Cancer Immunotherapy in 2022 - End of the Beginning
Assoc. Prof. Dr. Toh Han Chong

In the past over 10 years, the renaissance of cancer immunotherapy and its impact on cancer lives saved has been unprecedented. The first immune checkpoint inhibitor (ICI) anti-CTLA4lg was approved in 2011 and the first CAR T cell therapy approved in 2017. Since then, 6 more ICIs have been approved across many cancer indications. Tumour mutational burden and microsatellite instability form critical biomarkers for the use of ICIs. In 2022, we are seeing a rise in combination therapies involving ICI.

In cell therapy, CAR T cell therapy has made early important inroads in blood cancers. In solid tumours, challenges exist in reaching optimal clinical efficacy. Still, we see glimmers of hope. TCell Receptor and tumour infiltrating lymphocyte T cell therapy have shown some striking benefit in some patients refractory to further treatments with little further alternatives. Other exciting developments are in the therapeutic areas of T cell engager, antibody drug conjugates and therapeutic cancer vaccines. – where clinical benefits have been achieved. Central in the development of new immunotherapies and generally cancer treatment is a deeper dissection and understanding of the tumour microenvironment, to better elucidate mechanisms, biomarkers, resistance and new targets.

Immunotherapy now forms a vital pillar of cancer therapy with a trend towards more combinations showing greater impact. Challenges remain for cost of treatment and manufacturing capabilities. With more supersurvivors and complete remissions with such treatment even in advanced disease, we will surely see a better tomorrow for cancer patients.

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Precision Medicine in Lymphoid Malignancies
Prof. Dr. Richard Rosenquist Brandell

With the introduction of high-throughput sequencing technologies, the molecular landscape of major lymphoid malignancies was rapidly unraveled. While a few lymphoid neoplasms were characterized by a predominant gene mutation (e.g. Waldenström’s macroglobulinemia and hairy cell leukemia), the majority demonstrated a highly diverse genomic landscape with a high number of recurrent mutations. These studies have not only furthered our understanding of the onset and evolution of lymphoid malignancies, but also provided us with new biomarkers that can improve diagnostics, prognostication as well as guide clinical decision-making in lymphoid malignancies. In this lecture, I will highlight clinically relevant diagnostic, prognostic and predictive biomarkers that are used today in clinical diagnostics of chronic lymphocytic leukemia (CLL), one of the most frequent lymphoid malignancies, also as a basis for treatment decisions on
novel targeted therapies. I will also highlight future directions in this rapidly evolving field.

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ATLAS Project and the Future of International Collaborative Clinical Research in Asia
Dr. Mitsumi Terada
National Cancer Center Japan has initiated ATLAS (Asian Clinical Trials Network for Cancers) project since 2020 with financial support from AMED that is a Japanese public funding agency. The goals of ATLAS are to strengthen multilateral cooperation with ATLAS member institutions and improve the cancer clinical research capabilities; establish robust infrastructure for conducting oncology trials with high quality clinical data; deliver novel drugs and practice changing research results to cancer patients. To achieve this goal, construction of well-organized governance as a cooperative group such as EORTC, SWOG, or JCOG is urgently needed. We have just started to prepare the establishment of the ATLAS Board that is the guardian of ATLAS’s sustainability.

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Developing Affordable Cancer Therapeutics in Low and Middle Income Countries
Dr. Yolanda Augustin
Over half the world’s cancers occur in Low-and-Middle-Income Countries (LMICs), where cancer is a neglected disease. Despite significant advances in systemic anticancer therapy, many of these treatments are simple not available to the majority of the world’s cancer patients. For example, immunotherapy has changed the treatment paradigm for many cancer patients in developed countries, significantly prolonging survival and maintaining patient quality of life but at a cost that is currently unaffordable for many public health systems and patients in LMICs. We urgently need better solutions to treat patients more effectively, humanely and equitably.

Drug discovery and novel drug development takes on average 10-15 years to go from bench to bedside at an average cost of USD1 billion. Repurposing of ‘old’ drugs for new indications can shorten this pathway substantially with significant cost savings. One strong drug repurposing candidate is artesunate, an antimalarial derived from traditional Chinese medicine that also displays anticancer properties. This drug is currently the subject of our drug repurposing programme which includes a number of cancers relevant to Malaysian needs including colorectal cancer, cervical cancer, nasopharyngeal cancer and Acute Myeloid Leukaemia. Alongside drug repurposing, platform technologies such as molecular pharming – using plants to grow cancer drugs, in particular immunotherapies and targeted monoclonal antibodies can also reduce the cost of cancer drug development significantly.

Malaysia has the opportunity to lead on healthcare priorities facing LMICs, creating reverse linkage collaborations between Muslim countries through entities such as the Islamic Development Bank (which now has a Center of Excellence for Science, Technology and Innovation) in Kuala Lumpur and local governments. This also represents opportunities for technology transfer and halal sector manufacturing. Collaboration is urgently needed between academia, clinicians, patient advocate groups, governments, non-governmental stakeholders, regulators and responsible pharma to ensure equitable access to affordable cancer therapeutics for patients globally.

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Triple Negative Breast Cancer: Tumour Biology and Optimisation of Clinical Outcome
Dr. Mastura Md Yusof
Triple negative breast cancer are breast cancer types that are difficult to treat because they lack expression of the estrogen receptor (ER), progesterone receptor (PR) and Human Epidermal Receptor 2 (HER2) gene amplification. Intensive research, analysis by the Cancer Genome Atlas (TCGA) Research Network and the advent of high-throughput technology tools has expanded the classification of TNBC tumors into subgroups according to its gene expression profiles in order to identify the different molecular subtypes, novel TNBC biomarkers that can play both predictive and prognostic roles and enhance therapeutic strategies.

The “immune-activated,” subtype or tumours with defective BRCA pathway are amongst initial TNBC group with established genetic vulnerabilities that has allowed the addition of promising therapeutic approaches, including DNA-damaging agents (PARP inhibitors, platinum) as well as immunotherapy. The treatment of metastatic NBC (mTNBC) is currently transforming rapidly with better outcomes observed in clinical trials.

The recent success with immune checkpoint inhibitors (ICIs) targeting the programmed cell death receptor 1 and programmed death ligand 1 (PD-L1) and PARP inhibitors for germline BRCA mutation-associated breast cancers as well as other novel strategies in mTNBC treatment will change the course of this unique cancer subtype in the future.

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Updates on Treatment of Gastroesophageal Cancers
Prof. Dr. Ho Gwo Fuang
Management of gastric cancer has undergone significant changes over the past few years. TCGA classified gastric cancer into four molecular subtypes: chromosomal instability (50% of cases), MSI-H (21%), genomically stable (20%) and EBV-positive (9%). Molecular profiling is recommended prior to the start of systemic
Emeritus Prof. Dr. Liam Chong Kin
The receptor for hepatocyte growth factor, a tyrosine kinase that is encoded by the mesenchymal epithelial transition factor (MET) oncogene, plays a crucial role in cancer growth, invasion and metastasis. Oncogenic MET alterations can act as a primary driver of tumorigenesis, with tumor dependence on MET signaling for cancer initiation and progression, a phenomenon that is called ‘oncogene addiction’. MET exon 14 (METex14) skipping mutations and MET amplification have been identified as alterations that can convert MET into a primary oncogenic driver in non-small cell lung cancer (NSCLC). MET amplification is also an acquired resistance mechanism in 10-15% of patients with epidermal growth factor receptor (EGFR)-mutated non-small cell lung cancer (NSCLC) treated with EGFR tyrosine kinase inhibitors (TKIs). Targeted therapies are available or are in development to target NSCLC harbouring METex14 skipping mutations or MET amplification. Both of these MET alterations are biomarkers predictive of sensitivity to MET inhibition in NSCLC. The level of MET amplification potentially has an impact on the efficacy of targeted treatment, with patients with highly amplified tumours expected to show a better response. METex14 skipping mutation is now an actionable oncogenic driver in advanced/metastatic NSCLC with selective MET inhibitors such as tepotinib and capmatinib showing durable clinical activity in advanced NSCLC patients with this MET alteration.
Testing for MET amplification often requires tissue analysis using fluorescent in-situ hybridisation because tissue and especially circulating tumour DNA next-generation sequencing underestimates MET amplification. Combining a selective MET inhibitor with first- or third-generation EGFR TKI on development of acquired EGFR TKI resistance due to MET amplification is a treatment strategy with promising efficacy. 707. 10.25163/angiotherapy.639C

Exploiting New Cancer Drug Targets that Drive Drug Resistant and Metastatic Disease
Prof. Dr. Andrew D Westwell
Continuing progress in the field of cancer therapeutics will require the development of novel agents against previously unexplored drug targets driving resistant and metastatic disease. In a collaboration between the School of Pharmacy and the European Cancer Stem Cell Research Institute at Cardiff University (Wales, U.K.), we have focused on drug discovery against the transcription factor complex protein Bcl3. As an emerging cancer drug target, driving cancer hallmarks in diseases of unmet medical need such as metastatic colorectal cancer, Bcl3 is ripe for therapeutic exploitation [1]. We have used computational drug design methods to virtually screen for inhibitors at the interface between...
Emerging Immuno-cellular Therapy in the Treatment of Diffuse Large B-cell Lymphoma
Prof. Dr. Bee Ping Chong

Diffuse large B cell lymphoma (DLBCL) is the most common aggressive lymphoma in adults. The R-CHOP immunochemootherapy protocol has been the first-line standard of care for DLBCL patients for decades and is curative in approximately two-thirds of patients. However, 30–40% of patients are refractory or relapsing and they second-line salvage therapy that consisted of platinum-based chemotherapy regimens followed by autologous hematopoietic stem cell transplantation with curative intent for transplant-eligible patients or palliative chemotherapy for transplant-ineligible patients. The overall response rate is ranged between 40 to 60%. The median survival is approximately 13 months and the prognosis is even poorer for those who do not respond to salvage chemotherapy. Therefore, there is still an unmet need for these patients. Better understanding of both molecular biology of lymphoma cells and the tumor microenvironment have initiated clinical trials exploring targeted therapy based on driver genetic alterations. In recent years there have been several new therapeutic agents approved for the treatment of relapsed/refractory DLBCL. These agents include monoclonal antibodies such as polatuzumab vedotin (targeting CD79a), tafasitamab and loncastuximab tesirine (targeting CD19), Selinexor (SINE inhibitor), anti-CD19 chimeric antigen receptor T-cell therapies and bispecific antibodies.

