increase scan speed by ~100×, miniaturise the scanning handpiece and add "smarts"—we have found ways to detect targeting with each pulse. Clinical studies for PIH, melasma, other indications are pending.

Upregulation of peptidyl arginine deiminase 4 in neutrophil in systemic lupus erythematosus

20

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Introduction: Systemic lupus erythematosus (SLE) is a common and chronic autoimmune disorder. Both genetic and environmental factors contribute to the highly complex pathogenesis of SLE, which has not been understood completely. Apart from the dysregulation of adaptive immunity such as abnormal B cell and T cell autoreactivity, the innate immunity also participates in the disease progress of SLE. As the most abundant effector cells of innate immunity, neutrophil has shown to be associated with the aetiology of SLE in terms of aberrant regulation of neutrophil extracellular traps (NETs). The formation of NETs involves citrullination of histones, which refers to the conversion of positively charged arginine into neutrally charged citrulline catalysed by peptidyl arginine deiminases (PADs). Among the five isozymes of PADs, PAD4 is mainly expressed in neutrophil and thought to be predominantly responsible for the citrullination of histones and correlated with chromatin decondensation during NETs formation. In previous studies, PAD4 in NETs formation and disease progression seems to be context-dependent in different lupus mouse models. Based on the conflicting results of PAD functions in mouse models, the role of PAD4 in neutrophil in SLE patients still needs to be elucidated. This study aimed to investigate the dysregulation and subcellular localisation of PAD4 in neutrophil in SLE patients.

Methods: mRNA and protein expression level of PAD4 in neutrophil were compared between normal controls and SLE patients by quantitative polymerase chain reaction and western blotting. The distribution of PAD4 were detected by immunofluorescence microscope.

Results: Neutrophil expresses the highest level of PAD4 among different immune cell types. mRNA expression level of PAD4 in SLE neutrophil is around 4 folds higher than in normal controls. Although the individual variation is large, protein expression level of PAD4 also significantly increases in SLE neutrophil. The immunofluorescence results show that PAD4 mainly localises in neutrophil nuclei both in normal controls and SLE patients.

Conclusion: Gene and protein expression level of PAD4 are significantly higher in SLE neutrophil. The distribution of PAD4 in neutrophil is mainly in nuclei. Next, NETs formation and more neutrophil functional assays will be conducted to dig out the mechanisms of PAD4 upregulation in SLE neutrophil and its effect on pathogenesis of SLE.

Interferon-alpha induces AIM2 inflammasome response in systemic lupus erythematosus patients

21

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Introduction: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease of diverse aetiologies, characterised by a loss of tolerance to self-antigens as well as elevated production of pathogenic type I interferons (IFN-I). The AIM2 inflammasome, expressed by myeloid cells, is a caspase-1 dependent multi-protein complex that oligomerises in the cytosol following sensing of double-stranded DNA. In response, it secretes pro-inflammatory cytokines interleukin 18 (IL-18) and IL-1beta. Outside infection context, we have previously identified augmented AIM2 inflammasome response in SLE patients. We further demonstrated that SLE serum is capable of inducing AIM2 inflammasome activity in healthy monocytes. This study aimed to verify if serological IFN-I in SLE serum is responsible in dysregulating AIM2 inflammasome response in lupus patients. To elucidate the underlying molecular mechanisms behind the observed aberration, RNA sequencing is employed.

Methods: First, IFN-alpha receptor 2 on healthy monocytes were blocked with either anti-IFNAR2 antibody or isotype control and cultured overnight in SLE serum. Enzyme-linked immunosorbent assay was used to measure the production level of IL-18 and IL-1beta. Four sample groups: untreated healthy monocytes (group A), IFN-alpha–treated healthy control (HC) monocytes (group B), HC serum–treated HC monocytes (group C), and SLE serum–treated HC monocytes (group D) were submitted for RNA-Seq comparisons.

Results: Blockade of IFN-I signalling pathway with anti-IFNAR2 antibody significantly reduced AIM2 inflammasome responses in healthy monocytes after SLE serum treatment as compared with isotype control. RNA-Seq data identified a total of 360 differentially expressed, overlapping genes between group A versus group B and group C versus group D. Reactome pathway analysis identified cytokine signalling and interferon signalling pathways to be predominantly mobilised. Seventeen protein-coding genes were predicted to be associated with AIM2 based on STRING analysis.

Conclusion: Our results showed that IFN-alpha is in part responsible for elevating AIM2 inflammasome response in SLE monocytes. Subsequently, potential downstream molecular targets of the upregulated pathways based on RNA-Seq will be experimentally verified for their association with AIM2 inflammasome components.

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Toll-like 4 receptor signalling modulates long non-coding RNA LINC02207 isoforms expressions in systemic lupus erythematosus

22

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Introduction: Systemic lupus erythematosus (SLE) is a systemic autoimmune disease with multiple mechanisms for disease development. Recent studies showed that long non-coding RNAs (lncRNAs) play various roles from gene expression regulation to RNA-protein interaction for controlling cell functions. In our previous study, RNA sequencing analysis was preformed to explore gene expression profiles of lncRNAs of leukocytes among normal individuals and SLE patients. One of the candidate genes, lncRNA LINC02207, has been identified to be overexpressed in SLE patients' neutrophils, which are the most abundant type of white blood cells and important for innate immune responses. Two isoforms, 201c and 203, were identified and overexpression in SLE patients. We would like to investigate the factors contributing to differential expressions of these isoforms.

Methods: Normal individuals' and SLE patents' neutrophils were isolated. The neutrophils were treated with different stimulate toll-like receptor (TLR) stimuli such as lipopolysaccharide (LPS), CpG, and R837 with different time points. The RNAs were extracted and isoforms expressions of LINC02207 were examined by real-time quantitative polymerase chain reaction. We also developed HL-60 cell line model, which can be induced to neutrophil-like cells by all-trans retinoic acid. It was used to study the signalling pathway for controlling isoforms expressions.

Results: Among these stimuli, LPS treatment in normal human neutrophils induced the expressions of isoforms 201c and 203 expressions. As LPS activates TLR4 signalling, it suggests TLR4 may play role in regulating the LINC02207 expressions. Lipopolysaccharide-induced HL-60 cells were treated with different TLR4 signalling inhibitors. Our results agree with previous observations.

Conclusion: In neutrophils, the expressions of two isoforms of LINC02207 were enhanced by LPS via TLR4 pathway. HL-60 cell line model study also agrees with the observation. We will further characterise the functions of these isoforms in neutrophils in SLE patients.

23

Human leukocyte antigen–B*5801 screening to avoid allopurinol-induced severe cutaneous adverse reactions in Chinese patients with chronic kidney disease: a prospective study

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Introduction: Allopurinol is a common drug for treatment of gouty arthritis and complicated hyperuricaemia in patients with chronic kidney disease but also one of the most frequent causes of adverse drug reactions accounting for 5% of all cases of severe cutaneous adverse reactions (SCAR) with mortality rate as high as 26%. There is no effective test to predict the occurrence of SCAR. Human leukocyte antigen (HLA) is a leukocyte surface marker that is involved in immune-mediated diseases. Certain HLA genes have been implicated in drug-induced hypersensitivities.

Methods: Adult chronic kidney diseases subjects who need allopurinol but not previously received were recruited. Genotyping was performed from patients' peripheral blood to determine if they carried the HLA-B*58:01 allele. Those tested positive for HLA-B*58:01 were advised not to take allopurinol and an alternative medication were given; those tested negative were advised to take allopurinol, if indicated. We followed up all subjects for 2 months after allopurinol started to monitor symptoms. Retrospective data were retrieved from the hospital database to identify patients with allopurinol-induced Steven-Johnson syndrome/toxic epidermal necrosis based on clinical presentation and skin biopsy in the past.

Results: A total of 201 subjects were recruited and nine samples were excluded due to protocol violation. Twenty-eight (15%) samples were tested HLA-B*58:01 positive among 192 samples. For patients without HLA-B*58:01, 72% (118/164) have received allopurinol. No SCAR was reported in both groups. According to our local data, the incidence of allopurinol-induced SCAR was 0.39% in the past 5 years (2006-2010).

Conclusion: Use of HLA-B*58:01 screening can prevent allopurinol-induced SCARs by prospectively identifying susceptible subjects at genetic risk and it is a cost-effective preventive measure.

24

Long non-coding RNA LINC02207 in systemic lupus erythematosus

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Introduction: Systemic lupus erythematosus (SLE) is known as an immune complex-mediated disease with multiorgan involvement and diverse clinical presentations. Its pathogenesis depends on genetic, immune, and environmental factors. The heterogeneity of the disease makes it difficult to diagnose, treat and monitor. Recently, long non-coding RNA (lncRNA) was found to be involved in immune dysregulation in rheumatic diseases, especially SLE. We previous showed that LINC02207 lncRNA was overexpressed in SLE neutrophils. Its expression level also correlated with the overexpression of important cytokines such as interleukin 18 and interleukin 18-RAP. We observed the expression of three of its splice variants in SLE neutrophils. Here, the role of these three isoforms was studied.

Methods: Real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR) was used to assess differential expression of three major isoforms of LINC02207 named LINC02207-201, 226 and 203, which were identified from Ensembl and RefSeq. Whole blood sample of 48 SLE patients and 24 normal controls were collected. Neutrophils were isolated from their serum by Ficoll-Paque method, and their RNA was extracted by TRIzol. Real-time RT-qPCR was done on the three targeted isoforms of LINC02207 separately. The correlation between these isoforms with SLE status, nephritis status and other important clinical parameters such as SLEDAI, complement level, and neutrophil level were evaluated.

Results: The expression levels of all three splice variants showed significant elevation in SLE neutrophils samples when compared with normal controls. 226 and 203 were the major isoforms that contribute to the overexpression of the gene LINC02207. Among them, 226 was correlated with lupus nephritic status. Clinically, expression level of 203 was positively correlated with blood neutrophil level.

Conclusion: Two major lncRNA transcripts, LINC02207-226 and LINC02207-203, were positively correlated with SLE status, especially lupus nephritis. These candidates will be further evaluated for their functional importance and their potential as biomarker for SLE.

25

Role of orosomucoid 1–like protein 3 on cigarette smoke–induced airway inflammation, mucus hypersecretion, and activation of the unfolded protein response in human airway epithelial cells

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Introduction: Orosomucoid 1–like protein 3 (ORMDL3), a transmembrane protein localised in endoplasmic reticulum (ER), is strongly linked with childhood-onset asthma. Recent study suggested that ORMDL3 is also associated with chronic obstructive pulmonary disease, in which cigarette smoke (CS) is the major risk factor. We aimed to investigate the effect of ORMDL3 on CS-induced inflammatory responses, mucus hypersecretion, ER stress, and activation of the unfold protein response (UPR).

Methods: The expression of ORMDL3 was manipulated in primary normal human bronchial epithelial (NHBE) cells using siRNA technologies. Successful knockdown of ORMDL3 was confirmed at both mRNA and protein level. Cigarette smoke medium (CSM) was directly applied to NHBE cells for 24 hours (n=4). The inflammatory and mucin markers as well as the activation of the UPR were assessed by quantitative polymerase chain reaction, enzyme-linked immunosorbent assay, and western blot assay.

Results: Cigarette smoke medium caused upregulation of ORMDL3 expression at both mRNA and protein level. ORMDL3 knockdown reduced CSM-induced interleukin 8 release and MUC5AC gene expression. Silencing ORMDL3 also led to reduction of ER stress via inhibition of UPR pathways activating transcription factor 6 (ATF-6) and inositol-requiring enzyme (IRE)1alpha after CSM exposure.

Conclusion: The current findings provide evidence of the inducible nature of ORMDL3 in bronchial epithelial cells after CS exposure and suggest UPR pathways of ATF-6 and IRE1alpha through which ORMDL3 may be linked to CS-induced airway injury.

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26

Serum growth differentiation factor 15 levels are closely associated with the progression of non-alcoholic fatty liver disease

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Introduction: Growth differentiation factor (GDF) 15, a divergent member of the transforming growth factor-β superfamily, is synthesised as an inactive precursor, which is subsequently cleaved and secreted as a disulphide-linked mature protein with a molecular weight of 24.5 kDa. Growth differentiation factor 15 is a stress-induced cytokine and is correlated with cancer, cardiovascular and kidney diseases. However, the association of GDF15 with non-alcoholic fatty liver disease (NAFLD) remains unclear and the major source of GDF15 during the development of NAFLD is unidentified.

