

ORAL PRESENTATIONS • DISCOVERY SCIENCE**[OPS 1]****OVERCOMING CD8+ T CELL-MEDIATED CYTOTOXICITY RESISTANCE IN PANCREATIC CANCER CELLS THROUGH HISTONE DEACETYLASE INHIBITION****Wynne Sim¹, Wei Meng Lim², Ling Wei Hii², Shew Fung Wong^{1,3}, Chee Onn Leong³**¹*School of Medicine, International Medical University, Bukit Jalil, Kuala Lumpur, Malaysia*²*School of Pharmacy, International Medical University, Bukit Jalil, Kuala Lumpur, Malaysia*³*Institute for Research, Development and Innovation, International Medical University, Bukit Jalil, Kuala Lumpur, Malaysia*

Pancreatic Ductal Adenocarcinoma (PDAC) is a lethal disease accounting for over 340,000 global deaths from more than 350,000 global cases yearly. PDAC is notoriously refractory to conventional therapies, including surgery, chemotherapy and radiotherapy. Immunotherapies, including the utilisation of Cytotoxic T Lymphocytes (CTLs) to eliminate PDAC represent a promising strategy, but efforts to maximise immunotherapy have been limited by CTL-mediated cytotoxicity resistance (CTLR). Transcriptomic profiling and connectivity map demonstrated that Histone Deacetylase Inhibitors (HDACi) could potentially overcome CTLR. The main objective of this study was to determine whether HDACi can overcome CTLR, thus allowing the harnessing of immunotherapy for PDAC treatment.

BXPC3 and SW1990 parental and CTL-resistant cell lines were treated with HDACi Belinostat, Dacinostat, Droxinostat, Panobinostat, Givinostat, Vorinostat and Trichostatin-a together with CTLs at different ratios (CTLs: PDAC ratios of 1:1, 2:1, 4:1, 8:1, 16:1). To compare with conventional cytotoxic drugs used in PDAC treatment, gemcitabine and 5-fluorouracil were included in the assay. Cell viability was measured using CellTiter-Glo Luminescent Assay.

The results showed that HDACi can successfully reverse CTLR in BXPC3 and SW1990 CTL-resistant cell lines. Further, the use of HDACi resulted in increase in sensitivity of PDAC cells towards CTLs across all cell lines. In CTL-resistant cell lines, Givinostat caused the greatest improvement in CTL sensitivity with approximately 80% reduction in cell viability. Between BXPC3 and SW1990 cell lines, the BXPC3 cell line was also demonstrated to be more sensitive. The results also demonstrated that Gemcitabine and 5-Fluorouracil neither increased PDAC sensitivity to CTLs nor caused reversal of CTLR.

The results of this study support the utilisation of HDACi in combination with conventional immunotherapeutic drugs to overcome potential CTLR. Even when CTLR is absent, the inclusion of HDACi is favoured as HDACi can sensitise PDAC towards CTLs and enhance CTL-mediated killing.

ORAL PRESENTATIONS • DISCOVERY SCIENCE**[OPS2]****ANTIBIOFILM ACTIVITIES OF STINGLESS BEE HONEY AGAINST HUMAN PATHOGENIC BACTERIA*****Renee Chin, Wen Jie Ng****Department of Allied Health Sciences, Universiti Tunku Abdul Rahman, Jalan Universiti, 31900 Kampar, Perak, Malaysia*

Biofilms play a significant role in the pathogenesis and transmission of human diseases, particularly those associated with inert surfaces, such as implants and indwelling medical devices. These microbial aggregates are more difficult to eradicate than free living cells due to better protection against macrophages and antibiotics. *Bacillus cereus*, *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are commonly associated with the formation of biofilms which could potentially lead to nosocomial bacteraemia. Stingless bee honey is gaining increased attention and popularity in Malaysia, due to its acclaimed higher bioactivities, as evidenced in several studies. Hence, this study aimed to investigate the antibiofilm effects of stingless bee honey.

Biofilms of six bacterial isolates including *B. cereus*, *E. coli*, *E. faecalis*, *K. pneumoniae*, *P. aeruginosa* and *S. aureus* were cultivated in microtitre plates. The inhibitory effect on biofilm formation and eradication of established biofilm were evaluated after the treatment of stingless bee honeys produced by *Geniotrigona thoracica* and *Heterotrigona itama* with different moisture contents. Although it was found that each bacterial species exhibited various sensitivities towards different types of stingless bee honey, stingless bee honeys with lower moisture content were shown to exhibit higher percentage of biofilm inhibition and biofilm eradication than unprocessed raw honey. Scanning electron microscope (SEM) also revealed morphological changes of bacteria due to the action of stingless bee honey. Overall, all tested honey samples were found to be effective against biofilm, suggesting stingless bee honey as a prospective therapeutic agent for chronic infections.