Biomarkers in Clinical Trials: From Cancer Researcher and Cancer Patient Perspective
Dr. Magdalena B. Wozniak

Biomarkers have been used for diagnosis, assessment of disease risk, prognosis, prediction, as well as treatment response and safety at virtually every stage of drug discovery and development. In an era of targeted therapies, and precision medicine, clinical trials have adapted their designs to bring new medicines to the right patient population and improve treatment outcomes. In my presentation, I will provide an overview of the landscape of biomarkers discovery at various stages of drug development with specific examples to illustrate diagnostics and therapeutic monitoring. Biomarkers also play an important role in the detection and management of patients with breast cancer. Biomarkers that aid in the diagnosis, prognosis, and prediction of breast cancer are crucial for early detection and appropriate disease control throughout treatment. As a breast cancer survivor, I will share my experience of how biomarkers affected my treatment decision making during my cancer journey and discuss challenges in biomarker and clinical development from cancer researcher and cancer patient perspectives.

Cancer-Associated Fibroblasts as Potential Therapeutic Targets in Head and Neck Cancer
Prof. Dr. Ian Paterson

It is now recognised that non-malignant components within the tumour microenvironment (TME) also influence tumour development and progression. Cancer-associated fibroblasts (CAFs) are often the most abundant cell type within the tumour stroma. Here, they actively participate in the reciprocal communication between tumour cells and other host cells in the TME to create a tumour-permissive microenvironment in a number of epithelial tumours, including those of the head and neck. CAFs share many characteristics with fibroblasts found within healing wounds and demonstrate a perpetually “active,” alpha-smooth muscle actin positive phenotype. In this talk, I will describe the functional role of CAFs in head and neck cancers (oral and nasopharyngeal carcinomas) and discuss the signalling pathways involved in CAF activation. Finally, the possibility of targeting CAFs therapeutically will be discussed.

Targeted Delivery of Cisplatin Using RGD-Modified ZIF-90 as Delivery Vehicle

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Introduction: Chemotherapy is one of the most effective therapy available to treat cancers, but the side effects make it less favourable. Therefore, we aimed to effect selective and targeted delivery of cisplatin to cancer cell via covalent-modification of zeolitic imidazolate framework-90 (ZIF-90) with RGD peptide, a tripeptide that was known for its ability to recognise integrins on cell surface. Due to overexpression of integrins by cancer cell, RGD peptide on the surface of ZIF-90 would be able to guide the delivery of the cancer drug (cisplatin) encapsulated in the pores of ZIF-90, to the intended recipient. Thus, side effect to normal cells will diminish. Methods: Nano-sized ZIF-90 encapsulated cisplatin (RGD⊂Cis⊂nZIF-90) was prepared by in-situ encapsulation followed by covalent modification with RGD peptide. The drug loading amount and drug released was profiled, and MTT assay on MRC-5 (human fetal lung fibroblast cells) and A549 (lung adenocarcinoma) cells was conducted. Results: Powder XRD confirmed the formation of ZIF-90 and covalent bond to RGD peptide was confirmed with NMR and IR showing formation of imine bond between imidazolcarbaldehyde linker of ZIF-90 and the RGD peptide. The cisplatin loading was measured to be 24.8% using UV-vis and sustained released behaviour at pH 5 was observed with maximum released (92%) observed after 24 hours. The MTT assay showed that RGD⊂Cis⊂nZIF-90 nanoparticle was more toxic towards A549 (IC50 8.79 μg/mL) than MRC-5 (IC50 31.07 μg/mL). The selectivity index was found to be 3.5 suggesting that there is selectivity towards A549 cell in the presence of RGD peptide. Conclusion: RGD-modified nano-sized ZIF-90 encapsulated cisplatin has been successfully synthesized and determined to have good selectivity and toxicity towards lung cancer cell over normal cell. This may lead to more selective and improve performance of cancer therapy with little side effect.

Keywords: ZIF-90, RGD peptide, cisplatin, selective chemotherapy, cancer drug delivery

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ODD03

Molecular Modelling Analysis of Squamocin as Potential BCL-XI Anti-apoptotic Protein Inhibitor

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Introduction: Tropomyosin receptor kinase-C (TrkC) has been reported to be overexpressed in cancer and regulates its survival and metastasis. In addition, tumour microenvironment is immunosuppressive and associated with high expression of immunosuppressive mediators including regulatory T cells (Tregs), TGF-β and myeloid-derived suppressor cells. This study was aimed to study the antitumour efficacy of TrkC-targeting dinitrophenol (DNP) conjugate (IYIY-DNP) combined with Cyclophosphamide (CYP) for immunotherapy. CYP is an FDA-approved anticancer drug and has been reported to selectively suppress Treg cells at low dose. Methods: Female Balb/c mice was immunised with DNP-KLH (Keyhole Limpet Hemocyanin) to stimulate anti-DNP antibodies. After immunisation, TrkC expressing-4T1 cells were implanted, and tumour bearing mice at the size of 60-80mm³ were randomly divided to group of 10 mg/kg IYIY-DNP (LV), 25 mg/kg CYP (LP), 10 mg/kg IYIY-DNP + 25 mg/kg CYP for treatment on every alternative day, for five cycles (n=8 for each group including control saline). Tumour size were recorded using calliper on every two days, for 30 days. Results: No toxicity was observed on mice treated with all groups throughout 14 days of observation. Mice treated with low dose of 25 mg/kg CYP displayed delayed tumour growth by 6.29%, however, mice treated with 10 mg/kg IYIY-DNP + 25 mg/kg CYP delayed tumour growth by 36.14%, both compared to saline-treated mice. The average area under the tumour growth curve vs. day post treatment for 10 mg/kg IYIY-DNP + 25 mg/kg CYP group is significantly smaller compared with saline-treated group (P<0.05). Conclusion: These findings suggest that combination therapy of IYIY-DNP and CYP is able to decrease the tumour growth preclinically and have a potential to be developed to become new immunotherapy treatment strategy.

Keywords: Tropomyosin Receptor Kinase C (TrkC), 4T1 cells, Cyclophosphamide (CYP), Immunotherapy

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Introduction: BCL-XL is one of the prosurvival proteins that is overexpressed in certain cancers and implicated as a chemoresistance factor. It is known from in-vitro studies that certain acetogenins, including squamocin (SQ), have potent anticancer properties. However, its inhibitory effect towards BCL-XL has not been established. In this study, we evaluated the interaction and binding affinity of SQ into BCL-XL protein in molecular docking and dynamics. Methods: Autodock Vina was used to perform molecular docking of SQ with BCL-XL protein. The tested ligand was also compared with ABT-737, a known BCL-XL inhibitor. The best conformation with lowest binding energy and interaction with key active residues of BCL-XL receptor was chosen. The complex was further subjected to a molecular dynamic study for 100ns simulation using FF19SB forcefield in Amber 22. Molecular mechanics/Poisson-Boltzmann surface area (MM/PBSA) energy calculations, Root mean square deviation (RMSD) and Fluctuation of protein conformation (RMSF) of the complex were performed. Results: Interaction of SQ with the active site of BCL-XL protein revealed a better binding affinity of -11.9 kcalmol$^{-1}$ compared to -9.179 kcalmol$^{-1}$ of ABT-737. The SQ/BCLXL complex was found to be stable throughout 100ns simulation time. Furthermore, hydrogen bonding patterns between key residues of ARG139 and SER 109 with SQ ligand were detected, and energy profiles reflect the stability of the bound complex. Conclusion: Squamocin is a potential inhibitor of the BCL-XL protein based on the in-silico study. However, a further experimental study is needed to validate its action toward the development of a new drug for cancer therapy. Keywords: Cancer, Squamocin, BCL-XL protein, Molecular docking, Molecular dynamic

ODD04
Cytotoxicity Effects of Selected Flavonoids in Human OvarianCancer Cells
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Introduction: Ovarian cancer is the most lethal gynaecological malignancy in women. The use of chemotherapy for ovarian cancer treatment is effective in many patients, but it has been associated with serious side effects. Therefore, attention is given to natural compounds, including flavonoids as alternative drugs in cancer treatment and prevention. Objective: This study aims to determine the cytotoxicity of seven flavonoids, namely apigenin, biochanin A, flavone, fisetin, galangin, myricetin and 6-hydroxyflavone against two epithelial ovarian cancer cell lines (CAOV-3 and SKOV-3). Method: The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was carried out to determine the cell viability after being treated with selected flavonoids and carboplatin at 24, 48 and 72 h. The experiment was then followed by an AO/PI double staining assay using a fluorescent microscope to elucidate the morphological changes of cell death. Result: A total of four flavonoids, namely flavone, galangin, 6-hydroxyflavone and biochanin A showed highly cytotoxic against CAOV-3 cells at 24 h with lower IC50 value, ranging from 33.9 to 37.91 µgml$^{-1}$ compared with carboplatin 41.63 µgml$^{-1}$. Galangin has the lowest IC50 value among all at 48 h (26.16 µgml$^{-1}$) and 72 h (23.9 µgml$^{-1}$). Meanwhile, apigenin, 6-hydroxyflavone, flavone, biochanin A and myricetin exhibited less IC50 value than carboplatin (69.13 µgml$^{-1}$) of 36.55, 38.67, 38.78, 39.61 and 57.48 µgml$^{-1}$, respectively, at 24 h of SKOV-3 cells treatment. Apigenin and biochanin A were detected to be highly cytotoxic in SKOV-3 cells at 48 and 72 h, respectively. The morphological changes of treated cells with flavonoids exhibited the presence of cell membrane blebbing and chromatin condensation indicating induction of apoptosis. Meanwhile, secondary necrosis was also seen in all flavonoids treatment after 24 h. Conclusion: This study shows that apigenin, myricetin, flavone, 6-hydroxyflavone and biochanin A can be potential therapeutic drugs in ovarian cancer. Keywords: Flavonoid, CAOV-3, SKOV-3, Ovarian cancer
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ODD05
Elucidating the transcriptional regulation and chemosensitivity efficacy of Terminal Differentiation Induced Non-Coding RNA (TINCR) driver regulator in Triple-Negative Breast Cancer (TNBC)
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Objective: To determine the transcriptional regulation and chemosensitivity efficacy of TINCR driver regulator in TNBC. Method: In vitro experiments were performed on TTDA cells treated with TINCR overexpression and chemosensitivity using MTT assay. Results: TINCR overexpression showed increased chemosensitivity to chemotherapeutic drugs. Conclusion: TINCR may serve as a potential target for TNBC treatment.