Methods: We enrolled a cross-sectional cohort of obese patients who underwent bariatric surgery in the First Affiliated Hospital of Jinan University from 2017 to 2019. Patients with hepatitis B virus infection, aged <18 years, or without NAFLD were excluded. A total of 152 patients were included in our study. Clinical parameters and biochemical markers for glucose and lipid metabolism and liver injury were measured. Hepatic fat content was determined by magnetic resonance imaging. Stage of NAFLD is assessed by liver histology using NAFLD Activity Score. Liver and omentum fat were collected. We also established a diet-induced animal model for NAFLD with the use of choline deficient and methionine restricted L-amino acid diet with 60 kcal% fat. Dynamic changes of serum GDF15 levels during the development of NAFLD were measured and correlation between serum levels of GDF15 and alanine aminotransferase, aspartate transaminase, as well as histological score of NAFLD were explored. Correlation of serum GDF15 levels with basic and biochemical parameters, and the histological spectra of NAFLD in the human and mouse studies were analysed.

Results: In our human study, serum GDF15 level was positively correlated with insulin resistance and liver injury markers. In our mouse study, there was a stepwise increase in the serum level of GDF15 during the progression of NAFLD and was most significantly associated with steatosis when compared with inflammation, ballooning, and fibrosis. Overall, serum level of GDF15 was positively correlated with hepatic fat content. Hepatocytes contribute to the induced GDF15 expression in NAFLD.

Conclusion: Serum GDF15 levels are closely associated with progression of NAFLD in both humans and mice, and hepatocytes is the major source of induced GDF15 expression in NAFLD.

Potential therapeutic role of interleukin 9 in a mouse lung cancer model

27

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Introduction: Lung cancer is still the leading cause of cancer-related death. Mounting evidence indicated that interleukin 9 (IL-9) was linked to cancer. However, its role in lung cancer is still controversial. In this study, we aimed to explore the therapeutic role of IL-9 in lung cancer and to elucidate its mechanism.

Methods: Mouse Lewis lung carcinoma (LLC) cells and human lung cancer cell lines (H1975, A549, HCC827) were purchased from ATCC. Western blot was used to detect the IL-9 receptor expression. MTT assay was conducted to explore the effect of IL-9 on human lung cancer cell lines. Lewis lung carcinoma mouse model was established by subcutaneous injection of 500 000 LLC cells. At day 7, the mice were randomised and divided into control and IL-9 group. In IL-9 arm, mice were treated with IL-9 intraperitoneally (50 ng/mouse, 3 times/week). The body weight of mice and tumour volume were measured every other day.

Results: Interleukin 9 receptor was found in all human lung cancer cell lines, but not in LLC. Interleukin 9 inhibited cell proliferation in human lung cancer cell lines. After incubation of 50 ng/mL IL-9 for 48 hours, about 20% to 40% reduction of cell growth was observed. Moreover, IL-9 suppressed the tumour growth in LLC mouse model. The relative tumour volume in the IL-9 treatment arm was 69% that of the control group at the end of treatment (P=0.0295).

Conclusion: Interleukin 9 slightly inhibited growth of human lung cancer cell lines only possibly due to endogenous expression of IL-9 receptor in those cell lines. In contrast, IL-9 suppressed tumour growth in LLC mouse model. The underlying mechanisms remain to be elucidated.

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28

Targeting PIN1 as a potential novel therapeutic strategy against activated B cell–like subtype of diffuse large B cell lymphoma

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Introduction: Diffuse large B cell lymphoma (DLBCL) is the most common subtype of non-Hodgkin lymphoma and activated B cell–like subtype of DLBCL (ABC-DLBCL) is associated with the worst prognosis. Activated B cell-like subtype of DLBCL is characterised by constitutive activation of the nuclear factor (NF)–κB signalling pathway that is essential for the survival of cancer cells. Molecular targeting drug, ibrutinib, blocking NF-κB activation by inhibiting Bruton's tyrosine kinase shows promising clinical results in patients with ABC-DLBCL. However, most of the responses were partial and acquired ibrutinib resistance has been observed in ABC-DLBCL cells. As the peptidyl-prolyl-isomerase PIN1 has been found to enhance the oncogenic activity of NF-κB thereby promoting cancer cell proliferation. Therefore, we hypothesise that inhibition of PIN1 may be a potential therapeutic strategy against ABC-DLBCL cells through suppression of PIN1-mediated NF-κB activation.

Methods: Knocking-down PIN1 expression was performed by infection of ABC-DLBCL cells with lentiviral particles expressing shRNA against PIN1. Cell proliferation was determined by MTT assay. Protein expression of numerous PIN1-interacted oncogenic proteins was analysed by western blotting.

Results: PIN1 depletion by lentiviral shRNA-PIN1 particles suppressed cell proliferation in ABC-DLBCL cells and downregulated numerous PIN1-interacted oncogenic proteins including β-catenin, cyclin D1, and NF-κB. In addition, nuclear fraction of NF-κB was reduced in PIN1-depleted cells. Notably, PIN1 depletion showed a greater antiproliferative effect compared with ibrutinib treatment especially on those ibrutinib-resistant ABC-DLBCL cell lines. Most importantly, treatment of various ABC-DLBCL cell lines with PIN1 inhibitors (ATRA and KPT-6566) resulted in the decrease of cell viability in a dose-dependent manner.

Conclusion: Genetic (shPIN1) and chemical (ATRA and KPT-6566) inhibition of PIN1 induced an antiproliferative effect on ABC-DLBCL cells through suppression of PIN1-mediated NF-κB activation. Thus, this study provides new insights for treatment of ibrutinib-resistant ABC-DLBCL cells.

31

Safety and efficacy of lithium carbonate as the second-line antithyroid drug in the treatment of Graves' disease: a retrospective case series in Hong Kong Chinese patients

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Introduction: When thionamides become contra-indicated in Graves' disease, definitive treatment with total thyroidectomy or radioactive iodine (RAI) should be considered. Lithium can work as the second-line antithyroid drug by inhibiting thyroid hormone release while waiting for definitive treatment of hyperthyroidism. We aimed to review its safety and efficacy in the treatment of Graves' disease.

Methods: Case records of patients with Graves' disease who received lithium carbonate as the second-line antithyroid drug from 2010 to 2019 were reviewed.

Results: Twenty two patients who received lithium carbonate as the second-line antithyroid drug were identified. Contra-indications of thionamides included: thionamide allergy (9 cases), carbimazole related hepatotoxicity (5 cases), carbimazole related agranulocytosis (3 cases), and severe neutropenia or significant liver derangement at baseline (5 cases). The median lithium dose and the median lithium levels were 750 (interquartile range [IQR]=500-750) mg per day and 0.4 (IQR=0.28-0.55, toxic if >1.6) mmol/L, respectively. None of the patients developed lithium toxicity or side-effects. Twelve patients achieved a median reduction in free thyroxine (fT4) level by 10 (IQR=3-37) pmol/L after lithium therapy. Free thyroxine level rose in seven patients and all of them had been treated with thionamide prior to lithium therapy. Overall 85% of cases achieved free thyroxine level <1.5 times upper limit of normal prior to definitive treatment. Eighteen patients received RAI (median dose=370 MBq) while four underwent total thyroidectomy as definitive treatment. Out of 18 patients, 15 (83%) developed post-RAI hypothyroidism.

Conclusion: Lithium carbonate is a safe second-line antithyroid drug. It is effective in ameliorating hyperthyroidism or preventing major rise in thyroid hormone level which may make subsequent definitive treatment risky. Radioactive iodine has got a high success rate in patients with Graves' disease pretreated with lithium therapy.

32

Systemic therapy treatment outcomes for recurrent hepatocellular carcinomas following liver transplants in Hong Kong

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Background: Clinical outcome of hepatocellular carcinoma (HCC) recurs following liver transplant is poor. Recurrences are treated with surgical resection until they are no longer resectable or if disseminated recurrences occur. The use of systemic therapies in this clinical contest is complex because of the organ transplant and immunosuppressive environment, together with potential complex drug interactions between the immunosuppressants and anti-cancer therapies. Sorafenib was the main treatment of choice before the availability of more options in recent years. All the clinical trials of systemic therapies for advanced HCC excluded patients with any history of organ transplant. The safety and efficacy of the application of systemic medical therapies in this clinical setting is therefore largely unknown. Hence, retrospective data on real-life clinical settings are needed to inform best practices.

Methods: This paper is a retrospective cross-sectional study of consecutive adult patients with recurrence of HCC following liver transplant for the indication of treatment of HCC in Queen Mary Hospital from January 2008 to January 2018. Demographic data and baseline characteristics were described. The primary outcome of overall survival of the whole population and those treated with sorafenib or other systemic therapies were described. Secondary outcome of adverse events and tolerability of sorafenib were described.

Results: Forty three consecutive patients with a recurrence of HCC following liver transplant were identified from 2008 to 2018. Among this population (n=43) the median survival from diagnosis of recurrence was 17 months (95% confidence interval [CI]=11.3-22.7 months). Univariate analysis shows that early recurrence within 12 months of transplant was associated with a significantly worse median survival of 10 months (95% CI=8.5-11.4 months) compared with 26 months (95% CI=18.8-33.2 months) when recurrences occurred after 12 months from transplant (P<0.001). Among those who had sorafenib as the first systemic therapy (n=34), median survival from recurrence was 14 months (95% CI=7.3-20.7 months).

Conclusion: Treatment efficacy and adverse events and tolerability of sorafenib were comparable with those in the setting of advanced HCC without transplant. The overall survival of patients with HCC recurrence following liver transplant was poor, despite treatment with sorafenib. Early recurrence within 1 year from transplant was the most significant risk factor of poor survival.

33 Exploring the utility of auto-antibodies in immune checkpoint blockade for advanced cancers

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Introduction: The presence of autoantibodies in advanced cancers may serve as biomarkers of efficacy and toxicity in patients who receive immune checkpoint inhibitors (ICI).

Methods: Patients treated by immune checkpoint anti-PD1 and/or anti-CTLA4 antibodies, and tested for autoAbs (antinuclear antibodies [ANA], antithyroid antibodies, antiadrenal antibodies, anti-skin antibodies, and rheumatoid factor [RF]) were retrospectively identified. We explored autoAbs as prognostic and predictive markers of response and treatment-related immune-related adverse events (IRAE). Patients with underlying rheumatological or autoimmune diseases were excluded.

Results: Between 1 January 2018 and 30 September 2019, 48 patients (26 men and 22 women) with advanced malignancies received one or more doses of ICI and underwent testing for ANA. Median age was 61.55 years. Objective response was 35.4% (1 complete response and 16 partial responses), giving a median overall survival of 1.18 years (95% confidence interval=0.73 to not available). Thirty one patients had ANA testing before immunotherapy, while 17 received testing after treatment. Positive ANA, defined as a titre of ≥1/160, was found in 27%. Three of 32 patients showed elevated RF, while antithyroglobulin, antithyroid peroxidase and anti-skin antibodies were detected in three, six and five patients, respectively. Positivity for ANA, and other autoAbs was not predictive of treatment response (P=0.74, P=1.0) or IRAE (P=0.81, P=0.23) although the presence of any autoAbs showed a trend towards improved survival. Two patients with repeated measurements of ANA showed serial increase in titres corresponding to the onset of immune adverse events. Immune-related adverse events occurred in 19 patients and included acute kidney injury, hepatitis, neuropathy, uveitis, thyroiditis, pemphigoid, and hypocortisolism. None developed rheumatological symptoms. Out of four patients, three with biochemical thyroiditis had detectable antithyroid antibodies, while none of 25 patients had detectable adrenal antibodies at baseline.

Conclusion: Autoantibodies in cancer patients on ICIs do not predict response or toxicity but maybe associated with improved prognosis and the pathogenesis of IRAE.

34 Exercise improves cardiac dysfunction via fibroblast growth factor 21–sirtuin3 signalling in type 2 diabetic mice

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Introduction: Physical exercise is an effective non-pharmacological intervention for diabetic heart diseases. However, the underlying molecular mechanisms remain poorly defined. Fibroblast growth factor 21 (FGF21), a peptide hormone with multiple salutary effects on obesity-related cardiometabolic complications, has been identified as an exercise-responsive factor. Nevertheless, the roles of FGF21 in cardiac function under physical exercise remain unknown. Therefore, this study aimed to investigate whether FGF21 signalling is involved in exercise-induced improvement in cardiac function in type 2 diabetic mice.

Methods: Fibroblast growth factor 21 knockout (FGF21 KO) and wild-type (WT) type 2 diabetic mice were subjected to treadmill running for 6 weeks. Cardiac mitochondria were isolated after measurement of heart function. Muscle-specific beta klotho (KLB, the co-receptor of FGF21) knockout and WT diabetic mice were also performed exercise training to address the role of FGF21 signalling in the protective effects of exercise on type 2 diabetic hearts.