ORAL PRESENTATIONS • DISCOVERY SCIENCE**[OPS3]****THE PURSUIT OF NEURONAL HYPEREXCITABILITY IN HIGH-DENSITY SURFACE ELECTROMYOGRAPHY RECORDINGS OF AMYOTROPHIC LATERAL SCLEROSIS PATIENTS****Weng Kit Chan¹, Abdul Malik Musa¹, James Bashford^{1, 2}**¹*Institute of Psychiatry, Psychology and Neuroscience, King's College London, 16 De Crespigny Park, London SE5 8AB, UK*²*Maurice Wohl Clinical Neuroscience Institute, King's College London, 5 Cutcombe Rd, London SE5 9RT, UK*

Electrophysiology is an important method in reaching diagnostic certainty in amyotrophic lateral sclerosis (ALS), as evidenced by the Awaji Criteria that assigns equal weightage to both electrophysiological evidence and clinical evidence. High-density surface electromyography (HDsEMG) is a non-invasive electrophysiological technique of recording fasciculation potentials over long durations. Bashford and colleagues (2019) developed the Surface Potential Quantification Engine (SPiQE) to automatically detect and quantify fasciculation potentials in HDsEMG recordings. Subsequently, they introduced Active Voluntary Identification (AVID) as a semi-automated strategy to exclude voluntary potentials from the main fasciculation analysis.

This project aimed to i) compare the fasciculation parameters as detected and quantified by SPiQE, with the voluntary parameters as excluded by AVID; and ii) identify neuronal hyperexcitability (i.e. myokymic or neuromyotonic discharges) from HDsEMG recordings in ALS patients.

30-minute HDsEMG data recordings taken from 20 ALS patients in a previous longitudinal study were analysed in MATLAB across two domains. In the time domain, fasciculation parameters were quantified using SPiQE, and compared with voluntary parameters excluded by AVID. In the frequency domain, specifically designed scripts were utilised in search of neuronal hyperexcitability.

The voluntary and fasciculation parameters were significantly different ($p < 0.001$), with muscle parameters showing different trends across time ($p = 0.001$). Fasciculation doublets, triplets, quintuplets, and multiplets – all in the range of myokymic discharge, were successfully detected. The significantly different parameters across muscle groups suggest variable rates of motor unit decline, as seen clinically in the split hand phenomenon of ALS. We provide the first known discovery of myokymic discharges in ALS patients using HDsEMG analysis, while also further validating the AVID strategy as a complement to SPiQE fasciculation analysis. Future research may expand on this novel approach to detect abnormal electrophysiological patterns in a variety of diseases other than ALS.

ORAL PRESENTATIONS • DISCOVERY SCIENCE**[OPS4]****THE ROLE OF P53 GAIN-OF-FUNCTION MUTATIONS IN MODULATING THE MIR-200 FAMILY AND EPITHELIAL-MESENCHYMAL PLASTICITY IN BREAST EPITHELIAL CELLS***Vickie Wong, Zong Yang Yee, Chooi Ling Lim, Chee Onn Leong**School of Medicine, International Medical University, No. 126, Jalan Jalil Perkasa 19, Bukit Jalil, 57000 Kuala Lumpur, Malaysia*

Our study aimed to investigate how the miR-200 family is modulated by p53 gain-of-function mutations (R175H and R273H) during epithelial-mesenchymal plasticity (EMP) in the MCF10A breast epithelial cell line. EMP plays an important role in cancer metastasis as it allows cells to gain invasive features and ultimately reattach at a secondary tumour site. The microRNA (miR)-200 family is known to enhance EMP by regulating post-transcriptional gene expression, targeting specific base-pairing of messenger RNA (mRNA). Therefore, they have been demonstrated to regulate the expression of transcriptional factors (TFs) involved in the EMP programme namely E-cadherin, Vimentin and ZEB1. Recently, there have been discoveries on the association between p53 protein and miR-200 family and eventually EMP.

Quantitative real-time PCR was employed to investigate the basal levels of miR-141, miR-200a and miR-429 in MCF10A cells overexpressed with the p53 R175H and R273H mutants. Next, we transfected MCF10A parental cells with miR-200 family mimics and measured EMP markers using Western Blot. The morphology of the cells was observed using microscopy. Finally, we transfected miR-200 family inhibitors into MCF10A cells overexpressed with p53 R273H mutants and used Western Blot to measure EMP markers. Once again, morphology of these cells was observed using microscopy.