Keywords: TINCR, TNBC, chemosensitivity, transcriptional regulation
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Introduction: Previous baseline analysis (GSE58812) displayed dysregulation of TINCR in TNBC metastasis and death patients and high TINCR expression was shown to have shorter disease-free survival in TNBCs (HR: 2.69, p-value: 0.0044). However, the underlying mechanism of TINCR in driving poor outcome TNBC is unclear. Therefore, the study aimed to elucidate the transcriptional regulation and potential chemo-sensitisation effects of TINCR in TNBC cell line model. Methods: Ensembl, PROMO, KM plotter and Promoter 2.0 Prediction Server databases was utilised to predict TINCR promoter region and transcription binding sites. TINCR promoter region was predicted by input query FASTA TINCR sequence into Promoter 2.0 Server. The highest score of the predicted site was chosen for PROMO putative transcription factors (TFs). Predicted TFs were analysed further of their prognostic effects in TNBC on KM plotter. TNBC cell line model MDA-MB-231 were transduced with shTINCR and shNT via lentiviral transduction packaging vectors. Functional assays such as growth, proliferation, migration, wound-healing, and drug sensitivity assay (MTT) were carried out. Results: Three TFs were prognostically relevant in TNBCs such as the CEBPB, ELK1, and ARNT. RqPCR showed downregulation of CEBPB and ARNT whereas ELK1 was upregulated in TINCR-depleted MDA-MB-231 cell line. Characterisation of MDA-MB-231 shTINCR cell line showed slower rate of cell proliferation, growth rate, and migration of cells compared to MDA-MB-231 shNT control. This preliminary analysis narrowed down three potential driver mechanism of TINCR in TNBC however, RqPCR revealed contradicting outcome for ELK1. MTT drug assay showed 2-fold efficacy chemo-sensitisation to Cyclophosphamide by TINCR. Conclusion: Silencing TINCR and potential TINCR-TFs may suppress tumour progression and chemoresistance. Therefore, TINCR and TINCR-TFs expression may potentially serve as a significant predictive value and prognostic marker for TNBC patients. Further insights into the functional and clinical implications of the molecular cross talk between TINCR, CEBPB, ARNT, and ELK1 may also provide therapeutic benefit for TNBC patients.

Keywords: Triple negative breast cancer, LncRNA, TINCR, Transcription factors

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ODD06

Phytochemical Analysis and Evaluation of Antioxidant and Antibacterial Activities of Crude and Fractionated Flower Extracts of Clitoria ternatea

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Introduction: Clitoria ternatea (CT), (fam: Fabaceae), also known as Butter pea flower, is a medicinal plant used to treat various diseases. This plant contains a variety of plant metabolites including flavonoids. The phytochemicals present in flower extracts have been reported to possess various pharmacological activities that are beneficial for protecting the body against various diseases. The aim of the present work was to analyse, separate, and identify the phytochemicals and evaluate the antioxidant potential and antibacterial activities of flower extracts of CT. Methods: CT flower was dried, powdered and macerated in methanol to obtain methanolic crude extract. The process continued by successive fractionation using solvents of different polarities to obtain n-hexane fraction, chloroform fraction, ethyl acetate fraction, and aqueous extract fraction. The phytochemicals present in the extracts were separated by TLC and HPLC methods. Antioxidant and antibacterial activities were evaluated by DPPH and in vitro well diffusion methods, respectively. Results: Phytoconstituents present in CT were separated and some flavonoids were also identified in chloroform and ethyl acetate fractions. Antioxidant and antibacterial results showed that ethyl acetate fraction was found to exhibit good antioxidant activity (IC50 value 0.11 mg/ml) and border antibacterial effect. Conclusion: The ethyl acetate extract of CT was found to be potent and could be used to isolate pure compounds that might be helpful in the development of antibacterial pharmaceutical entities in the future.

Keywords: Clitoria ternatea, Butter pea flower, Antibacterial, Antioxidant, Extract

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ODD07

Asymmetric Total Synthesis of (-)-Swainsonine from Inexpensive and Commercially Available Starting Material, Ascorbic Acid

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CONFERENCE ABSTRACTS
**Introduction:** Swainsonine is a natural and synthetic alpha-mannosidase II inhibitor which has been shown to inhibit various types of cancer growth in vitro and in vivo. Our initial study was to synthesise (-)-swainsonine from commercially available D-isoascorbic acid, yet unsuccessful, although following published protocol. Hence, we envisioned this challenge associated with aldehyde-lactol tautomerism by exploiting another novel route.

**Methods:** Acetonide protected diol 1 was first prepared from D-isoascorbic acid via a three-step procedure. It was then selectively acetylated, and further Swern oxidation yielded aldehyde 2. Wittig olefination was carried out to afford olefinic ester 3 with ease. Subsequent deacetylation and azide substitution gave imino ester 6, which readily produces (-)-swainsonine in 4 steps. Purification of the products was carried out using the classical column chromatography Si-gel G60 (230-400 mesh, Merck). The 1H and 13C NMR spectra were registered in CDCl 3 with Joel Resonance ECZ4005 400 MHz (1H) and 100 MHz (13C) using TMS as the internal standard. Results: The imino ester 6 was obtained as a colourless oil with an overall yield of 2% from D-isoascorbic acid.

**Conclusion:** The present study illustrated the total synthesis route of (-)-swainsonine.

**Keywords:** Swainsonine, Cancer, Swern oxidation, Wittig olefination, Aldehyde-lactol tautomerism

**ODD08**

**Development and Characterisation of Zerumbone-Superparamagnetic Iron Oxide Nanoparticle Co-Loaded Nanostructured Lipid Carriers as A Potential Treatment for Breast Cancer**

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**Introduction:** Zerumbone (ZER), having significant medicinal value, is poorly soluble in water, and this poor solubility of ZER has been overcome by loading into nanostructured lipid carriers (NLC), but clinical trials with this compound have been rarely reported due to lack of selectivity. Hence, using superparamagnetic iron oxide nanoparticles (SPION) could be a potential approach to achieve specific delivery in various treatments. This study aims to develop and characterize an improved drug delivery system for ZER. **Method:** ZER and SPION were loaded into NLC (ZSN) using hot ultrasonication. This formulation was characterised by particle diameter, polydispersity index (PDI), zeta potential, encapsulation efficiency, loading capacity, state of lipid modification, lipid interaction, morphology, in vitro drug release, haemocompatibility, and stability. ZSN was also evaluated on its in vitro cytotoxicity, cellular uptake, internalisation, and magnetic targeting. **Results:** ZSN was milky brownish with an average diameter of 140.32 ± 1.14 nm, PDI of 0.18 ± 0.02, and zeta potential -13.37 ± 0.61 mV. ZSN was shown to be homogeneous, spherical, uniformly dispersed, haemocompatible, and stable over 3 months of storage. Over 72 hours, 38.88 ± 6.61% and 41.25 ± 4.90% of ZER were released from acidic and physiological conditions, respectively. At 48 hours of treatment, ZSN was time-dependently cytotoxic and apoptotic, with half maximal inhibitory concentration (IC50) values of 1.94 ± 0.19 and 2.55 ± 0.93 μg/mL in MCF-7 and MDA-MB-231 cells, respectively. The apoptotic effect was more significant in MDA-MB-231. In terms of the internalisation pathway, ZSN was internalised primarily by caveolae-mediated endocytosis and active energy-dependent process in MCF7 and MDA-MB-231 cells, respectively. The process was time-dependent with half maximum uptake (Km) value of 25.19 ± 1.50 minutes for MCF-7 cells and 6.07 ± 0.54 minutes for MDA-MB-231 cells. The magnetic characteristics of SPION were sufficient to direct ZSN. Under a 2T magnetic field, localised targeting occurred at the cellular level, resulting in a 19.85% ZSN uptake in MCF-7 and 6.07 ± 0.54 minutes for MDA-MB-231 cells.

**Conclusion:** ZSN is a potent ial magnetic guiding multi-modal therapeutic system for breast cancer treatment.

**Keywords:** Zerumbone, Superparamagnetic Iron Oxide Nanoparticle, Nanostructured Lipid Carrier, Nanoparticle, Specific Targeting

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The Effects of Repeated Exposure of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) on Bhas 42 Cell Line at Human Physiologically Relevant Doses

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Introduction: Heterocyclic amines (HCAs) are among the major groups of food carcinogens produced by high-temperature cooking of proteinaceous food. This study aims to examine the effects of two of the most abundant HCAs, 2-amino-1-methyl-6-phenylimidazo-[4,5-b]pyridine (PhIP) and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) on Bhas 42 cells’ viability and proliferation upon repeated exposure at human physiologically relevant concentrations. Methods: Cytotoxicity and cell proliferation of PhIP- and MeIQx-treated Bhas 42 cells were assessed using the alamar blue assay for 48 hours and 8 days, respectively. In both assays, Bhas 42 cells were exposed to PhIP or MeIQx at concentrations of 10^{-7}, 10^{-8}, 10^{-9}, and 10^{-10} M, respectively, which were diluted in three different vehicle concentrations of DMSO, namely 0.003%, 0.03%, and 0.1%. In the cell proliferation assay, the cells were repeatedly exposed to all sets of treatments every 48 hours for 8 days. Results: For cytotoxicity assay, all concentrations of PhIP exhibited a significant toxic effect in 0.003% and 0.03% DMSO with reductions of cell viability up to 19.1% and 75.6%, respectively whereas MeIQx showed toxic effects only in 0.03% DMSO, specifically at the two highest doses with cell viability reduced up to 90.4% and 75.0%, respectively. In the cell proliferation test, all concentrations of PhIP and MeIQx in all vehicle concentrations did not cause any toxic effects on Bhas 42 cells. Conclusion: The current study shows that at human physiologically relevant concentrations, the toxic effect of PhIP and MeIQx on Bhas 42 cells decreased when the cells were exposed to these carcinogens for a longer duration compared to when exposed in a short period of time regardless of DMSO concentrations. Therefore, further research on the carcinogenic potential of those HCAs may need to take these findings into account for better elucidation of their mechanism of action.