Results: Treadmill exercise significantly alleviated diabetes-induced impairments in cardiac function and mitochondrial function in WT mice, whereas such a cardiac protective role of exercise was abrogated in FGF21 KO mice. Furthermore, muscle KLB KO mice were also refractory to the cardioprotective effects of exercise on type 2 diabetic heart, suggesting FGF21 signalling is essential for exercise-induced improvement of diabetic cardiomyopathy. We next identified the critical mitochondrial deacetylase sirtuin3 (Sirt3) was selectively downregulated in FGF21 KO mice under exercise intervention. Restoration of cardiac Sirt3 by using adeno-associated virus 9 vectors alleviated mitochondrial dysfunction and diabetic cardiomyopathy in FGF21 KO mice, which suggests cardiac Sirt3 is obligatory for beneficial effects of FGF21 on cardiac function.

Conclusion: Our study uncovers a novel role of FGF21-Sirt3 signalling cascade for exercise-induced cardiac benefits via improvements of mitochondrial functions.

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Interaction between genetic variants and haemoglobin A1c on the risk of sight-threatening diabetic retinopathy

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Introduction: Diabetic retinopathy (DR) is a multifactorial disorder arising from the complex interplay of environmental and genetic factors. Gene-environment interaction may partially account for the unexplained heritability of previously identified DR-susceptibility variants. Haemoglobin A1c (HbA1c) has been suggested as a critical modifiable environmental factor for DR. Whether HbA1c modifies the genetic susceptibility to sight-threatening DR (STDR) was investigated in this study.

Methods: Sixty nine single nucleotide polymorphisms (SNPs) showing strong association signals ($P<0.0005$; $r^2<0.9$) in previous genome-wide association studies of DR were genotyped in 1051 STDR cases and 2042 non-DR controls. Their associations with STDR were examined by logistic regression analysis with adjustments for age, sex, body mass index, duration of diabetes, the presence of hypertension, and HbA1c. Haemoglobin A1c <7% has been suggested as an optimal glycaemic control of diabetes. Those SNPs showing nominal associations with STDR were further examined for their interactions with HbA1c (dichotomised as <7% vs ≥7%, or as a continuous variable) on the risk of STDR.

Results: Four SNPs were nominally associated with STDR, including COL5A1 rs59126004 (P=0.034; odds ratio [95% confidence interval]=0.84 [0.71-0.98]) and CREB5 rs11765845 (P=0.036; 0.87 [0.76-0.99]). COL5A1 rs59126004 showed significant interaction with dichotomised HbA1c on the risk of STDR (P_{int}=0.00173). In the stratified analysis, COL5A1 rs59126004 was associated with a significantly reduced risk of STDR in patients with adequate glycaemic control (HbA1c <7%: P=0.000176; odds ratio=0.58, 95% confidence interval=0.44-0.77) but was not associated with STDR in patients with inadequate glycaemic control (HbA1c ≥7%). The interaction between this SNP and continuous HbA1c was also found to be associated with the risk of STDR (P_{int}=0.029). CREB5 rs11765845 also appeared to show significant interaction with continuous HbA1c on the risk of STDR (P_{int}=0.018).

Conclusions: Our results suggest potential interactions between HbA1c and some DR-susceptibility variants on the risk of STDR. These findings may provide new insight into the pathophysiological mechanism of DR. Further studies are warranted to validate our findings.

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Role of G protein-coupled receptor 110 in the pathogenesis of obesity

37

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Introduction: Obesity is a major chronic disease giving rise to numerous metabolic complications, including type 2 diabetes, cardiovascular diseases, and even cancers. G protein-coupled receptor 110 (GPR110) is an orphan receptor which is involved in neuronal development and hepatocarcinogenesis. However, the role of GPR110 in metabolic diseases remains unexplored.

Methods: Global GPR110 knockout (KO) mice were generated and subjected to standard chow (STC) or high-fat diet (HFD) feeding. Body weight and body composition of mice was measured. Food intake, locomotor activity, and energy expenditure were determined by comprehensive lab animal monitoring system. Glucose tolerance and insulin sensitivity was examined by glucose tolerance and insulin tolerance tests. Serum lipid profiles were checked using biochemical methods.

Results: The GPR110 KO mice gained less weight during HFD feeding, but similar weight during STC feeding which was attributed to lower fat weight in the GPR110 KO mice after HFD feeding. Consistently, the GPR110 KO mice were more resistant to HFD-induced glucose intolerance and insulin resistance when compared with their wild-type littermates. While food intake was similar between GPR110 KO and wild-type mice placed on either STC or HFD, GPR110 KO mice had higher locomotor activities during the dark phase when fed with either STC or HFD which was consistent with higher energy expenditure in these mice.

Conclusion: These findings suggest the involvement of GPR110 in the pathogenesis of obesity which is potentially mediated through control of locomotion.

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Metabolic factors and adipokine levels are associated with fibrosis evolution in chronic hepatitis B patients on nucleoside analogue therapy

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Introduction: Nucleoside analogues are the first-line antiviral therapy in chronic hepatitis B (CHB). Nucleoside analogue therapy can result in fibrosis regression. However, fibrosis regression is not universal and metabolic factors have been increasingly identified to influence the clinical course of CHB. We performed a prospective study to determine the impact of adipokine levels and metabolic factors on longitudinal fibrosis evolution in patients with CHB on nucleoside analogue therapy.

Methods: On-treatment patients with CHB were prospectively recruited for paired transient elastography at baseline and 3 years. Fibrosis staging was defined according to the European Association for the Study of the Liver–Asociacion Latinoamericana para el Estudio del Higado guidelines. Progression and regression of fibrosis were defined as increment or decrement of one or more fibrosis stages from baseline respectively. Hepatic steatosis was defined as controlled attenuation parameter (CAP) ≥248 dB/m. A panel of plasma adipokines including fibroblast growth factor 21 (FGF21), adipocyte fatty acid binding protein (AFABP), and adiponectin were measured by enzyme-linked immunosorbent assay kits (Antibody and Immunoassay Services, The University of Hong Kong).

Results: In total, 404 patients with CHB (72.0% male; mean age 58.5 years) were recruited. The mean treatment duration for CHB was 72.3 (±43.9) months. Hepatitis B virus DNA was undetectable (≤20 IU/mL) in 92.3% of patients at baseline and 93.9% of patients at follow-up. The baseline AFABP, adiponectin and FGF21 levels were 96.5 (28.4-172.3) pg/mL, 10.4 (6.7-17.1) μg/mL and 12.6 (7.2-37.0) pg/mL, respectively. Fibrosis progression and regression were observed in 72 (17.8%) and 88 (21.8%) patients, respectively. Treatment factors including nucleoside analogue type, treatment duration, and hepatitis B virus DNA were not associated with fibrosis progression/regression. Higher baseline CAP was associated with fibrosis regression, but was non-significant after controlling for other parameters. In a separate multivariate model, longitudinal decrease in CAP was independently associated with fibrosis regression (odds ratio=1.009, 95% confidence interval=1.001-1.016; P=0.022) and longitudinal increase in body mass index was independently associated with fibrosis progression (odds ratio=1.212, 95% confidence interval=1.009-1.456; P=0.04). Lower level of AFABP was independently associated with fibrosis regression among patients with advanced fibrosis at baseline (odds ratio=1.005, 95% confidence interval=1.001-1.010; P=0.018) and higher level of adiponectin was independently associated with fibrosis regression in patients with diabetes (odds ratio=1.296, 95% confidence interval=1.108-1.516; P=0.001). Fibroblast growth factor 21 was not associated with fibrosis evolution.

Conclusion: Longitudinal increase in body mass index and steatosis were significant independent predictors of fibrosis evolution in on-treatment patients with CHB. Adiponectin and AFABP were predictive of fibrosis regression in specific subgroups, further demonstrating the role of metabolic factors in influencing CHB-related treatment outcomes.

39

Mechanosensitive ion channel Piezo1 controls cell fate determination of bone marrow mesenchymal stem cells

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Introduction: Bone marrow mesenchymal stem cells (MSCs) are non-haematopoietic multipotent stem cells which can be differentiated into osteoblasts and bone marrow adipocytes. The inverse relationship between MSC-derived adipogenesis and osteoblastogenesis forms a balance to maintain a homeostatic state. Disruption of this balance due to ageing or obesity will lead to osteoporosis or marrow adiposity. However, the molecular machineries controlling MSCs fate remain poorly understood. Piezo1 is a mechano-sensitive ion channel transducing mechanical stimuli into intracellular biochemical signals, which is highly expressed in bone marrow MSCs. In this study, we investigated the role of Piezo1 in controlling MSCs lineage commitment.

Methods: Piezo1$^{flox/flox}$ mice were crossed with platelet-derived growth factor receptor alpha (PDGFRa, a marker of MSCs) Cre transgenic mice to generate PDGFRa-Piezo1 knockout (KO) mice. Femurs and tibias were isolated from PDGFRa-Piezo1 KO mice and wild-type (WT) controls with or without treadmill exercise. The isolated femurs and tibias were subjected to micro-computed tomography analysis and osmium tetroxide staining to visualise bone volume and bone marrow adipocytes, respectively. Mesenchymal stem cells isolated from WT and KO bone marrows were differentiated into osteoblasts and adipocytes in vitro.

Results: The PDGFRa-Piezo1 KO mice had lower body weight but normal peripheral fat content. The decreased body weight of KO mice was due to their reduced bone volume and bone density. Marrow adiposity was also observed in KO mice. In addition, exercise-induced increase of bone volume and decrease of marrow adiposity was abolished in KO mice. In vitro, KO MSCs are more prone to differentiate into adipocytes rather than osteoblasts.

Conclusion: Our study uncovers the critical role of Piezo1 in controlling the cell fate determination of MSCs by sensing mechanical stimulation.

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40

Detection of hepatitis B virus covalently closed circular DNA and integrated hepatitis B virus DNA in hepatitis B surface antigen–negative hepatocellular carcinoma patients

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Background: Occult hepatitis B infection (OBI), denoted by the presence of hepatitis B virus (HBV) DNA in the liver or blood in individuals with undetectable circulating hepatitis B surface antigen (HBsAg), is a risk factor for hepatocellular carcinoma (HCC). The current diagnosis of OBI is based on the detection of HBV DNA using four sets of polymerase chain reaction (PCR) targeting to four HBV regions (four-region PCR). The aim of this study was to detect HBV covalently closed circular DNA (cccDNA) and integrated HBV DNA in the liver in HCC patients with OBI.

Methods: "Four-region PCR" was used to detect OBI in 90 HBsAg-negative HCC patients. Covalently closed circular DNA and integrated HBV DNA were detected by "over-gap PCR" and Alu-PCR, respectively.

Results: Using the "four-region PCR", we identified OBI in 62/90 (69%) HBsAg-negative HCC patients. Of the 62 HCC patients with OBI, 29 (47%) and 43 (69%) had detectable cccDNA and integrated HBV DNA, respectively. A higher proportion of patients with integrated HBV DNA was found in patients with undetectable cccDNA (29/33; 88%) than in those with detectable cccDNA (14/29; 48%; P=0.001). Some hotspot HBV DNA integration sites near cancer-related genes such as TERT and KMT2B were identified. Majority (39/43; 91%) of OBI patients with HBV DNA integration did not have cirrhosis.

Conclusion: Among 62 HCC patients with OBI, 47% had detectable cccDNA and 69% had detectable integrated HBV DNA. While the detection of cccDNA in OBI patients indicates the risk of HBV reactivation, the detection of integrated HBV DNA, which indicates the risk of HCC, should not be overlooked. The presence of integrated HBV DNA near HCC-related genes in non-cirrhotic patients, even when cccDNA was undetectable, suggests that OBI induces HCC via HBV DNA integration in HBsAg-negative patients.

Inducing beige adipocyte from human-induced pluripotent stem cells without gene transfer

41

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Introduction: Obesity is now becoming a pandemic, with potentially disastrous consequences for human health. In contrast to white adipocytes that stores energy, beige adipocytes are capable of dissipating energy in the form of heat. Both clinical and animal studies have pointed to the therapeutic potential of adipocyte beiging in obesity. Therefore, reliable and safe strategies to induce human beige adipocytes are priorities for this therapeutic strategy. Here we present a differentiation method in which the human-induced pluripotent stem cells (hiPSC) were induced to functional beige adipocytes without gene transfer.