Based on our findings, we discovered that the overexpression of miR-200 family was only associated with the presence of p53 R273H mutant. Further investigation demonstrated that the p53 R273H mutant enhanced E-cadherin and downregulated ZEB1 and vimentin expression with the overexpression of miR-200 family members, specifically miR-141 and miR-429. When these miRNAs were inhibited, the effect was abrogated, demonstrating a dependency of the p53 R273H mutant on miR-141 and miR-429 in the modulation of EMP markers.

In conclusion, our findings suggested that the p53 R273H mutant regulates miR-200 family expression levels to promote EMP in the MCF10A breast epithelial cell line.

ORAL PRESENTATIONS • DISCOVERY SCIENCE**[OPS5]****ASSOCIATION BETWEEN PERIODONTAL DISEASE WITH BLOOD PRESSURE, AND VASCULAR FUNCTION MEASURED WITH FLOW MEDIATED DILATATION AND PULSE WAVE VELOCITY:
A SYSTEMATIC REVIEW***Kelten Clements¹, Eleanor Murray², Tomasz Guzik²**¹School of Dentistry, University of Glasgow, Glasgow Dental Hospital & School, 378 Sauchiehall Street, Glasgow, G2 3JZ**²Institute of Cardiovascular and Medical Sciences, University of Glasgow, BHF Glasgow Cardiovascular Research Centre, 126 University Place, Glasgow, G12 8TA*

This review aimed to critically appraise the literature investigating the association between periodontal disease and blood pressure, measurements of arterial vascular function: flow mediated dilatation (FMD) and pulse wave velocity (PWV). This will help inform future research studies utilising these methods and healthcare workers treating patients with cardiovascular disease about the need to account for periodontal disease.

Literature was searched in multiple databases that had been published between the year 2000 and May 2021 in the English language. Studies were sought that compared FMD, PWV, and blood pressure values between groups of patients with and without periodontitis. The relevant data was collected from the studies and summarised into 3 tables. The risk of bias was assessed using a modified Newcastle-Ottawa quality assessment scale, that had been adapted for use with cross-sectional studies.

A total of 2761 titles and abstracts were screened, of these 75 full text articles were assessed for eligibility. 45 articles met the eligibility criteria and were included in this review. Of these included studies 37 were cross-sectional, 5 were case-control and 4 were cohort studies. The studies that were included were conducted in 21 different countries across Asia, Europe and America with the sample size ranging from 32 to 36,110. Thirty-five (78%) of the studies included in this review showed a positive association between periodontal disease with impaired FMD, higher PWV and blood pressure values. The scores from the modified Newcastle-Ottawa quality assessment scale ranged from 3/10 to 10/10.

This systematic review supports the hypothesis of an association between periodontal disease with impaired FMD, higher PWV and elevated blood pressure values which increases the risk of cardiovascular disease compared to patients without periodontal disease.

ORAL PRESENTATIONS • DISCOVERY SCIENCE**[OPS6]****SPATIAL PROTEOME PROFILING OF DISTINCT CANCER CELL SUBPOPULATIONS WITHIN PANCREATIC DUCTAL ADENOCARCINOMA (PDAC): A PILOT STUDY**

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Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest malignancies in the world with a very low survival rate due to late diagnosis at advanced stage and high treatment resistance because of tumour heterogeneity. Tumour heterogeneity is caused by the presence of distinct subpopulations of cancer cells with different sensitivity to chemotherapy drugs leading to chemoresistance. This study aimed to identify the proteome profiles of distinct cancer cell subpopulations in PDAC to facilitate an understanding of PDAC biology and allow biomarker and therapeutic target discovery in the future.

In brief, a total of six formalin-fixed paraffin embedded (FFPE) blocks derived from six PDAC patients were collected from the UKM Medical Centre (UKMMC) tissue archive. The subpopulation of cancer cells from infiltrating invasive and far from invasive regions were identified by light microscope. The identified regions were microdissected by laser capture microdissection (LCM), underwent protein extraction, digestion by trypsin enzyme and ran through mass spectrometer followed by data analysis with MaxQuant software.

We discovered that there were distinct proteome profiles within each subpopulation of cancer cells, but no significant pathways identified due to low protein numbers. We recommended the use of molecular imaging approach to better characterise cancer cell subpopulations in PDAC. Besides, fresh frozen tissue instead of FFPE can be used to increase sampling quality in future.