Keywords: Cytotoxicity, Heterocyclic amines, PhIP, MeIQX, Bhas 42 cells

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ODD10

RNA Sequencing Analysis of NTCU-Induced Lung Squamous Cell Carcinoma in Mice

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Introduction: Lung squamous cell carcinoma (SCC) is associated with a high mortality rate because of its assemblage of DNA mutations causing poor therapeutic responses. Therefore, elucidating the molecular events that underpin the pathobiology of lung SCC is essential to the development of effective therapies. Methods: We induced lung SCC in N-nitroso-tris-chloroethylurea (NTCU)-treated mice and RNA sequenced the resultant lung tumours. Cleaned sequence reads were aligned to the Mus musculus genome (GRCm38/mm10) using Spliced Transcripts Alignment to a Reference (STAR). Then, DESeq2, an R package, was used to normalise per-gene read counts before Gene Set Enrichment Analysis (GSEA). The functional impact of single nucleotide polymorphisms (SNPs) was assessed using the Protein Variation Effect Analyzer (PROVEAN) and Sorting Intolerant From Tolerant (SIFT). Pathogenic DNA variants were defined as having SIFT and PROVEAN scores <0.05 and -2.5, respectively. The final list of SNP-affected genes was analysed using g:Profiler to pinpoint overrepresented pathways. Adjusted p-values <0.05 were considered statistically significant. Results: The transcriptomic analysis revealed that the top five pathways potentially driving lung SCC development were cholesterol biosynthesis, keratinization, activation of gene expression by SREBP (SREBP), formation of the cornified envelope, and neutrophil degranulation. These pathways are plausibly involved in cancer development; for instance, cholesterol is an activator of tumorigenic signaling pathways and is required for increased membrane synthesis during cell proliferation. However, the association of the pathways with lung SCC has not been extensively reported. The SNP analysis revealed ‘homophilic cell adhesion via plasma membrane adhesion molecules’ as the most enriched gene ontology. The dysregulation of homophilic cell adhesion is a well-known contributor to cancer cell migration and metastasis. Conclusion: Our combinatorial analysis of differentially expressed genes and deleterious SNPs revealed novel, interacting biological pathways that may contribute to lung SCC.
ADAR1 Dependency in Oral Squamous Cell Carcinoma

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Introduction: Our recent genome-wide CRISPR knockout screen revealed that the Adenosine deaminase acting on RNA-1 (ADAR1) gene is essential for the survival of a majority of oral squamous cell carcinoma (OSCC) cell lines. Given that deleting ADAR1 caused severe lethality in OSCC, targeting ADAR1 could have therapeutic benefits. ADAR1 catalyzes Adenosine-to-Inosine RNA editing, and suppresses dsRNA sensing-triggered cell death. ADAR1 has two isoforms, the constitutively expressed P110 and the interferon-inducible P150. It is also an interferon-stimulated gene (ISG) that is highly expressed in cancers including OSCC. In this study, we aimed to determine the molecular mechanisms underlying ADAR1 dependency which may afford an opportunity to develop better treatment strategies for OSCC. Methods: ADAR1 dependency is validated by competitive co-culture assay, apoptosis and colony formation assay using single-guide RNA (sgRNA) knockout in OSCC cell lines (ORL-48, ORL-214, ORL-174, ORL-195, SCC9 and BICR10). Changes in protein expression upon gene(s) knockout are determined by western blotting. Results: We confirmed that ADAR1 depletion significantly increased apoptosis by flow cytometry and induced colony formation in selected cell lines. For cell lines that are ADAR1-less dependent (BICR10 and ORL-174), IFN-β treatment increased the expression of ADAR1 and other ISGs, sensitizing these cells to cell death. Notably, overexpression of ADAR1-P150 but not ADAR1-P110, significantly rescued cell lethality in ADAR1 depleted cells suggesting that the ADAR1-P150 is important in the survival of OSCC. We also showed that cell death is mediated by dsRNA sensors protein kinase R (PKR) and melanoma differentiation-associated protein 5 (MDA5) whereby knocking-out either one of these genes did not reverse cell lethality, but co-deleting both genes partially rescued cell death in ADAR1-dependent cell lines. Conclusion: The present findings revealed that the interferon-inducible P150 of ADAR1 is essential for OSCC survival. Activation of the dsRNA-sensing pathways such as PKR and MDA5 underlies this dependency.

Keywords: ADAR1 dependency, dsRNA sensing, CRISPR knock-out, Interferon-stimulated gene, OSCC

OTM01

OTM02

Identification and Evaluation of Tumour-homing Peptides Specifically Targeting Metastatic Breast Cancer Cells

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Introduction: Molecular probes are crucial for early breast cancer diagnosis. Metastatic breast cancer is associated with high mortality and recurrence rates. An active-targeted strategy using homing peptides is an effective approach to diagnose and treat breast cancer as it encompasses prominent specificity and activity through cellular membranes and have lesser toxicity than the vastly used small molecule inhibitors. Method: Bioinformatics analysis was carried out to find peptides homing breast cancer cells and to predict the peptide sequence binding to receptors found on metastatic breast cancer cells. Total of 4 peptides were designed based on their binding affinity with highly expressed receptors by breast cancer cells and synthetised for imaging detection purpose. These tumour-homing peptides (THPs) were labelled with fluorescein isothiocyanate (FITC) and were tested on metastatic breast cancer cell lines (MDA-MB-231, MDA-MB-453, T47D & MCF-7) and a normal human breast cell line (MCF-10A). MTT assay was performed to evaluate FITC-labelled THPs and potency in cells exposed to these peptides for up to 24 hours. The binding efficacy of the THPs was then confirmed by fluorescence imaging and detection in human metastatic breast cancer cells. Results: Identified positive THPs were termed as MBC-BT1, MBC-BT2, MBC-BT3 & MBC-BT4. After incubation with increasing concentrations of the FITC-labelled THPs for MTT assay, the dose selected for succeeding tests were fixed at 12.5 µg/mL. FITC-labelled MBC-BT1, MBC-BT2, MBC-BT3 & MBC-BT4 peptides bind to MDA-MB-231 breast cancer cells based on fluorescence imaging after 30 minutes of incubation of these peptides with MDA-MB-231 cells. These peptides had no binding to normal human breast cancer cell line MCF-10A. Conclusion: The findings of this study produce high potential peptide-based
diagnostic tracers for metastatic breast cancer detection and identification of unique cell surface profile of highly metastatic breast cancer cells, thereby provide new targets for therapeutic intervention.

**Keywords:** Homing, Peptide, Metastatic breast cancer

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**OTM03**

**An Effort to Establish the Concept of Adaptive Therapy In Vitro Using Non-Small Cell Lung Cancer Cells Treated with Afatinib**

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**Introduction:** Drug resistance in cancer becomes a barrier to cancer cure and they are insensitive towards a drug, which requires patients to change to a new set of treatments. It was reported that minor pre-existing drug resistance is present in patients during diagnosis. Although changing treatment options is the only way to eradicate drug-resistant cells, a new theory called Adaptive Therapy (AT) focuses on survival competition between sensitive and resistant cells in treatment-free period. During this period, sensitive cells will outgrow resistant cells for space. Thus, AT possesses the potential to delay the emergence of resistance. This in vitro study was done with sensitive and resistant non-small cell lung cancer (NSCLC) cell lines against afatinib. Continuous (96 hours exposed) and intermittent treatment (24 hours, followed by a 72-hour treatment-free period) in a direct co-culture of 0.1% resistant cells the advantage to be dominant.  

**Results:** The sensitive cell line has a lower IC50 value than the resistance cell line (0.3 nM against 300 nM). At 96- and 192-hours, concentrations at 1.0 and 2.0 nM showed higher cell counts for intermittent than continuous by 5000 cells, and qPCR showed gene expression of resistance cells are higher than sensitive cells.  

**Conclusion:** This preliminary study showed that AT was not able to be established to delay the emergence of NSCLC resistance in vitro. One of the shortcomings is that the resistant cells proliferate 24 hours faster than sensitive cells, and this does not reflect the reported scenario in patients and opposes one of the parameters to achieve AT. Thus this study gave resistant cells the advantage to be dominant.

**Keywords:** Drug resistance, Continuous therapy, Adaptive therapy, Non-small cell lung cancer

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**OTM04**

**Gene Signature for Predicting Homologous Recombination Deficiency in Triple-Negative Breast Cancer**

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**Introduction:** Recently, PARP inhibitors have been approved for treatment of triple negative breast cancer in patients with germline or somatic alterations in BRCA1 or BRCA2. Whilst many mechanisms of action have been proposed, it is likely that the response in this group is due to deficiencies in the homologous recombination repair pathway. Thus, a biomarker that is able to identify patients who may have deficiency in homologous repair despite a lack of mutations in BRCA1 or BRCA2 would have significant clinical utility.  

**Methods:** We designed a nearest-centroid classifier for homologous recombination deficiency (HRD) in Asian TNBCs from the Malaysian Breast Cancer (MyBrCa) cohort using an RNA-seq gene expression dataset of 100 genes (HRD100). The classifier was trained on data from 94 TNBC samples from the MyBrCa cohort, and validated in an
The classifier was also validated on the NanoString nCounter platform, as well as using FFPE instead of fresh frozen tissue. **Results:** The HRD100 classifier identified samples with strong HRD mutational signature at an AUROC of 0.892 in the MyBrCa training dataset, as well as 0.783 and 0.713 in MyBrCa and TCGA validation datasets, respectively. Analysis of the 100 genes in the HRD100 classifier using the NanoString nCounter platform showed a concordance rate of 98% (CI: 95-100%) with RNA-seq gene expression analyses, and a concordance rate of 87% (CI: 73-100%) between FFPE and fresh frozen tissue. **Conclusion:** Taken together, gene expression using these 100 selected genes may identify triple-negative breast cancer patients with homologous recombination deficiency who may benefit from treatment with PARP inhibitors or platinum chemotherapy. **Keywords:** Triple-negative breast cancer, Homologous recombination deficiency, Gene expression signature, PARP inhibitors