Methods: Oil red O and immunofluorescence staining were used to examine the cell morphology. Important markers of hiPSC, mesodermal cell, preadipocyte progenitors, and mature beige adipocytes were investigated to record the process of differentiation by quantitative polymerase chain reaction and western blot. We also conducted lipolysis, glucose uptake, and oxygen consumption rate studies to analyse the functions of these hiPSC-derived beige adipocytes.

Results: Our hiPSC-derived beige adipocytes displayed beige-specific morphology with multiple small cytoplasmic lipid droplets and markers such as CD137 and UCP1. Moreover, these cells displayed the adipose functions including lipolysis, glucose uptake, and increased oxygen consumption in response to thermogenic-related stimuli T3 and CL314243.

Conclusion: The hiPSC-derived beige adipocytes from our differentiation approach have similar characteristics to human beige adipocytes. These results provide a platform for further drug screen and population studies.

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Risk factors of community-acquired pneumonia in patients with spondylarthritis

42

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Objectives: To compare the risk of community-acquired pneumonia (CAP) in spondylarthritis (SpA) and non-specific back pain (NSBP), and to identify the risk factors for developing CAP in SpA.

Methods: A total of 2906 patients with SpA from nine rheumatology centres and 2526 patients with NSBP from an orthopaedic unit were reviewed from the Clinical Management System in Hong Kong. Incidence of CAP leading to hospital admission and demographic data including age, sex, smoking and drinking status, conventional disease-modifying antirheumatic drugs (c-DMARDs), individual biological DMARDs (b-DMARDs), micro-organisms, and co-morbidities were identified. Dosage and duration of b-DMARDs were also recorded. Risks of CAP in SpA were compared with that in NSBP using propensity score matching method. Multivariate cox regression model was used to identify the risk factors in SpA. Results were expressed in hazard ratio (HR).

Results: Community-acquired pneumonia was found in 242 patients with SpA and 139 patients with NSBP. Increased risk for CAP were found in the following groups with SpA: all subgroups (HR=2.76, P<0.001), without use of DMARDs (HR=4.87, P<0.001), with co-morbid psoriasis (PsO; HR=3.36, P<0.001), and without co-morbid PsO (HR=2.60, P<0.001). The use of b-DMARD did not increase the risk of CAP. A decreased HR was found with use of c-DMARDs (HR=0.24, P<0.001). Identified risk factors include smoking (HR=1.69, P<0.001), alcohol use (HR=1.54, P=0.02), co-morbid PsO (HR=1.98, P<0.001), steroid use (HR=2.14, P<0.001), malignancy (HR=2.39, P<0.001), renal impairment (HR=1.48, P=0.03), and chronic lung disease (HR=4.43, P<0.001). The two most common organisms identified were influenzae and pneumococcus.

Conclusion: Influenzae and pneumococcus vaccination should be considered in all patients with SpA.

Adipocytes regulate osteogenesis in a novel lipoatrophic mouse model

43

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Introduction: Substantial clinical evidence demonstrating a complex relationship between adiposity and osteoporosis suggests that adipose tissue plays an important role in bone metabolism. However, the exact function of adipocytes and mechanisms underlying its impact on bone health are yet incompletely understood due to marked discrepancies in human and animal studies. In this study, the effect of adipocytes on osteogenesis was explored using a novel lipodystrophic mouse model.

Methods: We generated adipose tissue-specific murine double minute 2 knockout (KO) mice with Cre/loxP system. Knockout mice displayed gradually loss of peripheral adipose tissue and bone marrow adipocytes until nearly 80% to 100% of total fat reduction at 12-week-old mice.

Results: Micro-computed tomography revealed that bone mineralisation density of femur from 12-week-old KO mice was significantly higher than the wild-type (WT) mice. Interestingly, the absence of adipocytes in KO mice exhibited markedly lower serum levels of C-terminal telopeptides of type I collagen (bone resorption marker) than WT mice, whereas the N-terminal propeptides of type I collagen (bone formation marker) remained unaffected. No differences were observed in the ex vivo differentiation of osteoblasts and osteoclasts between WT and KO mice. In addition, adipocyte-conditioned media treatment of isolated haematopoietic stem cells from bone marrow enhanced the differentiation of osteoclasts.

Conclusion: These results indicate that secreted factors from adipocytes may promote osteoclastogenesis of haematopoietic stem cells. In summary, mature adipocytes suppress osteogenesis by enhancing the formation of osteoclasts, which eventually lead to low bone mass. Further studies are required to find out the novel factor and its detailed underlying mechanism in promoting osteoclastogenesis.

Incidence of cancer in patients taking valsartan or amlodipine for ≥1 year

44

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Introduction: In July 2018, five generic valsartan products suspected to contain nitrosodimethylamine were recalled. Subsequently, nitrosamines have been found in other drugs produced by chemical synthesis. As valsartan is widely used for hypertension, heart failure and nephropathy, we investigated whether valsartan increases cancer risk relative to amlodipine, a commonly used antihypertensive drug.

Methods: Patients who took valsartan or amlodipine for at least 1 year between 1 January 2003 and 30 June 2009 were identified using the Clinical Data Analysis and Reporting System of the Hong Kong Hospital Authority. Patients with previously diagnosed cancers were excluded. Patients were followed until cancer diagnosis, death, loss to follow-up or 30 June 2019, whichever occurred first. Results were analysed using R version 3.6.1. The incidence and 95% confidence interval (CI) of cancer were estimated using Poisson regression.

Results: Among 5639 valsartan users and 4386 amlodipine users, 1001 and 855, respectively, had cancer diagnosed during a median follow-up period of 10.97 and 12.12 years. The incidences of cancer are 161.82 (95% CI=138.89-188.54) per 10 000 person-years and 160.84 (95% CI=137.98-187.49) per 10 000 person-years, respectively.

Conclusion: Valsartan does not appear to increase cancer incidence when compared with amlodipine.

Adipose tissue–targeted liposomal drug delivery system for treatment of obesity

45

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Introduction: Obesity is now becoming a pandemic, with potentially disastrous consequences for human health. However, there are no drugs or combinations of drugs for treating obesity that are both safe and highly effective. One possible approach for solving this problem is to deliver anti-obesity drugs to adipose tissue accurately. Previous papers reported that prohibitin targeting peptide (PTP)–modified nanoparticles are selectively targeted to adipose tissue. Herein, we used PTP-modified liposomes to encapsulate triiodothyronine (T3) to achieve adipose selective transport.

Methods: DSPE-PEG-PTP was synthesised and PTP-conjugated liposomes were prepared. Then T3 was loaded in liposomes to prepare T3-PTP-Lip. Transmission electron microscopy, dynamic light scattering, and high-performance liquid chromatography were used to characterise T3-PTP-Lip. Live imaging and fluorospectrophotometry were used to measure the adipose tissue targeting ability. Pharmacokinetic analysis was used to investigate the residence time of T3-PTP-Lip. The effect of T3-PTP-Lip on the metabolism of mice and adipose tissue was measured by metabolic cage and seahorse bioanalyser, respectively.

Results: Mass spectrometry and nuclear magnetic resonance spectroscopy results confirmed the successful synthesis of DSPE-PEG-PTP. Transmission electron microscopy and dynamic light scattering showed that T3-PTP-Lip was formed with a particle size of 105 nm. Compared with conventional liposome, T3-PTP-Lip displayed significantly enhanced enrichment in adipose tissue. Pharmacokinetic analysis showed that T3-PTP-Lip had a high residence time in adipose tissue. In addition, compared with other groups, T3-PTP-Lip had a higher ability to enhance the metabolism rates of mice and adipose tissue.

Conclusion: The selective delivery and efficient action of T3-PTP-Lip suggests that it might serve as a prototype for anti-obesity drugs.

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Cardiovascular outcomes in trials of new antidiabetic drug classes

46

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Introduction: Whether a specific new antidiabetic drug class reduces cardiovascular events better is uncertain because of few direct comparative trials. We therefore compared new antidiabetic drug classes with respect to cardiovascular outcomes by using network meta-analysis.

Methods: Cardiovascular outcome trials or renal outcome trials evaluating cardiovascular outcomes of glucagon-like peptide-1 receptor agonists (GLP-1 RAs), sodium-glucose co-transporter 2 (SGLT-2) inhibitors, or dipeptidyl peptidase-4 (DPP-4) inhibitors in patients with type 2 diabetes were searched up to 30 July 2019. Network meta-analysis was performed using R statistics.

Results: Fifteen trials were finally included. Compared to placebo, GLP-1 RAs and SGLT-2 inhibitors significantly reduced major adverse cardiovascular events (odds ratio [OR]=0.88, 95% confidence interval [CI]=0.83-0.93 and OR=0.88, 95% CI=0.82-0.94), cardiovascular mortality (OR=0.89, 95% CI=0.80-0.99 and OR=0.82, 95% CI=0.73-0.92), all-cause mortality (OR=0.90, 95% CI=0.83-0.96 and OR=0.84, 95% CI=0.77-0.92), and renal composite outcome (OR=0.81, 95% CI=0.75-0.88 and OR=0.59, 95% CI=0.52-0.67), respectively. Furthermore, both of the two drug classes reduced major adverse cardiovascular events (OR=0.88, 95% CI=0.80-0.96 and OR=0.88, 95% CI=0.79-0.97), all-cause mortality (OR=0.88, 95% CI=0.79-0.99 and OR=0.83, 95% CI=0.73-0.94), and renal composite outcome (OR=0.78, 95% CI=0.66-0.92 and OR=0.57, 95% CI=0.47-0.68) more than DPP-4 inhibitors. Only SGLT-2 inhibitors reduced hospitalised heart failure when compared with GLP-1 RAs (OR=0.73, 95% CI=0.63-0.85), DPP-4 inhibitors (OR=0.64, 95% CI=0.55-0.75), and placebo (OR=0.68, 95% CI=0.61-0.77).

Conclusion: Both GLP-1 RAs and SGLT-2 inhibitors show favourable cardiovascular efficacy and safety profiles while DPP-4 inhibitors do not. Sodium-glucose co-transporter inhibitors reduce cardiovascular events, hospitalised heart failure, renal events, and deaths the most, so they are the preferred treatment for most patients with type 2 diabetes while GLP-1 RAs can be considered thereafter as an add-on therapy.

47

Efficacy and safety of $P2Y_{12}$ inhibitors in patients with acute coronary syndrome: a network meta-analysis

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Introduction: Whether newer $P2Y_{12}$ inhibitors are more efficacious and safer than clopidogrel in acute coronary syndrome (ACS) is uncertain. We used network meta-analysis to compare their effects on clinical outcomes.

Methods: Randomised controlled trials (RCTs) comparing clopidogrel, prasugrel, ticagrelor, or cangrelor, in combination with aspirin in ACS patients were searched up to 20 October 2019. Those reporting major adverse cardiovascular events (MACE), bleeding events, and deaths were included. Statistical analysis was performed using R.

Results: Sixteen RCTs with altogether 78176 patients were included. Prasugrel significantly reduced MACE (odds ratio [OR]=0.80, 95% confidence interval [CI]=0.68-0.94) and myocardial infarction (OR=0.75, 95% CI=0.63-0.99) when compared with clopidogrel. Prasugrel and ticagrelor both reduced cardiovascular mortality (OR=0.85, 95% CI=0.75-0.97 and OR=0.82, 95% CI=0.73-0.93) and definite or probable stent thrombosis (OR=0.49, 95% CI=0.38-0.63 and OR=0.72, 95% CI=0.57-0.90), respectively. Prasugrel reduced definite or probable stent thromboses more than ticagrelor (OR=0.69, 95% CI=0.51-0.93). In contrast, prasugrel increased the risk of thrombolysis in myocardial infarction (TIMI) major bleeding when compared with clopidogrel (OR=1.26, 95% CI=1.02-1.55) while increased the risk of TIMI minor bleeding when compared with clopidogrel (OR=1.44, 95% CI=1.16-1.77) and ticagrelor (OR=1.32, 95% CI=1.01-1.72), respectively. Cangrelor showed no additional benefits but had more TIMI minor bleeds than clopidogrel (OR=1.47, 95% CI=1.01-2.16).

Conclusions: Ticagrelor is a safe antiplatelet drug of choice for most ACS patients. Prasugrel improves ischaemic outcomes further and is suitable for those at low bleeding risk.

48

Optimal duration of dual antiplatelet therapy after drug-eluting stents implantation: a network meta-analysis of randomised controlled trials

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Introduction: Although there is a trend of recommending shorter duration of dual antiplatelet therapy (DAPT) after drug-eluting stent (DES) implantation, the optimal duration is as controversial as ever. We performed a network meta-analysis incorporating the latest trials to assess the risks and benefits of different DAPT durations.

Methods: Randomised controlled trials (RCTs) comparing different DAPT durations after DES implantation and reporting frequencies of cardiovascular and bleeding events were searched up to 30 June 2019. Data analysis was performed using R statistics.

Results: Seventeen RCTs with altogether 46684 patients were finally included. Extended DAPT significantly reduced the incidence of myocardial infarction and definite/probable stent thrombosis compared with 12-month DAPT (odds ratio [OR]=0.54, 95% confidence interval [CI]=0.45-0.66, P<0.001 and OR=0.47, 95% CI=0.32-0.69, P<0.001), and short-term DAPT (OR=0.52, 95% CI=0.41-0.67, P<0.001 and OR=0.47, 95% CI=0.31-0.72, P<0.001), respectively. Short-term DAPT increased the risk of repeat revascularisation (OR=1.23, 95% CI=1.06-1.43, P=0.006) compared with 12-month DAPT. In contrast, extended DAPT resulted in more major bleeds than short-term DAPT (OR=2.00, 95% CI=1.36-2.92, P<0.001) and 12-month DAPT (OR=1.43, 95% CI=1.07-1.91, P=0.016).

Conclusion: Extended DAPT over 12 months, if well tolerated, decreases myocardial infarction and stent thrombosis but increases major bleeds. Short-term DAPT should be considered for most patients after DES implantation; however, the possibility of increased repeat revascularisation should not be ignored. The optimal DAPT therapy should therefore be individualised after weighing up the bleeding risk and ischaemic benefits for that patient.

49

Association between gout and depression: The United States National Health Nutrition and Examination Survey 2007-2016

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Introduction: Acute gout is painful and can lead to anxiety and depression. Whether gout leads to chronic depression is unclear.

Methods: A total of 25 016 adult participants who responded to the 9-item Patient Health Questionnaire in the United States NHANES (National Health Nutrition and Examination Survey) 2007-2016 were included in this analysis. The diagnosis of gout was based on self-reporting. Results were analysed using SPSS complex sample module version 25. Logistic regression was used to study the association between gout and depression. Odds ratio (OR) and 95% confidence interval (CI) were estimated.

Results: Although there was increased prevalence of depression among participants with gout (without gout vs gout: 7.9% [7.4%-8.5%] vs 9.8% [7.8%-12.4%]; P=0.003), gout was not independently associated with depression in both univariate (OR=1.27, 95% CI=0.99-1.63) and multivariate analysis (OR=1.15, 95% CI=0.84-1.56). Multivariate analysis showed that female sex (OR=1.75, 95% CI=1.51-2.04), education (college graduate vs <9th grade: OR=0.47 [95% CI=0.34-0.64]; college/associate degree vs <9th grade: 0.71 [0.56-0.91]; high school vs <9th grade: 0.74 [0.59-0.92]; 9-11th grade vs <9th grade: 0.94 [0.77-1.14]), alcohol consumption (OR=1.18, 95% CI=1.03-1.35), body mass index (OR=1.02, 95% CI=1.01-1.03), as well as the co-morbidities of gout, including diabetes (OR=1.23, 95% CI=1.03-1.35), hypertension (OR=1.52, 95% CI=1.27-1.82), and coronary heart disease (OR=1.63, 95% CI=1.12-2.38) were associated with depression.

Conclusions: Gout is not independently associated with depression. However, physicians should still watch out for depression in patients with gout.

50

Efficacy of Janus kinase inhibitors or biologic disease-modifying antirheumatic drugs in psoriatic arthritis patients: a network meta-analysis

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Introduction: Novel therapeutic agents, which include Janus kinase inhibitors and biologic disease-modifying antirheumatic drugs, are more efficacious than placebo in psoriatic arthritis (PsA) patients. However, there were limited studies to compare efficacy between different novel therapeutic agents.

Methods: We searched for randomised controlled trials using ISI Web of Science, Scopus, Medline, Cochrane library, Clinicaltrials.gov, and Embase. Studies reporting the proportion of PsA patients that achieved ACR20 response were included. They were stratified into two groups: (1) biologic-naïve; and (2) intolerant to tumour necrosis factor inhibitor (TNFi). Results were analysed using R version 3.6.1 with "netmeta" version 1.1-0. Odds ratio (OR) and 95% confidence interval (CI) were estimated using random-effects model. P-rank scores were used to estimate the best therapeutic option for a specific group of PsA patients.

Results: In this network meta-analysis, 31 studies were included; 29 were in biologic-naïve and nine were in TNFi-intolerant populations. Golimumab was the best option in biologic-naïve patients (P-rank score 0.9324), superior to abatacept (OR [95% CI]=0.14 [0.06-0.36]), adalimumab (0.33 [0.18-0.62]), apremilast (0.21 [0.11-0.38]), certolizumab (0.40 [0.17-0.94]), guselkumab (0.39 [0.20-0.76]), ixekizumab (0.33 [0.16-0.66]), tofacitinib (0.30 [0.14-0.66]), and ustekinumab (0.28 [0.14-0.56]). However, golimumab was not significantly more efficacious than etanercept (0.94 [0.39-2.22]), filgotinib (0.62 [0.21-1.82]), and secukinumab (0.57 [0.30-1.06]). Apremilast was the best option in TNFi-intolerant patients (P-rank score: 0.8479), but there were no significant differences in efficacy between apremilast and other therapeutic agents.

Conclusions: All novel therapeutic agents show similar efficacy in PsA patients with TNFi failure. Golimumab is more efficacious than abatacept, adalimumab, apremilast, guselkumab, ixekizumab, tofacitinib, and ustekinumab in biologic-naïve patients.

51

Comparative effectiveness of Janus kinase inhibitors and biologic disease-modifying antirheumatic drugs in rheumatoid arthritis patients with an inadequate response to tumour necrosis factor alpha inhibitors: a network meta-analysis

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Introduction: Tumour necrosis factor alpha inhibitor (TNFi) was the first class of biologic disease-modifying antirheumatic drugs (bDMARDs) available for the treatment of rheumatoid arthritis (RA). Therefore, many studies have compared the efficacies of other bDMARDs and Janus kinase (JAK) inhibitors in patients with an inadequate response to TNFi. However, head-to-head comparisons among other bDMARDs and JAK inhibitors are lacking. Therefore, we conducted a network meta-analysis to compare the efficacies of bDMARDs and JAK inhibitors in patients with an inadequate response to TNFi.

Methods: We searched literature using Medline, clinicaltrials.gov, and Embase. For inclusion, randomised controlled trials must include RA patients with an inadequate response to TNFi and report the proportion of patients who achieved ACR20 response in week 12/24. Results were analysed using R version 3.5.1 with 'netmeta' 4.9-2. Odds ratio (OR) and 95% confidence interval (CI) were estimated using random-effects model.

Results: Twelve trials were included in this analysis. Tocilizumab was superior to other bDMARDs and JAK inhibitors in ACR20 response (OR=0.39, 95% CI=0.20-0.78 for abatacept; 0.26 (0.12-0.54) for baricitinib; 0.30 (0.14-0.64) for filgotinib 100 mg; 0.44 (0.21-0.93) for filgotinib 200 mg; 0.28 (0.13-0.61) for golimumab; 0.48 (0.24-0.99) for rituximab; 0.27 (0.13-0.53) for sarilumab; 0.22 (0.10-0.49) for tofacitinib; and 0.47 (0.22-0.98) for upadacitinib. Tocilizumab remained superior to baricitinib (0.28 [0.09-0.81]), filgotinib 100 mg (0.26 [0.09-0.71]), and sarilumab (0.32 [0.12-0.83]) in ACR50 response. Tocilizumab was superior to upadacitinib in ACR70 response (0.17 [0.03-0.90]).

Conclusion: All bDMARDs and JAK inhibitors have comparable efficacies in RA patients with an inadequate response to TNFi. However, tocilizumab is better than other bDMARDs and JAK inhibitors in terms of ACR20 response.

52

Cardiovascular safety of Janus kinase inhibitors in patients with rheumatoid arthritis: a meta-analysis

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Introduction: Janus kinase (JAK) inhibitors are a new class of medication approved for treating rheumatoid arthritis. As JAKs are involved in lipid metabolism, we investigated the cardiovascular safety of JAK inhibitors.

Methods: Literature search was conducted using Medline, ISI Web of Science, Scopus, Embase and clinicaltrials.gov. Randomised controlled trials reporting major cardiovascular events or hypertension were included. Results were analysed using RevMan 5.3.5. Odds ratio (OR), mean difference, and 95% confidence interval (CI) were estimated using random-effects model.

Results: A total of 23 studies were included in this meta-analysis. Janus kinase inhibitors were not associated with major cardiovascular events (OR=0.94, 95% CI=0.57-1.57). Tofacitinib was not associated with hypertension (OR=1.53, 95% CI=0.96-2.43). However, JAK inhibitors were associated with an increase in high-density lipoprotein (OR=10.11, 95% CI=8.03-12.19 mg/dL) and low-density lipoprotein cholesterol (OR=7.99, 95% CI=7.14-8.85 mg/dL), respectively.

Conclusion: Although JAK inhibitors were not associated with increased major cardiovascular events, they were associated increased low-density lipoprotein and high-density lipoprotein. Tofacitinib was not associated with hypertension. Physicians should monitor the lipids of patients taking JAK inhibitors.

53

Blood and urine inorganic and organic mercury levels in the United States: The United States National Health and Nutrition Examination Survey 1999-2016

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Introduction: Mercury is an environmental hazard. Therefore, we studied recent trends in the blood level of organic and inorganic mercury in the United States.

Methods: A total of 56 445 participants that had blood mercury and urine mercury measurements in NHANES (The United States National Health and Nutrition Examination Survey) 1999-2016 were included. The organic mercury level was obtained by subtracting the inorganic mercury level from the total mercury level. Results were analysed using SPSS complex sample module version 25. Pregnant women and children aged <20 years were analysed as subgroups.

Results: Blood inorganic mercury level and urine mercury level have been decreasing between 1999 and 2016 (P<0.001). Blood inorganic mercury level decreased from (geometric mean [95% confidence interval]) 0.31 (0.31-0.31) μg/L in 1999-2000 to 0.21 (0.21-0.22) μg/L in 2015-2016 (P<0.001). Urine mercury level decreased from 0.75 (0.71-0.80) μg/L in 1999-2000 to 0.16 (0.16-0.17) μg/L in 2015-2016 (P<0.001). In contrast, blood organic mercury level increased from 0.08 (0.07-0.10) μg/L to 0.17 (0.16-0.18) μg/L during 1999-2016. Blood organic mercury increased significantly (P<0.001) from 0.03 (0.02-0.03) μg/L to 0.07 (0.06-0.07) μg/L in children aged <20 years and from 0.14 (0.09-0.21) μg/L to 0.36 (0.16-0.83) μg/L in pregnant women.

Conclusion: A steady decline was observed in both blood inorganic mercury level and urine mercury level over the period 1999-2016. However, blood organic mercury levels have been increasing, which is of particular concern for pregnant women and children. Environmental pollution by inorganic mercury has been decreasing. The increase in organic mercury may be related to changes in diet, such as increased consumption of seafood.

54

Ankylosing Spondylitis Disease Activity Score is associated with both the extent and intensity of diffusion-weighted magnetic resonance imaging spinal inflammation in active axial spondylarthritis

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Objective: To investigate the relationship between Ankylosing Spondylitis Disease Activity Score (ASDAS) and intensity of spinal inflammation measured by apparent diffusion coefficient (ADC) in magnetic resonance imaging (MRI) in participants with active axial spondylarthritis (SpA).

Methods: Participants with axial SpA and back pain were recruited. Clinical, demographic, biochemical, and imaging data were collected. The ASDAS was calculated based on C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). Inflammatory lesions were identified in short tau inversion recovery images and the corresponding ADC maps to determine the maximum ADC (ADCmax), normalised maximum ADC, mean ADC (ADCmean), and normalised mean ADC by two independent readers. Spondyloarthritis Research Consortium of Canada (SPARCC) spine and sacroiliac (SI) joint MRI indexes were determined. Univariate and multivariate linear regression models were used to determine the associations between of ASDAS with ADC values, SPARCC spine, and SI MRI scores.

Results: Eighty two participants had identifiable ADC lesions. Multivariate analyses using ADCmax and SPARCC spine MRI as independent variables showed associations with ASDAS-CRP (B=0.27, P=0.02 for ADCmax; B=0.32, P=0.01 for SPARCC) and ASDAS-ESR (B=0.24, P=0.03 for ADCmax; B=0.36, P<0.01 for SPARCC). Using ADCmean and SPARCC spine MRI as independent variables also showed an association with ASDAS-ESR (B=0.22, P=0.05 for ADCmean; B=0.36, P<0.01 for SPARCC) and a tendency to associate with ASDAS-CRP (B=0.21, P=0.07 for ADCmean; B=0.34, P<0.01 for SPARCC).