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**OTM05**

**Induction of in vitro Cytotoxicity in High-Risk Oral Leukoplakia Using a Cancer Vaccine**

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**Introduction:** Patients with oral leukoplakia diagnosed with moderate-severe oral epithelial dysplasia (OED) have an increased risk of developing oral cancer. However, chemoprevention agents targeting epithelial cells are ineffective in preventing malignant transformation and disease recurrence. Emerging evidence indicates that host immunity can impact premalignant disease progression, but the immune profile of oral leukoplakia has not been studied. In this study, we characterized the immune profile of high-risk oral leukoplakia with a long-term aim of identifying immunotherapeutic strategies to intercept cancer development. **Methods:** The immune profile of moderate-severe OED was determined by transcriptomic analysis of 125 immune signatures reported in cancer. To determine the utility of a cancer vaccine targeting MAGED4B, we evaluated MAGED4B expression by immunohistochemistry on oral leukoplakia tissues. Then, in-vitro T cell-based immunogenicity studies were performed using patients’ blood samples to evaluate antigen-specific immune responses. **Results:** Immune profiling demonstrated the induction of both immune surveillance and immune suppression mechanisms in moderate-severe OED. Notably, three distinct immune subtypes were identified: (1) immune cytotoxic; (2) non-cytotoxic; and (3) non-immune reactive. Patients progressed to cancer appear to lack cytotoxic T cells responses, suggesting that restoring T cell immunity via cancer vaccine may intercept malignant development. Next, we evaluated the feasibility of activating patients’ immune responses using a cancer vaccine targeting MAGED4B. We demonstrated that moderate-severe OED significantly over-expressed MAGED4B. Our T cell-based immunogenicity studies showed that MAGED4B-specific CD8+ T cells could be expanded in-vitro and become activated as indicated by increased interferon gamma secretion and CD38 expression. Importantly, these CD8+ T cells demonstrated antigen-specific killing of tumour cells expressing MAGED4B. **Conclusion:** Our series of studies demonstrated that discrete immune responses are present in high-risk leukoplakia, and antigen-specific immune response can be harnessed to induce cytotoxic responses in these lesions. **Keywords:** Oral leukoplakia, Immune profile, Oral epithelial dysplasia, Cancer vaccine

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**OTM06**

**Characterisation of Primary Cilia (PC) Expression in Oral Cancer Cell Lines with Different Metastatic Potentials**

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**Introduction:** Primary cilia (PC), a sensory organelle that exists on the surface of most cells are found in various cancers including prostate, renal, lung, ovarian, and breast cancer. PCs are critical signalling hubs for the maintenance of cell homeostasis through various signalling pathways including Hedgehog, Wnt, Hippo, and ROR2 pathways. Dysregulation of these pathways, therefore, lead to tumorigenesis. While most PC studies focused on cancer related molecular pathway, little is known about intraflagellar-transport 20 (IFT20), a component of IFT machinery that is required...
for ciliogenesis. Therefore, PC changes and its associated ciliary signalling in oral cancer cells were investigated. **Methods:** Immortalized normal oral keratinocytes (OKF6-TERT2 cell) and two OSCC cell lines, HSC-2 (non-metastatic OSCC) and HSC-3 (highly metastatic OSCC) cells were cultured in media containing low serum (2%) and high serum (20%) concentrations of foetal bovine serum (FBS). Cells were subjected to serum-starvation for 24, 48, 72 hours and 5 days. For each culture condition, the incidence, length and association of PC with cell proliferation were assessed by fluorescence microscopy. Further, the expression of the ciliogenesis gene, IFT20, was determined by quantitative-RT-PCR. **Results:** An increase in the percentage of ciliated HSC-2 and HSC-3 cells cultured in low and high serum media was observed compared to OKF6-TERT2 cells. Cilia length was longer in HSC-2 and HSC-3 cells compared to OKF6-TERT2 cells, suggesting cilia length abnormalities in oral cancer cells. The number of ciliated cells was increased, and proliferation was decreased in both HSC-2 and HSC-3 cells cultured in low serum and high serum media at all time points. Furthermore, mRNA transcript levels of IFT20 increased in HSC-2 and HSC-3 cells as compared to OKF6-TERT2 cells. **Conclusion:** These findings indicate that an increase of PC and the presence of ciliary defects in oral cancer cells is associated with the upregulation of IFT20. **Keywords:** Primary Cilia (PC), Oral Squamous Cell Carcinomas (OSCCs), Oral Cancer, Intraflagellar-transport 20 (IFT20) 716-717. 10.25163/angiotherapy.6329C

**OTM07**

The Expression of YKL-40 Protein in Oral Potentially Malignant Disorders (OPMD) and Oral Squamous Cell Carcinoma (OSCC)

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**Introduction:** Oral squamous carcinoma cell (OSCC) is the most common type of oral cancer, which results in significant morbidity and mortality. In OSCC, the progression from normal mucosa to different grades of dysplasia and to invasive carcinoma is characterized by an increased formation of angiogenesis. There is much evidence that indicated YKL-40, a secreted glycoprotein, was highly elevated in cancers and associated with tumour angiogenesis. Being a potent angiogenic factor capable of stimulating development and vascularization of tumour during carcinogenesis, the role of YKL-40 in oral dysplasia and its progression remains unknown. Hence, this study aims to determine the expression of YKL-40 in OSCC and its potential role in the oral carcinogenesis. **Methods:** Secreted YKL-40 protein levels were screened by enzyme-linked immunosorbent assays (ELISA) in serum samples from 16 healthy donors, 14 oral precancer and 22 OSCC patients as well as conditioned media collected from oral epithelial dysplasia (OED) and OSCC cell lines. In addition, we employed immunohistochemical (IHC) detection in 75 oral tissue samples consisting of 20 normal oral mucosa (NOM), 35 OED and 20 OSCC cases. To determine the effect of YKL-40 on vascular endothelial angiogenesis, migration assay and tube formation assay of human umbilical vein endothelial cell (HUVEC) was performed. **Results:** YKL-40 protein expression in serum was the highest in OSCC followed by oral dysplasia and normal samples. Similar trend was seen in cell lines whereby OSCC exhibited higher YKL-40 expression as compared to dysplasia cell lines. IHC results showed that YKL-40 was highly expressed across the epithelium in OSCC, and OED compared to NOM tissue. Additional of exogenous recombinant YKL-40 prompted HUVEC migration and tube formation compared to control. Notably, YKL-40 also enhanced migration of HUVEC induced by conditioned medium of OSCC cells. **Conclusion:** These findings provide novel insights into angiogenic activities of YKL-40 in oral cancer development. **Keywords:** Oral squamous carcinoma cell, Oral epithelial dysplasia, Angiogenesis 717. 10.25163/angiotherapy.6330C

**OTM08**

Preliminary Study of BRF2 Expression in Subset of Head and Neck Cancers

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**Introduction:** Head and neck cancers (HNC) are abnormal cell proliferation in the mucosal surfaces involving several anatomical sites such as oral cavity, sinonasal cavity, pharynx, and larynx. In Malaysia, HNC especially involving the nasopharyngeal cancer is the fourth most common cancer, with total new cases of 2,222 detected in 2020. Determination of the potential biomarkers for
early diagnosis of this disease is crucial. Transcription factor II B (TFIIB)-related factor 2 (BRF2) is one of the important transcription factors, regulating the RNA Polymerase III (Pol III) gene transcription. Dysregulation of Pol III transcription mediated by BRF2 lead to abnormal cell growth and cancer progression. This study aimed to compile and analyse data on BRF2 expression related to HNC using online database and immunohistochemistry (IHC) analysis. **Methods:** Information regarding BRF2 protein and specific mRNA transcripts were retrieved from Human Protein Atlas (HPA), Oncomine, GEPIA, and eBioPortal databases. IHC analysis was performed on tissue microarrays (TMA) containing normal and a subset of HNC involving the lip tissues using BRF2 and PCNA antibodies as comparison. **Results:** HPA revealed overexpression of BRF2 protein in the nuclear region, detected in 25-75% cancer cells of all HNC cases (n=499) with staining intensity ranging from moderate to strong. In Oncomine, BRF2 shown differential expression between normal vs tumour tissues whereby 4/8 datasets showed upregulation of BRF2 transcripts. GEPIA also reported mRNA overexpression for BRF2 transcripts. GEPIA also reported mRNA overexpression for BRF2 (8.6 vs 5.32) in head and neck squamous cell cancer (HNSC) compared to normal tissue. IHC staining revealed varying degree of BRF2 expression in HNC involving the lip as compared to normal tissue controls. **Conclusion:** Compiled data suggest that BRF2 is commonly overexpressed in HNC studied. Further study is essential to evaluate BRF2 expression in more HNC subsets as tissue distribution is heterogenous and involved large anatomical sites.

**Keywords:** RNA Polymerase III, BRF2, Head and Neck Cancers, Oral Cancers, Biomarkers

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**PDD01**

**Synergistic Growth Inhibitory Effect of Hydroxyurea and SRJ23 Combination on T-ALL Jurkat Cells**

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**Introduction:** T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive haematologic neoplasm that accounts for about 15% and 25% of children and adults, respectively of all types of acute lymphoblastic leukemia. Treatment has not shown positive curative sustenance and is cost-effective. SRJ23, a bicyclic lactone, is a novel semi-synthetic derivative of andrographolide while hydroxyurea (HU) is a known inexpensive anticancer agent causing suppression of DNA synthesis by inhibiting ribonucleoside diphosphate reductase and inhibits GDP-GTP exchange to induce apoptosis. We aim to evaluate the inhibitory effect of HU in combination with SRJ23 on the Jurkat cell line and determine its efficacy. **Methods:** T-ALL (Jurkat cells) were seeded and treated with different concentrations of SRJ23 (0.1, 1, 10, 100 µM) and HU (7.8, 15.6, 31.25, 62.5, 125, 250, 500, and 1000 µM). After 96 hours of incubation, the MTT assay was used to assess the in vitro growth inhibition of the combination towards the cells. An apoptosis assay was also performed by flow cytometry technique using Annexin V-FITC and Propidium Iodide (PI) double staining assay. **Result:** The dose-response curves showed a reduction of cell viability with a synergistic effect with a combination of concentration of 10 µM SRJ23 with 25 µM HU (combination index of 0.53) or 250 µM HU (combination index of 0.40). The combination induced enhanced apoptosis when compared with single-agent alone. **Conclusion:** The combination of HU and SRJ23 had a remarkable synergistic effect inhibiting T-ALL Jurkat cells by inducing apoptosis. The combination needs to be tested further both in vitro and in vivo in order to assess how well it works as a potential novel targeted therapy.

**Keywords:** T-ALL, SRJ23, Hydroxyurea, Synergistic effect, MTT, Apoptosis

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**PDD02**

**The In Vitro Cytotoxic Evaluation and Apoptotic Effects of Histone Deacetylase (HDAC) Inhibitory Drugs on Anaplastic Large Cell Lymphoma (ALCL) Cell Lines**

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**Introduction:** Anaplastic large cell lymphoma (ALCL) is a subtype of aggressive peripheral T-cell lymphoma (PTCL). ALCL develops from T-lymphocytes (T-cells) and approximately 10-20% of all T-
Introduction: Jacareubin is a plant-derived natural xanthone that has a strong inhibitory effect on human breast cancer cells. According to a previous study, Jacareubin is less toxic and more effective than the conventional medication, 5-Fu (IC50 17.01±0.23), with an IC50 of 6.28±0.47. (Sun et al., 2016). Strong interactions were discovered in the binding pocket of aromatase (3EQM) by an initial molecular docking study on Jacareubin. However, there is still a dearth of knowledge regarding how this structure would affect the binding strength of these compounds. We propose that changes to the main structure of Jacareubin with a more varied array of substituents will significantly affect inhibitory activity of the xanthonoids. Methods: The Grover, Shah, and Shah (GSS) reaction is used to synthesis xanthonoids in a single pot. It involves phenol-benzoic acid condensation followed by direct cyclization of the benzophenone intermediate to produce the xanthone product. Nuclear Magnetic Resonance (NMR), Gas Chromatography-Mass Spectrometry (GC-MS), and Fourier Transform Infrared Spectroscopy (FTIR) are used to analyze newly synthesized compounds. The biological activity of the synthetic xanthonoids is evaluated against breast cancer cell lines via MTT assay. Results: There are nine xanthones and seven Jacareubin derivatives that have been synthesized. The molecular docking study shows that the derivatives have higher binding energy (-8.3 kcal/mol to -9.8 kcal/mol) than the Jacareubin (-9.4 kcal/mol) which the value of IC50 is 6.28±0.47μM. The IC50 value for 8-fluoro-1,3-dihydroxy-9H-xanthen-9-one (-8.3 kcal/mol) and 9-fluoro-5-hydroxy-2,2-dimethylpyrano[3,2-b]xanthen-6(2H)-one (-9.8 kcal/mol) are 3 μM and 60 μM respectively. Conclusion: The study showed that the IC50 value of 9-fluoro-5-hydroxy-2,2-dimethylpyrano[3,2-b] xanthen-6(2H)-one was higher than the Jacareubin due to how intensely this derivative interacts with aromatase binding sites (3EQM). Aromatase and ligand have a binding energy of -9.8 kcal/mol. Thus, this Jacareubin derivative may prevent human breast cancer from spreading throughout the body.

Keywords: Xanthone, Jacareubin, Gemcitabine, Breast cancer, Aromatase.

PDD04
Preliminary Study of Structure-Activity Relationship of Xanthone and Jacareubin Derivatives in Inhibiting Aromatase Activity via in Vitro and In-Silico Approaches
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Introduction: Jacareubin is a plant-derived natural xanthone that has a strong inhibitory effect on human breast cancer cells. According to a previous study, Jacareubin is less toxic and more effective than the conventional medication, 5-Fu (IC50 17.01±0.23), with an IC50 of 6.28±0.47. (Sun et al., 2016). Strong interactions were discovered in the binding pocket of aromatase (3EQM) by an initial molecular docking study on Jacareubin. However, there is still a dearth of knowledge regarding how this structure would affect the binding strength of these compounds. We propose that changes to the main structure of Jacareubin with a more varied array of substituents will significantly affect inhibitory activity of the xanthonoids. Methods: The Grover, Shah, and Shah (GSS) reaction is used to synthesis xanthonoids in a single pot. It involves phenol-benzoic acid condensation followed by direct cyclization of the benzophenone intermediate to produce the xanthone product. Nuclear Magnetic Resonance (NMR), Gas Chromatography-Mass Spectrometry (GC-MS), and Fourier Transform Infrared Spectroscopy (FTIR) are used to analyze newly synthesized compounds. The biological activity of the synthetic xanthonoids is evaluated against breast cancer cell lines via MTT assay. Results: There are nine xanthones and seven Jacareubin derivatives that have been synthesized. The molecular docking study shows that the derivatives have higher binding energy (-8.3 kcal/mol to -9.8 kcal/mol) than the Jacareubin (-9.4 kcal/mol) which the value of IC50 is 6.28±0.47μM. The IC50 value for 8-fluoro-1,3-dihydroxy-9H-xanthen-9-one (-8.3 kcal/mol) and 9-fluoro-5-hydroxy-2,2-dimethylpyrano[3,2-b] xanthen-6(2H)-one (-9.8 kcal/mol) are 3 μM and 60 μM respectively. Conclusion: The preliminary study showed that the IC50 value of 9-fluoro-5-hydroxy-2,2-dimethylpyrano[3,2-b] xanthen-6(2H)-one was higher than the Jacareubin due to how intensely this derivative interacts with aromatase binding sites (3EQM). Aromatase and ligand have a binding energy of -9.8 kcal/mol. Thus, this Jacareubin derivative may prevent human breast cancer from spreading throughout the body.

Keywords: Xanthone, Jacareubin, Gemcitabine, Breast cancer, Aromatase.

PDD04
Synthesis of PEGylated Liposome Co-loaded with Doxorubicin Hydrochloride and Tumour Suppressor miR-145 Mimics for Its Anti proliferative Effect Against Triple Negative Breast Cancer
In vitro
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Introduction: Jacareubin is a plant-derived natural xanthone that has a strong inhibitory effect on human breast cancer cells. According to a previous study, Jacareubin is less toxic and more effective than the conventional medication, 5-Fu (IC50 17.01±0.23), with an IC50 of 6.28±0.47. (Sun et al., 2016). Strong interactions were discovered in the binding pocket of aromatase (3EQM) by an initial molecular docking study on Jacareubin. However, there is still a dearth of knowledge regarding how this structure would affect the binding strength of these compounds. We propose that changes to the main structure of Jacareubin with a more varied array of substituents will significantly affect inhibitory activity of the xanthonoids. Methods: The Grover, Shah, and Shah (GSS) reaction is used to synthesis xanthonoids in a single pot. It involves phenol-benzoic acid condensation followed by direct cyclization of the benzophenone intermediate to produce the xanthone product. Nuclear Magnetic Resonance (NMR), Gas Chromatography-Mass Spectrometry (GC-MS), and Fourier Transform Infrared Spectroscopy (FTIR) are used to analyze newly synthesized compounds. The biological activity of the synthetic xanthonoids is evaluated against breast cancer cell lines via MTT assay. Results: There are nine xanthones and seven Jacareubin derivatives that have been synthesized. The molecular docking study shows that the derivatives have higher binding energy (-8.3 kcal/mol to -9.8 kcal/mol) than the Jacareubin (-9.4 kcal/mol) which the value of IC50 is 6.28±0.47μM. The IC50 value for 8-fluoro-1,3-dihydroxy-9H-xanthen-9-one (-8.3 kcal/mol) and 9-fluoro-5-hydroxy-2,2-dimethylpyrano[3,2-b] xanthen-6(2H)-one (-9.8 kcal/mol) are 3 μM and 60 μM respectively. Conclusion: The preliminary study showed that the IC50 value of 9-fluoro-5-hydroxy-2,2-dimethylpyrano[3,2-b] xanthen-6(2H)-one was higher than the Jacareubin due to how intensely this derivative interacts with aromatase binding sites (3EQM). Aromatase and ligand have a binding energy of -9.8 kcal/mol. Thus, this Jacareubin derivative may prevent human breast cancer from spreading throughout the body.

Keywords: Xanthone, Jacareubin, Gemcitabine, Breast cancer, Aromatase.
**Introduction:** Doxorubicin hydrochloride (Dox-HCl) is a widely prescribed anti-cancer drug for the treatment of breast cancer. However, the clinical application of Dox-HCl is constrained, especially in triple-negative breast cancer (TNBC) due to its toxicity and the aggressiveness of the tumour. Meanwhile, microRNA-145 (miR-145) is known to be a tumour suppressor miRNA (tsmiRs) exhibiting anti-proliferative and anti-metastasis effect against various cancers. However, there is no evidence delineating the combination use of miR-145 mimics with Dox-HCl co-loaded in nanoparticles that increases the therapeutic efficacy on TNBC when compared to individual or combinational treatment without nano-carrier. The aim of this study is to synthesise and characterise Dox-HCl and miR-145 mimics co-loaded in PEGylated liposome and to investigate its in vitro anti proliferative activity against MDA-MB-231 cells. **Methods:** Dox-HCl and miR-145 mimics co-loaded in PEGylated liposomes were formulated according to composite central design. Response models were developed to investigate the correlation of formulation parameters toward nanoparticle size (d.nm) and encapsulation efficiency (EE%) of both Dox-HCl and miR-145 mimics. The ideal liposome formulation was further characterised for its in vitro stability, drug release, cellular uptake, and cellular toxicity. **Results:** Statistical analysis of the response models indicated that formulation 6 (F6) exhibited the highest desirability function ($D=0.934$) with optimum nanoparticle size (137.1±3.76 nm) and high EE% for both Dox-HCl (67.31±3.76%) and miR-145 mimics (87.85±4.60%). F6 displayed great stability over 60 days at 4°C with stable nanoparticle size and zeta potential ($\zeta=4.3\%$), comparable to vismodegib. Moreover, pancreatic cancer cells easily acquire resistance to gemicitabine, standard chemotherapy. Studies have indicated that andrographolide, a naturally occurring agent, exhibits various pharmacological properties, including anti-cancer and anti-inflammatory activities. In this study, the anti-pancreatic cancer stem cell activity of andrographolide and its derivative, Raspholide was evaluated in vitro. **Methods:** Pancreatic cells Capan-2 were treated with andrographolide, raspholide, as well as dimethylsulfoxide, gemicitabine, sodium chloride, vismodegib (positive control for anti-CSCs), and benzimidazole (anti-Ras agent). The effect of the treatment was assessed via a tumoursphere assay and flow cytometry analysis. **Results:** It was found that raspholide had a significant effect on the tumoursphere number reduction with a $p$-value less than 0.05 compared to andrographolide. Raspholide was also able to target the main cancer stem cell markers, CD24+, CD44+, and CD133+ with a value of 4.3%, comparable to vismodegib. **Conclusion:** Raspholide exhibits anti-cancer activity against pancreatic cancer stem cells. **Key Words:** Anti-pancreatic cancer stem cells, Andrographolide, Raspholide, Tumoursphere assay, CD markers 720. 10.25163/angiotherapy.6336C

**PDD06**

**Transforming Growth Factor-β (TGF-β) may Regulate Progression of Triple Negative Breast Cancer Intrinsic Subtypes via Differential Activation of TGF-β Isoforms**

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Introduction: Transforming growth factor-β (TGF-β) is known to play significant dual roles in cell development and pathophysiology but very cell-specific dependent. Due to these features, it has been concomitantly associated in the development of triple negative breast cancer (TNBC) via activation of TGF-β signaling pathway. In house baseline studies directed the involvement of TGF-β signaling in TNBC via differentially expression analyses and may be regulated by SETD1A, an epigenetic regulator. This study aimed to identify differentially expressed genes by SETD1A knockdown and elucidate the molecular mechanism of TGF-β signaling across isoforms.