Conclusion: The ASDAS is associated with both the extent and intensity of spinal inflammation in patients with detectable inflammatory lesions. Our results showed that ASDAS is an objective disease assessment tool.

Efficacy and safety of ticagrelor versus clopidogrel in patients with stable coronary artery diseases: a systematic review and meta-analysis

55

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Objective: Ticagrelor has been recommended for acute coronary syndrome with percutaneous coronary intervention, but its efficacy and safety comparative to clopidogrel in patients with stable coronary artery disease remains inconsistent.

Methods: This was a systematic review and meta-analysis. Embase and Medline was searched through November 2019. Studies were eligible when they compared ticagrelor and clopidogrel in patients with stable coronary artery disease regarding the outcomes of interest. Patient characteristics, treatment regimens, and outcome data were extracted. The primary outcome was major adverse cardiac events (MACE), and the secondary outcomes included major bleeding, cardiovascular death, and non-fatal myocardial infarction (MI). Random-effects model was used to combine the results across studies. Sensitivity analysis by including only randomised trials was conducted.

Results: Out of 231 citations, three studies (2 randomised trials and 1 cohort study) with 8827 patients (4353 on ticagrelor and 4474 on clopidogrel) were eligible. The median follow-up time ranged from 17 months to 24 months. Overall, compared with clopidogrel, ticagrelor did not show significant difference in reducing the risks of MACE (relative risk [RR]=0.87, 95% confidence interval [CI]=0.72-1.07), non-fatal MI (RR=0.67, 95% CI=0.24-1.88), cardiovascular death (RR=1.51, 95% CI=0.35-6.51), and major bleeding (RR=1.32, 95% CI=0.97-1.79). Similar results were also observed in sensitivity analysis.

Conclusion: This study did not provide evidence that ticagrelor was more effective or safer than clopidogrel in patients with stable coronary artery disease. However, the small number of included studies should be borne in mind when interpreting the results.

Ticagrelor reduced risk of infection compared with clopidogrel: a meta-analysis

56

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Introduction: Ticagrelor has antibacterial activity in vitro.[1] In the PLATO (Platelet Inhibition and Patient Outcomes) trial, ticagrelor reduced the incidence of pneumonia and improved lung function. We conducted a meta-analysis to investigate if ticagrelor reduces the risk of infections.

Methods: We searched Medline, Embase, Cochrane Library, and clinicaltrials.gov for randomised controlled trials comparing ticagrelor and clopidogrel that have reported the incidence of infection. The primary outcome was pneumonia. Secondary outcomes were upper respiratory tract infection (URTI), urinary tract infection (UTI), and sepsis. Odds ratios (ORs) and 95% confidence intervals (CIs) were combined with random-effects model in RevMan version 5.3.

Results: Out of 5231 citations, 13 trials with altogether 90679 patients were included. Ticagrelor was associated with a lower risk of pneumonia (OR=0.79, 95% CI=0.66-0.95) compared with clopidogrel, but no statistically significant difference was observed for URTI (OR=0.68, 95% CI=0.33-1.43), UTI (OR=1.06, 95% CI=0.75-1.49), or sepsis (OR=0.79, 95% CI=0.52-1.21).

Conclusion: Our meta-analysis provides further evidence that ticagrelor reduces the risk of pneumonia, when compared with clopidogrel. Our results did not show a reduction in URTI, UTI, or sepsis. Further research is needed to investigate the effect of ticagrelor on infections.

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57

Magnetic resonance imaging inflammation of facet and costovertebral joints is associated with restricted spinal mobility and worsened functional status

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Objective: To investigate the association of spinal inflammation on magnetic resonance imaging (MRI) in patients with various clinical, functional, and radiological outcomes in patients with axial spondylarthritis (SpA).

Methods: A total of 397 participants with axial SpA and back pain were recruited from 10 rheumatology centres. Clinical, biochemical, and radiological parameters were collected and participants underwent MRI of spine. Magnetic resonance imaging features including inflammatory lesions of facet joints and costovertebral joints, corner inflammatory lesions, and spondylitis were assessed. Bath Ankylosing Spondylitis Functional Index (BASFI), Bath Ankylosing Spondylitis Disease Activity Index, Bath Ankylosing Spondylitis Global Index, Bath Ankylosing Spondylitis Metrology Index (BASMI), and modified Stoke Ankylosing Spondylitis Spinal Score were measured. Multivariate linear regression models were used to determine the associations between MRI parameters and various clinical, functional, and radiological outcomes.

Results: Both BASMI and BASFI correlated well with inflammatory features in spinal MRI. Multivariate analysis showed that lumbar facet joint inflammation was independently associated with BASMI (regression coefficient B=0.12, P<0.001), lumbar spinal flexion (B=0.13, P=0.00), lateral spinal flexion (B=0.09, P=0.04), tragus-to-wall distance (B=0.16, P<0.001), and BASFI (B=0.14, P=0.01). Costovertebral joint inflammation was also associated with BASMI (B=0.08, P=0.05).

Conclusion: Inflammatory lesions of facet and costovertebral joints in MRI is associated with restriction in spinal mobility and functional impairment. These important yet commonly overlooked lesions should be reviewed in clinical practice in patients with SpA.

58

Identification of biomarkers by immunoprofiling for immunotherapy in advanced hepatocellular carcinoma

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Introduction: Checkpoint inhibitors targeting tumour-infiltrating T cells to trigger antitumour immune response have enamoured as the promising therapy. Investigation of different immune cell clusters may also yield crucial information for immunological changes. Guidance for precise immunotherapy as well as applicable response markers are currently limited. Henceforth, we are interested in establishing immunoprofiling for responders and non-responders to serve as predictors for immunotherapy in advanced hepatocellular carcinoma (HCC).

Methods: Peripheral blood mononuclear cells were collected from responders, non-responders, and treatment-naïve cohorts. Buffy coat samples were collected from red cross along with other research team.

Results: Responders showed a significantly lower T cells and regulatory T cells in peripheral blood, no significant differences were observed in NK cells. One-way analysis of variance in using PD-1, Tim-3 and OX40 as response predictor showed an effect on both T cells and NK cells. No significant differences were observed in CTLA-4 expression among cohorts on both T cells and NK cells. Pearson product-moment correlation coefficient was conducted between PD-1, Tim-3 and OX40. Tim-3 on T cells showed a significant positive correlation to the same expression count on NK cells (R=0.9747, P<0.001), while OX40 also showed a significant positive correlation to the expression count on NK cells (R=0.9892, P<0.001). No significance in either PD-1 expression or CTLA-4 expression were seen in T cells and NK cells.

Conclusion: Immunoprofiling revealed immunological changes in peripheral blood after exposure to immunotherapy PD-1, Tim-3 and OX40 may serve as biomarkers for response to immunotherapy in advanced HCC biomarkers on NK cells showed a strong correlation with the same markers on T cells.

59

Neutrophil serine proteinases exacerbate atherosclerosis by increasing intestinal permeability and endotoxinaemia in mice

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Introduction: Atherosclerosis is a chronic inflammatory disease responsible for fatal cardiovascular events. Neutrophil serine proteinases (NSPs), including neutrophil elastase (NE) and proteinse-3 (PR3), have been shown to be important players in metabolic inflammation. However, the pathophysiological roles of these NSPs in atherosclerosis and mechanisms underlying their actions remain to be defined.

Methods: We generated NE/PR3 double knockout mice and crossed the double knockout with ApoE-deficient mice (a well-established model with spontaneous development of atherosclerosis) to generate NE/PR3/ApoE triple deficient (TKO). The impact of genetic ablation or pharmacological inhibition of NE and PR3 on atherosclerosis was evaluated at the histological and molecular levels in mice.

Results: Western diet–induced atherosclerotic plaque formation, as determined by red oil O staining of aortic arch, was markedly reduced in TKO mice compared with $ApoE^{-/-}$ mice. The TKO mice also exhibited a significant reduction of smooth muscle proliferation and a marked alleviation of macrophage infiltration and vascular inflammatory response. Furthermore, western diet–fed $ApoE^{-/-}$ mice treated with recombinant human elafin, which is the endogenous inhibitor of NE and PR3, also exhibited significant reduction in atherosclerotic plaque formation and vascular inflammation. Mechanistically, genetic ablation or pharmacological inhibition of NE/PR3 with elafin obviously decreased western diet–induced endotoxinaemia, mediated by elevated expression of two tight junction proteins (occludin and Zo1), leading to reduced gut permeability.

Conclusion: Neutrophil serine proteinases contribute to vascular inflammation and atherosclerotic formation by increasing the intestinal permeability and endotoxinaemia. Pharmacological inhibitors of NSPs are promising drug candidates for therapeutic intervention of cardiovascular diseases.

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60

Incidence of hospitalised hypokalaemia in patients with indapamide prescription: a population-based study

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Introduction: Indapamide is a commonly prescribed thiazide diuretic for treating hypertension. In the literature, 40% of patients receiving diuretics experience hypokalaemia. We investigated the incidence of hypokalaemia as a cause of admission to public hospitals in Hong Kong.

Methods: Adult patients aged ≥20 years who had a prescription of indapamide for >180 days from 1 January 2006 to 31 December 2017 were identified using the Clinical Data Analysis and Reporting System. Patients who had hypokalaemia before indapamide prescription were excluded. They were followed until hospitalisation for hypokalaemia, death or end of prescription. Results were analysed using R version 3.6.0. The incidence and 95% confidence interval (CI) were estimated. These were further adjusted for age, or age and sex.

Results: Altogether, 97 878 patients were included with 226 098 person-years of observation. The incidence of hospitalised hypokalaemia was 24.3 (95% CI=22.7-26.1) per 10 000 person-years. The age-adjusted as well as age-and-sex-adjusted incidences were 25.1 (95% CI=23.3-27.0) and 24.9 (95% CI=23.2-26.8) per 10 000 person-years, respectively. Female had higher incidence than male (incidence rate ratio=1.40, 95% CI=1.29-1.52). No patients died of hypokalaemia during admission.

Conclusions: Indapamide-induced hypokalaemia requiring hospitalisation is uncommon, which may be due to awareness of this common adverse effect. By monitoring blood electrolytes, emergency admissions and fatalities arising from drug-induced hypokalaemia can be avoided.

61

Gut microbiome-derived lipopolysaccharide contributes to pathogenesis of murine lupus nephritis

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Introduction: Pathogenesis of lupus nephritis is complex, involving both genetic and environmental factors. Emerging evidence suggests that translocation of microbial products such as lipopolysaccharide (LPS) from the gut may enter the circulation and induce inflammatory responses at distant sites. This study investigated the role of LPS in the pathogenesis of murine lupus nephritis.

Methods: Eight-week-old NZB/W F1 mice were randomised to receive: (a) drinking water or (b) ampicillin and neomycin for 20 weeks. Intestinal mucosal permeability was investigated with LPS-FITC administration, and ZO-1 and occludin expression. Quantitative changes to the gut microbiota were assessed by 16S rRNA sequencing.

Results: Serum LPS level was significantly higher in NZB/W F1 mice with active nephritis compared with age-matched BALB/c mice ($P<0.05$). Histopathological features of active nephritis in NZB/W F1 mice were accompanied by increased LPS binding protein, CD14 and TLR-4 expression in proximal renal tubular epithelial cells. Mice with active nephritis also showed increased gut permeability to orally fed LPS-FITC, with decreased ZO-1 and occludin expression in the colonic epithelium. 16S rRNA analysis showed a progressive decrease in Gram-positive bacteria phyla Actinobacteria and Firmicutes and an increase in Gram-negative bacteria phyla Bacteroides and Proteobacteria as nephritis progressed. Antibiotic treatment significantly decreased serum LPS level, and attenuated abnormalities observed in both the colon and kidney ($P<0.05$, for both), and also proteinuria.

Conclusion: Our results demonstrate that progressive murine lupus nephritis is associated with increased gut-derived circulating LPS, which may contribute to renal tubulointerstitial inflammation.

62

Artificial intelligence–assisted real-time detection reduces missed lesions during colonoscopy: a retrospective and prospective study

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Introduction: A recent meta-analysis showed that up to 26% of adenomas are missed during colonoscopy. We investigated whether the use of artificial intelligence (AI) could reduce missed colonic lesions during colonoscopy.