Methods: Two TNBC cell line models (FEC-sensitive MDA-MB-468 and FEC-resistant Hs578T; FEC – 5-flourouracil, epirubicin, cyclophosphamide chemo cocktail) were transfected by SETD1A siRNA and RNA were subjected for the Nanostring Cancer Progression Panel. Differentially expressed mRNA and pathway analysis were performed using the nSolver Software. RqPCR were carried out to confirm downstream regulated genes. Wound scratch, colony forming, invasion assays were conducted to complement the gene mechanisms. Results: Differentially expressed genes (TGF-β1, SMAD1, ID2, ZEB1) narrowed down TGF-β signaling among top hit pathways. Each gene was found to be differentially expressed between the MDA-MB-468 and Hs578T cell lines. TGF-β1, TGF-β3, SMAD1, SMAD2 and ID2 genes were upregulated and TGF-β2 was suppressed by SETD1A knockdown in FEC-resistant TNBC (Hs578T). These genes were inversely expressed in MDA-MB-468 cell line. SETD1A knockdown inhibited proliferative effects in TNBC cell lines, complemented by downregulation of Ki-67 by colony forming assay. Reduction wound closure were observed upon SETD1A knockdown. Induction of invasion were seen in TGF-β2 and TGF-β3 in a recovery setup. Conclusion: TGF-β signaling maybe a key regulator of TNBC development via activation of epigenetic regulator SETD1A. TNBC may be regulated via intrinsic mechanisms differing between FEC-sensitive and FEC-resistant cells, indicating a potential personalised therapeutic intervention in the two intrinsic TNBC groups. However, further evaluation is warranted to extend the understanding of TNBC progression by TGF-β signaling activation.

Keywords: TNBC, TGF-β, SETD1A, Chemoresistance, Epithelial-Mesenchymal-Transition, Triple negative breast cancer
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PDD07
Differential Cell Cycle Arrest in Dihydroorotate Dehydrogenase (DHODH) Inhibition of Different Subtypes of Breast Cancer Cell Lines
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Introduction: Dihydroorotate dehydrogenase (DHODH) inhibitors hold a great potential for breast cancer treatment especially in combinatorial therapy due to their antitumour capability. Brequinar and teriflunomide, the two potent DHODH inhibitors, showed cell cycle arrest in several cancer cells. These inhibitors target DHODH, the key enzyme in the de novo pyrimidine biosynthesis pathway that generates nucleotides essential for cells to proliferate. While the treatment for breast cancer is closely associated with the molecular subtyping (ER/PR and HER2 receptors) that has major implications on the survival of breast cancer patients, yet, the effect on the distinct hormone receptor status by these inhibitors has not been fully investigated. Thus, this study aimed to investigate the differential effects of breast cancer subtypes on the phases of cell cycle preferentially arrested by DHODH inhibitors. Methods: Three breast cancer cell lines with different receptor status were selected to represent each subtype; namely the MCF-7 (ER/PR+/HER2-), SKBR-3 (ER/PR-/HER2+), MDAMB-231 (ER/PR-/HER2- , also referred to as triple negative) and one non-tumorigenic MCF-10A (ER/PR+/HER2- ) cells. Cells were cultured and treated with variable concentrations of inhibitors. Cell cycle analysis was performed by staining with propidium iodide and examined through BD FACSCanto II and analysed using Flow Jo software. Results: Results showed that MCF-10A, MCF-7 and MDAMB-231 were arrested at G1/S phase of the cell cycle by both inhibitors. Meanwhile, SKBR-3, the only cell line with HER2+, did not exhibit any distinct cell cycle phase arrest between both inhibitors and the control. Conclusion: The present study showed that the phase of cell cycle arrested in DHODH inhibition in breast cancer is...
**Introduction:** Turmeric and ginger are among the most commonly used food condiments that are studied for their use as chemopreventive agents. Bioactive compounds curcumin in turmeric and gingerol and shogaol in ginger have been shown to modulate multiple cell signaling pathways involved in carcinogenesis including apoptosis, cancer cell survival (PI3K/AKT), inflammation (NF-kB, IL-6, and TNF), and proliferation (EGFR and AP-1) pathways. Very few studies have explored the chemopreventive role of these bioactive compounds in cervical carcinogenesis. This research aims to elucidate the chemopreventive properties of curcumin, 6/10-gingerols, and 6/10-shogaols in different concentrations ranging from 3 to 100 µM. **Method:** MTT assay was used to determine the cell growth inhibition of curcumin, 6/10-gingerols, and 6/10-shogaols in different concentrations ranging from 3 to 100 µM. **Results:** Curcumin and shogaols are the most potent compounds against both cervical cancer cells. The decreasing order of inhibition effects of curcumin and ginger bioactive compounds on the growth of cervical cancer cells is as follows (mean ± SD, n=3): for HeLa cells; Curcumin (IC50=31.2 ± 1.7µM)> 10-shogaol (IC50=36.3 ± 3.2µM)> 10-gingerol (IC50=65.5 ± 1.5µM)> 6-shogaol (IC50=68.8 ± 2.4µM)> 6-gingerol (IC50=83.3 ± 10.5µM). For SiHa cells; Curcumin (IC50=54.5 ± 10.6µM)> 10-gingerol (IC50=65.5 ± 1.5µM)> 6-shogaol (IC50=68.8 ± 2.4µM)> 10-shogaol (IC50=36.3 ± 3.2µM)> 6-gingerol (IC50=83.3 ± 10.5µM). **Conclusions:** Our study shows that curcumin and 6/10-shogaols have higher inhibitory effects compared to 6/10-gingerols on the growth of both cervical cancer cells, HeLa and SiHa. The chemopreventive effect of 10-shogaol and 10-gingerol is most likely due to longer carbon chain length which plays a significant role in antiproliferative effects and initiating apoptosis. Further investigations will be conducted to determine the optimal formulation of curcumin in combination with 6/10-gingerol, and 6/10-shogaol compounds against cervical cancer cells.

**Keywords:** Cervical cancer cell, Chemoprevention, Curcumin, Gingerol, Shogaol

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peptides’ SVM score increased, whereas the mutant peptides showed a decreasing trend. Similarly, for the mutant peptides analysis, both the hemolytic probability and docking energy scores towards the FAS receptor showed an increasing pattern as the peptide length built up. **Conclusion:** This *in silico* prediction found shorter length pardaxin 6 peptide fragments derived from *Pardachirus Marmoratus* that could have good potential to be developed as anti-cancer agents in the future.

**Keywords:** *In silico* prediction, Pardaxin, Anticancer peptides, *Pardachirus marmoratus*-derived peptides, Cancer

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**PDD10**

**Cytotoxic and Anti-angiogenic Effects of Postbiotics Derived from *Pediococcus* spp. against CT26 Mouse Colon Carcinoma Cells**

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**Introduction:** The current pharmacological treatments against colorectal cancer (CRC) are often compromised by their side effects and cancer resistance. As the majority of CRC are sporadic in nature and associated with diet, current efforts have been directed towards alternative approaches which include probiotics. There is now evidence indicating the strain-dependent usefulness of probiotic-derived bioactive metabolites (i.e., postbiotics) against CRC. There are also reports on *Lactobacillus* and *Bifidobacterium* probiotics that inhibit tumour angiogenesis which drives the growth of CRC. As such, this study aimed at assessing the cytotoxic and anti-angiogenic potentials of cell free supernatant (CFS) fermented by two unique strains of lactic acid bacteria (LAB) isolated from fermented tapioca in *vitro*. **Methods:** LAB identification was performed by sequencing the 16S rRNA gene of the LAB and matching the consensus sequence from GeneBank. Cytotoxicity of the LAB were then screened by using Sulforhodamine B (SRB) assay. Immunocytostaining of CT26 cells treated with LAB-derived CFS was performed at their respective highest subtoxic concentration (IC50) for examination of the anti-angiogenic potential. Lastly, high performance liquid chromatography (HPLC) analyses was undertaken to determine the concentrations of four major short chain fatty acids (SCFA; i.e., acetate, butyrate, lactate and propionate) in the LAB-derived CFS. **Results:** The LAB were identified as *Pediococcus pentosaceus* LAB3 and *Pediococcus acidilactici* LAB4 (99% similarity), respectively. LAB4 emerged as the most potent *Pediococcus* sp. against CT26 cells with IC50=4.67±0.3%. Immunocytostaining of CT26 cells treated with LAB-derived CFS (especially LAB4) resulted in downregulation (≤6%) of pro-angiogenic vascular endothelial growth factor (VEGF) and upregulation (≤42%) of anti-angiogenic thrombospondin (TSP-1). HPLC analyses found LAB4-derived CFS to yield the highest concentration for lactate and propionate. **Conclusion:** The present findings imply the potential strain-dependent cytotoxic and anti-angiogenic properties of LAB-derived postbiotics against CRC. The potent cytotoxic and anti-angiogenic effects of LAB4 may be associated with the production of SCFA.

**Keywords:** Lactic acid bacteria, Colorectal cancer, Cytotoxicity, Angiogenesis, Short chain fatty acids

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**PDD11**

**Evaluation of the Cytotoxic Potential of the Crude Extracts from Marine Microalga *Isochrysis* sp.**

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**Introduction:** Breast cancer is the most diagnosed cancer worldwide. Due to the side effects and drug resistance of chemotherapeutic drugs, a search for alternative anticancer agents from natural sources, especially marine sources, is warranted. *Isochrysis* sp. is a marine microalga that produces a wide range of pharmaceutically important metabolites but there is a lack of data about the cytotoxic activities of this microalga. Hence, this research is planned to investigate the cytotoxicity of marine
**Isochrysis sp.** crude extracts against a human breast cancer cell line (MCF-7) and to evaluate its mode of cell death. **Method:** Freeze-dried *Isochrysis* sp. biomass was extracted with eight solvents with different polarities through sonication and maceration. The algal extracts were evaluated for their cytotoxic effect at 100 µg/mL concentration against the MCF-7 cell line using MTT assay. The most cytotoxic extract of *Isochrysis* sp. was further investigated for apoptosis induction in MCF-7 cells through flow cytometry and RT-PCR. **Results:** Among eight extracts, the ethanol extract of *Isochrysis* sp. reduced the cell viability of MCF-7 cells to 7.24 ± 0.47% after 72 hours of incubation, at a concentration of 100 µg/mL. The IC50 (half maximal inhibitory concentration) value was 13.37 ± 0.59 µg/ml after 24 hours in MCF-7 cells and >100 µg/ml in non-cancerous human lung fibroblast cells, MRC-5. Morphological observation under a light microscope revealed cell shrinkage, condensation of cellular contents, and membrane blebbing in treated MCF-7 cells compared to untreated cells. The Annexin V-FITC and PI staining analysis confirmed that the mode of cell death is mainly apoptosis. Cell cycle analysis revealed the accumulation of cells in the sub-G1 phase and G2/M arrest. An up-regulation of the proapoptotic Bax gene and tumor suppressor p53 gene was observed through RT-PCR. **Conclusion:** The data suggest that crude ethanolic extract from marine *Isochrysis* sp. has induced apoptosis in MCF-7 and may have potential therapeutic value for human breast cancer. **Keywords:** Apoptosis, Cytotoxic, *Isochrysis* sp., Marine microalgae, MCF-7