Methods: We developed a deep learning AI model for the real-time detection of colonic neoplasm with high accuracy. The AI model was first tested in the videos of tandem colonoscopy of the proximal colon (from caecum to splenic flexure) collected in our ongoing study. The gold standard was all lesions detected in the proximal colon by endoscopists in the tandem examinations with histological confirmation. We then validated the real-time AI model in prospective colonoscopy examinations in patients undergoing colonoscopy for various indications. Examinations were performed by both experienced endoscopists and trainees. The AI findings were displayed in a separate monitor and observed by an independent investigator, while the endoscopist was blinded to the real-time AI findings. The colon was divided into three segments (right-side, transverse and left-side colon) and segmental unblinding of the AI result would be provided after complete examination of each segment. If a lesion was detected by AI but not by the endoscopist, that segment would be re-examined. If no additional lesion was detected by AI, the endoscopist would proceed to examination of next segment. All polyps detected were removed for histological examination.

Results: In the retrospective review of 65 tandem colonoscopy videos of the proximal colon, there were 32 polyps (24 adenomas) missed in the first examination and were detected on second examination only. The AI detected 78.1% of these missed polyps (79.1% of missed adenoma) in the video of the first-pass examination and 100% of lesions were detected by AI on tandem examination. In the prospective study of 40 colonoscopy examinations (mean patient age 67 years; 50% male), the overall adenoma detection rate was 62.5% and a total of 113 polyps (59 adenomas) were detected with AI assistance. There were 33 (29.2% of total) missed polyps or 13 (22%) missed adenomas detected by AI. The AI detected at least one missed adenoma in 25% of the cases. Six (15.3%) suspected missed lesions detected by the AI were not identified on re-examination. Multivariate analysis showed that missed polyp(s) were more likely to located at proximal colon (adjusted odds ratio=1.26, 95% confidence interval=1.06-1.49, $P<0.01$), but not associated with the size and final histology of the lesions. Experienced endoscopists had fewer missed polyp(s) than trainees (adjusted odds ratio=0.79, 95% confidence interval=0.63-0.98).

Conclusion: Artificial intelligence–assisted real-time detection correctly identified 79% of the missed proximal adenoma which were detected in tandem examinations. It also missed fewer adenomas per examination, particularly in the right-side colon.

63

Cholinergic receptor nicotinic alpha 7 subunit mediates cigarette smoke–induced PD-L1 expression

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Introduction: Genetic variations including single nucleotide polymorphisms and copy number variations of the nicotinic acetylcholine receptors (nAChRs) are associated with the risk of lung cancer according to various genome-wide association studies. However, the underlying mechanisms remain unknown. Besides, it is still unclear why there is usually a better observed response to immune-checkpoint inhibitor in smokers compared with that in non-smokers with lung cancer. Here, we hypothesised that cigarette smoke (CS) and the carcinogen nicotine–derived nitrosamine ketone (NNK) can induce PD-L1 expression in lung epithelial cells, mediated by cholinergic receptor nicotinic alpha 7 subunit (*CHRNA7*) expression.

Methods: Immortalised normal bronchial epithelial cells derived from smokers or non-smokers were treated with CS or NNK. Both *CHRNA7* and PD-L1 mRNA expression levels were measured by quantitative polymerase chain reaction. Surface and endogenous PD-L1 expressions were determined by immunofluorescence staining and western blot analysis, respectively. The *CHRNA7*-specific small interfering RNA was used to knockdown gene expression.

Results: Both CS and NNK can induce the gene and protein expressions of PD-L1 in normal bronchial epithelial cells derived from both smokers and non-smokers. Knockdown of *CHRNA7* can abolish the induction of PD-L1 expression by NNK.

Conclusion: This preliminary data demonstrated the interactions of *CHRNA7* on PD-L1 expressions. Further investigation is warranted to elucidate the molecular actions of *CHRNA7* and even other nAChRs on the expressions of PD-L1.

64

Role of oncofetal protein *HMGA2* in the development of hepatitis B virus–associated hepatocellular carcinoma

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Introduction: Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related deaths worldwide. Hepatitis B virus (HBV) infection is one of the major risk factors for HCC, particularly in China and Hong Kong. Improvements in timely diagnosis and effective treatment remains urgent. However, the underlying molecular mechanisms in hepatocarcinogenesis are still largely unknown. Elucidating of these mechanisms may allow identification of new candidates for more effective targeted therapies. Several studies have implicated that activation of oncofetal protein *HMGA2* was involved in initiation and progression of cancers.

Methods: We analysed the expression level of *HMGA2* in tissue specimens obtained from patients with HBV-associated HCC by real-time polymerase chain reaction and western blot. The serum protein levels of *HMGA2* in patients with HBV-associated HCC and healthy controls were analysed by enzyme-linked immunosorbent assay. Loss of function studies were performed in vitro to evaluate the role of *HMGA2* in hepatocarcinogenesis and its potential as a molecular target for therapy.

Results: The mRNA and protein expression of *HMGA2* were significantly upregulated on 20 pairs of HCC tumour samples compared with their non-tumour counterparts (both $P<0.001$). Serum *HMGA2* protein levels in patients with HBV-associated HCC were significantly higher than healthy controls ($P<0.0001$). Functional analysis using RNAi-mediated knockdown of *HMGA2* revealed a suppression of cancer cell proliferation, suggesting that *HMGA2* plays a pivotal role in progression of HCC.

Conclusions: These findings suggest that *HMGA2* plays a pivotal role in HCC proliferation through its overexpression, and highlights its importance as a prognostic and potential therapeutic target in HCC.

65

Cardiac cell patches with decellularised placenta and human-induced pluripotent stem cell–derived cardiomyocytes for myocardial repair

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Introduction: Cardiac cell patches (CCP) has emerged as a new strategy for cardiac repair. Here, we investigated whether the extracellular matrix (ECM) from decellularised placenta can be used as natural scaffold material to create CCP for myocardial repair.

Methods and Results: Cardiac cell patches were created by seeding human-induced pluripotent stem cell–derived cardiomyocytes (hiPSC-CMs) onto decellularised rat placenta. The functional and electrophysiological properties of the CCP were characterised by in vitro analysis and optical mapping. The therapeutic efficacy of transplanting the CCP on infarcted myocardium was evaluated in a rat model of myocardial infarction (MI) and was compared with MI alone group and MI+ECM group. Cytokine profiling demonstrated that the ECM of decellularised placenta contains multiple growth and angiogenic factors. In vitro optical mapping showed that CCP exhibit synchronised electrical propagation. Compared to MI and MI+ECM groups, CCP significantly improved left ventricular function, left ventricular ejection fraction, and fractional shortening at 4 weeks follow-up. In vivo histological examination showed successful engraftment of hiPSC-CMs in the CCP at the infarct zone that decreased the infarct size, increased neovascularisation, as compared with MI and MI+ECM groups.

Conclusions: Our results demonstrated that ECM from decellularised placenta contains multiple growth and angiogenic factors, which enhance the survival of hiPSC-CMs in CCP after transplantation, thus providing a novel therapy for myocardial repair after MI.

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66

Mesenchymal stem cell preconditioning facilitates therapeutic efficacy of transplanted cells in myocardial infarcted heart

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Introduction: Human-induced pluripotent stem cell (hiPSC) is an attractive source for allogenic stem cell for cardiac repair but is limited by the immune rejection of the transplanted cells. Prior studies suggest that allogenic mesenchymal stem cell (MSC) preconditioning can migrate the immune rejection in solid organ transplantation. We sought to investigate whether immunomodulation with intravenous administration of hiPSC-derived MSCs ameliorates the immune rejection of intramyocardial transplantation of hiPSC-MSCs or hiPSC-derived cardiomyocytes (hiPSC-CMs) in mice model of myocardial infarction (MI).

Methods: We compared the therapeutic efficacy and cell survival of intramyocardial transplantation of hiPSC-MSCs (10×10^5) or hiPSC-CMs (10×10^5) in mice model of MI with or without intravenous administration of hiPSC-MSC preconditioning.

Results: Intravenous hiPSC-MSC preconditioning (5×10^5) increased the systemic and myocardial expression of T regulatory cells (Tregs), increased the survival of transplanted hiPSC-MSCs or hiPSC-CMs, decreased the infarct size, and improved left ventricular function at day 28 after MI compared with saline preconditioning. The hiPSC-MSC preconditioning with hiPSC-MSCs or hiPSC-CMs transplantation were also associated with increases in cardiomyocyte proliferation and vascular density, and decreases in endogenous cell apoptosis.

Conclusion: Intravenous hiPSC-MSC preconditioning improved the therapeutic efficacy of hiPSC-MSCs or hiPSC-CMs transplantation for the treatment of MI, which is a potential strategy to migrate the immune rejection of the transplanted allogenic stem cell for cardiac repair.

67 Point-of-care ultrasound augments physical examination learning in undergraduate medical students

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Introduction: Point-of-care ultrasound (POCUS) has been widely incorporated in clinical practice. Little is known about the impacts of provision of handheld POCUS devices on physical examination (PE) skills in medical students.

Methods: We describe an educational initiative consisting of POCUS workshop followed by allocation of POCUS devices to medical students for use in the subsequent 8 weeks. They were encouraged to use the devices to scan patients and correlate with PE findings. Mobile instant messaging group discussion platform was utilised to provide instantaneous feedback by instructors. At the end of the programme, self-reported electronic survey was performed to assess students' perceptions and feedback. Physical examination skills were assessed by means of clinical examination and their performance were compared with students from the previous academic year who served as historical controls.

Results: In 2019, 210 final-year medical students from The University of Hong Kong participated in the programme. After using the POCUS devices for 8 weeks, 46.3% submitted response to the end of programme electronic survey: 74.6% enjoyed using the POCUS devices, 50.0% found POCUS useful in validating PE findings, and 47.7% agreed that POCUS increased their confidence with PE. Also, 93.9% agreed that the programme should be incorporated into medical curriculum and 81.9% would like to keep the device for a longer period of time, ranging from 16 weeks (45.6%) to over 49 weeks (35.3%). Medical students undergoing the POCUS programme had a higher mean score for abdominal examination compared with the medical students from the previous academic year without the POCUS programme (3.65±0.52 vs 3.21±0.80, P=0.014), while there was no statistically significant difference in their mean score for cardiovascular examination (3.62±0.64 vs 3.36±0.93, P=0.203).

Conclusion: Our results indicate that POCUS programme including provision of handheld POCUS device for their personal uses improve students' attitude, confidence, and ability to perform PE.

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68 Bone density and quality in prediabetes among postmenopausal Chinese women: the role of fibroblast growth factor 21

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Introduction: Prediabetes is associated with a worse trabecular bone score (TBS). Fibroblast growth factor 21 (FGF21) has been reported to be implicated in bone metabolism, and its levels are raised in prediabetes and other insulin-resistant states. We compared the bone mineral density (BMD) and TBS between prediabetes and normoglycaemia, and studied the correlation of FGF21 with BMD and TBS.

Methods: Chinese postmenopausal women without type 2 diabetes were recruited from the Hong Kong Cardiovascular Risk Factor Prevalence Study between November 2016 and October 2018, and divided into prediabetes (fasting glucose ≥5.6 mmol/L or haemoglobin A1c ≥5.7%) and normoglycaemia. Both BMD and TBS were measured by dual-energy X-ray absorptiometry. Serum FGF21 levels were measured with an in-house enzyme-linked immunosorbent assay kit.

Results: A total of 258 participants were included (130 prediabetes and 128 normoglycaemia). Bone mineral density was comparable between prediabetes and normoglycaemia, while TBS was lower in prediabetes (1.27±0.07 vs 1.30±0.07, P=0.007), which remained significant after adjustment for age and body mass index. Fibroblast growth factor 21 did not correlate with BMD but inversely correlated with TBS. On stepwise multiple linear regression models, FGF21 showed an independent inverse correlation with TBS (standardised beta -0.13, P=0.031), and remained significant with the inclusion in the model of homeostasis model assessment of insulin resistance.

Conclusion: Among Chinese postmenopausal women, the bone quality was worse in prediabetes despite comparable bone density. Fibroblast growth factor 21 showed a significant independent correlation with TBS, suggesting its potential impact on the deterioration of the bone microarchitecture in prediabetes.

69

Statin use reduces incident hip fractures among older Chinese people with type 2 diabetes, independent of mean haemoglobin A1c and duration of diabetes

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Introduction: Statins are associated with reduced risks of hip fractures in the general population. People with type 2 diabetes have increased fracture risk related to its unique bone pathophysiology and level of glycaemia. We therefore studied the effect of statins on incident hip fractures in people with type 2 diabetes.

Methods: Chinese individuals with type 2 diabetes aged ≥60 years were identified from electronic health records in Hong Kong between 2008 and 2012 and observed for incident hip fractures. Participants were categorised into 'never users', 'long-term users', and 'new users' of statin. Cumulative defined daily doses (cDDD) of statins were calculated. Multivariable Cox regression analysis was used to calculate the adjusted hazard ratio (aHR) of incident hip fractures with the use of statins.

Results: Among the 60 825 participants, both 'long-term users' and 'new users' of statin had less incident hip fractures compared with 'never users' (aHR=0.57 and 0.60 respectively, both P<0.001). Comparison between 'never users' and 'new users' with propensity score matching for baseline characteristics revealed a comparable reduction in incident hip fractures among 'new users' (aHR=0.50, P<0.001). A dose-response relationship was observed, with more risk reduction in the quartiles with higher cDDD of statins (aHR=0.49 for Q2, aHR=0.37 for Q3, aHR=0.23 for Q4; all P<0.001 compared with 'never users'). These protective effects were independent of haemoglobin A1c and the duration of diabetes.

Conclusion: Statin use reduces incident hip fractures among older Chinese people with type 2 diabetes, in a dose-response manner.

70

Liver-specific adeno-associated virus 2/8 trimeric adiponectin gene transfer reduces β-amyloidosis and improves learning and memory in a mouse model of Alzheimer's disease

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Objective: Type 2 diabetes is a major risk factor of Alzheimer's disease (AD). Insulin resistance and inflammation are involved in the pathogenesis of both conditions. Adiponectin, an adipocyte-derived circulating adipokine, possesses anti-inflammatory and insulin-sensitising functions. We have reported that chronic adiponectin deficiency in aged mice developed AD-like pathologies and cognitive impairments. Here, we investigate whether overexpressing trimeric adiponectin by adeno-associated virus (AAV) delivery can reduce AD pathologies by reducing amyloid beta (Aβ) deposition and suppressing neuroinflammation.

Methods: To test this hypothesis, transgenic mice (5xFAD) that carried familial APP and PS mutations were given with liver-specific AAV carrying trimeric adiponectin expression plasmid (AAV-APNTri) by intravenous injection. Cognitive functions of these mice were evaluated by Morris water maze 4 months after injection. Enzyme-linked immunosorbent assay (ELISA) was performed to study the levels of Aβ peptides and cytokines in the mice brains. Western blotting and immunohistochemistry analysis were performed on brain and tissue cells. Microgliosis and cytokines levels were studied by ELISA and immunofluorescent staining.

Results: We demonstrated that liver-specific AAV overexpressed trimeric adiponectin found in the circulation. Spatial learning and memory function were significantly improved in AAV-APNTri-treated 5xFAD mice. Molecular studies indicated overexpressing trimeric adiponectin reduced Aβ40 and Aβ42 levels, Aβ-plaque loading, microgliosis, and astrogliosis. Neuroinflammatory response was also reduced as shown by reduction of proinflammatory cytokine levels.

Conclusion: Taken together, these results suggest overexpressing adiponectin is protective against Aβ deposition and neuroinflammation. Trimeric adiponectin exerts anti-inflammatory effects to the AD brains by suppressing microglial activation and proinflammatory cytokine secretion. We suggested that overexpressing trimeric adiponectin by liver-specific AAV delivery can be a potential treatment for AD.

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71

Adiponectin paradox in the association between circulating adiponectin levels and incident cancer in patients with type 2 diabetes

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Introduction: Although preclinical studies have demonstrated the beneficial cardiometabolic effects of adiponectin, in patients with diabetes, epidemiological studies have reported paradoxical elevation of circulating adiponectin levels to be associated with incident cardiovascular events, renal outcomes, as well as mortality. Since diabetes is also associated with an increased risk of cancer, we investigated prospectively the association between circulating adiponectin levels and incident cancer, using a cohort of exclusively individuals with type 2 diabetes.

Methods: Baseline serum adiponectin levels were measured in 5658 participants, recruited from the Hong Kong West Diabetes Registry. The associations of circulating adiponectin levels with incident cancer and cancer-related deaths were evaluated using multivariable Cox regression analysis.

Results: Over a median-follow up of around 6.5 years, 7.53% and 3% of participants had incident cancer and cancer-related deaths, respectively. Serum adiponectin levels were significantly higher in those who had incident cancer (9.8 ug/mL vs 9.1 ug/mL, P<0.001) and cancer-related deaths (11.5 ug/mL vs 9.3 ug/mL, P<0.001) compared with those without. Moreover, serum adiponectin level was independently associated with both incident cancer (hazard ratio=1.17, 95% confidence interval=1.03-1.32; P=0.013) and cancer-related deaths (hazard ratio=1.22, 95% confidence interval=1.03-1.45; P=0.025), after adjustments for other clinical variables including age, body mass index, smoking, and renal function at baseline.

Conclusions: In addition to cardiovascular and kidney diseases, adiponectin paradox can also be observed in the association with incident cancer in diabetes. An elevated serum adiponectin level, instead of being beneficial, could implicate an increased risk of incident cancer and cancer-related deaths in type 2 diabetes.

72

Artificial haemoglobin YQ23 increases circulating mesenchymal stem cells and promotes angiogenesis in a mouse model of hind limb ischaemia

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Introduction: Although endovascular revascularisation effectively improves tissue perfusion and oxygenation, only a minority of patients with critical limb ischaemia are eligible for the therapy. Haemoglobin-based oxygen carrier, a potential red cell substitute for emergency transfusion, is expected to deliver oxygen to the ischaemic tissue through critical stenotic arteries given its small size.

Methods and Results: We investigated the functional consequences of an engineered bovine derived haemoglobin, YQ23 that is a stabilised non-polymeric cross-linked tetrameric haemoglobin, in a murine model of hind limb ischaemia. After successful femoral artery ligation, single intravenous YQ23 injection instantaneously normalised oxy-haemoglobin concentration over the ischaemic limbs in a dose-dependent manner, despite markedly reduced tissue perfusion. The improved tissue oxygenation persisted up to 21 days, which was substantially longer than the plasma half-time of YQ23 (8.2-10.8 hours). In contrast, there was progressive improvement in tissue blood perfusion over the ischaemic limbs up to 61% of the healthy limbs in mice receiving YQ23 treatment from 7 days to 21 days, which resulted from enhanced arteriogenesis with increasing number of arterioles in the ischaemic limbs in a dose-dependent manner. While the number of circulating mesenchymal stem cells increased immediately after femoral artery ligation and returned back to baseline level from 7 days onwards in control mice, in mice receiving YQ23 treatment, the numbers of circulating mesenchymal stem cells: CD45-CD29+ cells, CD45-CD105+ cells, and CD45-CD106+ cells remained persistently elevated till 21 days in a dose-dependent manner.

Conclusion: Single intravenous YQ23 injection results in instantaneous and persistent normalisation of tissue oxygenation and subsequent improvement in tissue perfusion through arteriogenesis, thereby alleviating critical limb ischaemia in mice.

AUTHOR INDEX

Page No.

A
R Anderson 15

B
J Bhawalkar 15
L Bowes 15
M Bunting 41

C
A Chan 40
AKC Chan 12
C Chan 37
EW Chan 6
FHW Chan 7
H Chan 15
HHL Chan 17
HWF Chan 6
JCY Chan 17
KH Chan 7, 41
KY Chan 40
LYY Chan 9
MWM Chan 17
SCW Chan 33
TH Chan 28
TM Chan 7, 37
VSF Chan 15, 16, 17
EES Chang 11, 14
D Chau 20
HT Chau 36
VWK Chau 40
JY Chen 42
K Chen 29
L Chen 6
R Chen 12, 18
CW Cheng 20
S Cheng 26
Y Cheng 27
YY Cheng 40
LY Cheong 26, 28
BCY Cheung 17
BMY Cheung 13, 14, 28, 29, 30, 31, 32, 33, 34, 36
CYY Cheung 23, 42
KS Cheung 6, 37
M Cheung 40
S Cheung 40
TT Cheung 13, 14, 31, 32, 33, 36
TT Cheung 26
JWY Chiu 21, 22
PCK Chiu 6
YK Choi 11, 14
JX Chow 15, 16
SC Chow 35
WS Chow 23, 40, 41, 42
PH Chu 12
ETF Chui 33
HY Chung 27, 33, 35

D
J Deng 38
RX Dui 12

E
I Erenburg 15

F
T Fei 34
Y Fei 29, 30, 31, 32, 34
Q Feng 34

Page No.

Y Feng 19
Y Feng 42
CHY Fong 23, 40, 41, 42
PY Fong 27
CC Fu 37
J Fung 8, 25, 26

G
XF Gao 27
LL Geng 22

H
JJ Hai 40, 42
HC Han 42
S He 24
JCM Ho 8, 11, 19
PWL Ho 11, 14
SL Ho 11, 14
Hong Kong Society of Rheumatology 27
E Honore 26
D Huang 40
FY Huang 38
Z Huang 13, 18, 24
C Hui 37
RWH Hui 8, 25
XY Hui 26, 27, 29

I
MSM Ip 12, 18

J
M Jian 41
Y Jiang 39
LG Jin 22, 36

K
CF Ko 15, 16, 17
KL Ko 37
JSC Kwan 41
YK Kwan 6
GW Kwok 21, 22
HH Kwok 38
J Kwok 17
MHB Kwong 35
YL Kwong 35

L
CL Lai 26, 38
KN Lai 9
KY Lai 38
WH Lai 39, 42
CK Lam 30
DCL Lam 38
IKY Lam 16
JKY Lam 21, 40
KSL Lam 23, 25, 36, 40, 41, 42
SK Lam 8, 11, 19
CL Lau 27
CP Lau 40
CS Lau 10, 15, 16, 17, 31, 32, 33
SH Lau 42
YCC Lau 33
YM Lau 42
ACH Lee 21, 40
CH Lee 21, 23, 25, 40, 41, 42
HTA Lee 35
KH Lee 33
NCM Lee 38

Page No.

SC Lee 6, 7
YK Lee 42
CT Leung 11, 14
JCK Leung 9
KS Leung 6
RCY Leung 21, 22
WK Leung 6, 37
B Li 9
BCW Li 21, 22
H Li 9
HL Li 34
PH Li 10
VKS Li 40
YM Liang 12
SY Liao 39
HF Liu 11, 14
PT Liu 27
WH Liu 9
Z Liu 28
RCL Lo 26
SWY Lok 9
DTW Lui 40, 41, 42
TKL Lui 37

M
J Ma 15, 16, 17
OKF Ma 41
Y Ma 26, 28
JCW Mak 12, 18
LY Mak 8, 25, 26
K Man 26
D Manstein 15
CKF Mok 6
MYM Mok *7*

N
KKK Ng 23
KM Ng 42
RCL Ng 41
HY Nip 27

O
B Or 13, 14, 32

P
Y Pan 22, 36
SYY Pang 11, 14

R
K Rutkowski 10

S
WK Seto 6, 8, 25, 26, 38
PC Sham 23
YF Shea 6, 7
GKB Shing 21
ACK Shum 6
LQC Siew 10
CW Siu 40, 41, 42
ASC So 33
S Sun 39

T
ACP Tai 37
KCB Tan 40
N Tan 42

Page No.

SCW Tang 9, 17
V Tang 21
V Tang 36
I Thomas 10
J Ting 15
V Ting 35
E To 26
HHL Tsang 33
E Tse 20
HF Tse 39, 40
MF Tsoi 13, 14, 28, 29, 30, 31, 32, 33, 34, 36
VWM Tsui 37
FC Tzang 42

U
KL Ue 10
KC Un 40

W
B Wang 26, 28
Q Wang 36
S Wang 16, 17
W Wang 29
TJ Watts 10
CC Wong 35
CK Wong 40, 42
CY Wong 10, 33
DKH Wong 25, 26, 38
ICK Wong 6
RLC Wong 23
SM Wong 17
SY Wong 37
SYY Wong 16
WH Woo 22
YC Woo 21, 23, 40, 41, 42

X
A Xu 13, 18, 22, 23, 24, 25, 26, 27, 28, 29, 36, 42
L Xu 13, 18, 24
R Xue 9

Y
BP Yan 42
S Yan 8, 11, 19
THB Yan 33
X Yan 13, 18, 24
R Yang 13, 18, 22, 24
TCC Yau 21, 22, 35
CK Yeung 17
KMY Yeung 40
P Yeung 40
LW Yick *7*
L Ying 22
PS Yip 40
WH Yiu 9
J Yu 37
LM Yue 20
MAA Yuen 42
MF Yuen 8, 25, 26, 38
S Yung 37

Z
Y Zhang 12
Z Zhen 39
M Zhou 40
V Zuo 15