**PDD12**

**Phytochemical Composition and Anticancer and Cisplatin-sensitising Activities of Green Macroalgae Ulva sp. Extracts against Oesophageal Squamous Cell Carcinoma KYSE-150 Cells**

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**Introduction:** Oesophageal squamous cell carcinoma (ESCC) is an aggressive subtype of oesophageal cancer. Cisplatin is a standard chemotherapeutic agent for ESCC with limited treatment efficacy and chemoresistance occurrence. Thus, a novel anticancer candidate with a chemosensitising effect could be a promising ESCC therapy. Green macroalgae have exhibited cytotoxicity against several cancers preclinically, but their anticancer and chemosensitising effects against ESCC are unknown. This preliminary study investigated anticancer and cisplatin-chemosensitising potentials of two unidentified *Ulva* sp. extracts. **Methods:** *Ulva* sp. powder was macerated in ethanol and methanol by shaking for 48 h. The metabolites in the concentrated extracts were identified using phytochemical tests. Half-maximal inhibitory concentration (IC50) was determined using MTT assay. Among eight extracts, the ethanol extract of *Ulva* sp. was further investigated for chemosensitising potentials of two unidentified *Ulva* sp. extracts.

**Conclusion:** Both *Ulva* sp. extracts show anticancer activity against KYSE-150 cells by inducing apoptosis via ROS modulation. **Keywords:** Anticancer, Chemosensitising, *Ulva* sp., Cisplatin, Oesophageal squamous cell carcinoma

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**PDD13**

**Targeted Delivery of Cisplatin Using RGD-Modified ZIF-90 as Delivery Vehicle**

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**Introduction:** Cisplatin is a standard chemotherapeutic agent for ESCC with limited treatment efficacy and chemoresistance occurrence. Thus, a novel anticancer candidate with a chemosensitising effect could be a promising ESCC therapy. Green macroalgae have exhibited cytotoxicity against several cancers preclinically, but their anticancer and chemosensitising effects against ESCC are unknown. This preliminary study investigated anticancer and cisplatin-chemosensitising potentials of two unidentified *Ulva* sp. extracts.

**Results:** Both *Ulva* sp. extracts show anticancer activity against KYSE-150 cells by inducing apoptosis via ROS modulation. **Keywords:** Anticancer, Chemosensitising, *Ulva* sp., Cisplatin, Oesophageal squamous cell carcinoma

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**Introduction:** Chemotherapy is one of the most effective therapies available to treat cancers, but the side effects make it less favourable. Therefore, we aimed to effect selective and targeted delivery of cisplatin to cancer cell via covalent-modification of zeolitic imidazolate framework-90 (ZIF-90) with RGD peptide, a tripeptide that was known for its ability to recognise integrins on cell surface. Due to overexpression of integrins by cancer cell, RGD peptide on the surface of ZIF-90 would be able to guide the delivery of the cancer drug (cisplatin) encapsulated in the pores of ZIF-90, to the intended recipient. Thus, side effect to normal cells will diminish. **Methods:** Nano-sized ZIF-90 encapsulated cisplatin (RGD@Cis⊂nZIF-90) was prepared by in-situ encapsulation followed by covalent modification with RGD peptide. The drug loading amount and drug released was profiled, and MTT assay on MRC-5 (human fetal lung fibroblast cells) and A549 (lung adenocarcinoma) cells was conducted. **Results:** Powder XRD confirmed the formation of ZIF-90 and covalent bond to RGD peptide was confirmed with NMR and IR showing formation of ZIF-90 nanoparticle was more toxic towards A549 (IC50 8.79 μg/mL) than MRC-5 (IC50 31.07 μg/mL). The selectivity index was found to be 3.5 suggesting that there is selectivity towards A549 cell in the presence of RGD peptide. **Conclusion:** RGD-modified nano-sized ZIF-90 encapsulated cisplatin has been successfully synthesized and determined to have good selectivity and toxicity towards lung cancer cell over normal cell. This may lead to more selective and improve performance of cancer therapy with little side effect. **Keywords:** ZIF-90, RGD peptide, Cisplatin, Selective chemotherapy, Cancer drug delivery 724-725. 10.25163/angiotherapy.6344C
other hydrophobic interactions. The in silico simulation results showed very high PARP1-ligand complex stability for both compounds. **Conclusion:** SS_C2 and SS_C4 are new potential PARP1 inhibitors, which in turn provide a plausible explanation of their observed anticancer activities.

**Keyword:** Synclisia scabra, Isolation, Structural elucidation, In silico studies, Cytotoxicity, PARP1 inhibitor

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**PTM01**

MMP-9/MMP-2 Inhibitor Sensitises Human Oesophageal Squamous Cell Carcinoma towards Cisplatin and 5-Fluorouracil in Different Treatment Models

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**Introduction:** Oesophageal squamous cell carcinoma (ESCC) is an aggressive type of oesophageal cancer in Asia, with a high mortality rate due to the occurrence of chemoresistance. Upregulation of the endopeptidases matrix metalloproteinase (MMP)-9 and MMP-2 are known to contribute to chemoresistance. MMP-9 and MMP-2 overexpression was observed in ESCC. However, their roles in ESCC chemosensitivity are still elusive. This study evaluated the effect of MMP-9/MMP-2 inhibition on the chemosensitivity of ESCC cells towards cisplatin (CDDP) and 5-fluorouracil (5-FU) using co-treatment and pre-treatment models. **Methods:** ESCC cell line (EC109) was treated with different concentrations of CDDP, 5-FU or MMP-9/MMP-2 inhibitor (MMP-9/MMP-2i) to identify their half-maximal inhibitory concentration (IC50) using MTT assay. Using the IC50 of MMP-9/MMP-2i, co-treatment was conducted with the same concentration range of CDDP and 5-FU for 3, 6 and 24h. In pre-treatment model, MMP-9/MMP-2i (IC50) was added to the cells for 3 or 6 h before treating with the same concentration range of CDDP and 5-FU. **Results:** Inhibition of MMP-9/MMP-2 activity enhanced EC109 chemosensitivity towards CDDP and 5-FU, except for CDDP in co-treatment model. In the co-treatment model (24h), MMP-9/MMP-2i decreased the IC50 of 5-FU from >80 μM to >80 μM. In the pre-treatment model, the IC50 of CDDP was also reduced from 40 μM to 20 μM at 3 h pre-incubation and from 40 μM to 5 μM at 6 h pre-incubation. Meanwhile, IC50 of 5-FU remained at 1000 μM at 3 h pre-incubation but was reduced to 50 μM at 6 h pre-incubation. **Conclusion:** The inhibition of MMP-9/MMP-2 activity induces EC109 chemosensitivity towards CDDP and 5-FU in the pre-treatment and co-treatment models. These preliminary results indicate the potential use of MMP-9/MMP-2i to induce ESCC chemosensitivity and deserve further identification of the associated molecular mechanisms.

**Keywords:** Oesophageal squamous cell carcinoma, Matrix metalloproteinase, Chemoresistance, Chemosensitivity

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**PTM02**

Effects of Nicotinamide and Nilotinib on Telomerase Activity and Telomere Length in K562 Myeloid Cell Line

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**Introduction:** Blast phase of chronic myelogenous leukaemia (CML) has continued to exist as a challenging disease even though the advanced tyrosine kinase inhibitor therapy has been introduced. We study the effect of nicotinamide, nilotinib on telomerase activity, telomere length and TERT expression in the K562 myeloid cell line as an approach to enhance the existing therapy for CML. Yet the role of nicotinamide in tumorigenesis is controversial. We hypothesized that nicotinamide would enhance the effects of Nilotinib on K562 cells, hence reducing tumour growth and/or promoting tumour cell death. **Methods:** K562 cell line was treated with nicotinamide, nilotinib and combination of both for IC50 assay. Detection of telomerase activity was carried out using TRAP assay, while qPCR assay was used for detection of telomere length and TERT expression. **Results:** This study has shown the effect of nicotinamide, nilotinib...
and both substances in exhibiting the anti-proliferation ability on K562 cell line after 48 hours. The implicated mechanism involved to induce such an effect are not yet clear. All treated samples exposed by nicotinamide, nilotinib and both have been assessed as telomerase-positive suggesting that all treatments were most likely not able to repress telomerase activity in K562 cells. Data results in longer telomere length in all groups of treatments except for nicotinamide that have a slight decrease of telomerase activity thus decrease in number of telomere length. Expression of TERT in this study suggests that the effect of these substances on telomerase activity is necessarily dependent on its expression. However, no study has been done to investigate the effect of nicotinamide in combination with nilotinib on telomerase and telomere regulation. Conclusion: The effects of nicotinamide and nilotinib on telomerase-telomere mechanisms may be linked to apoptosis which may give evidence for further investigation, notably in PARP-1 regulation. Keywords: Nicotinamide, Nilotinib, Telomerase, Telomere, CML

PTM03
Barriers and Challenges in Chemotherapy-Induced Nausea and Vomiting (CINV) Management: A Systematic Review
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Introduction: Barriers and challenges in chemotherapy-induced nausea and vomiting (CINV) management are essential and must be identified for stakeholders to achieve high-quality cancer care. Unfortunately, these components were not routinely addressed, and to date no systematic review has evaluated these aspects. This review aimed to systematically gather and appraise such evidence. Methods: Pubmed, Ovid, Scopus, Cochrane Library, Wiley Online, and Web of Science were searched using the following keywords: challenges, prevention, CINV, chemotherapy, and their alternative keywords’ equivalent. Studies involved adult cancer patients receiving chemotherapy exclusively, the caretaker, and healthcare professionals handling cancer patients were included. The studies must be in English language and original primary studies. Data extraction form was developed based on the PRISMA guide. The Joanna Briggs Institute (JBI) Critical Appraisal tool was used to assess the quality of the studies. Results: From 1,170 related articles retrieved, 37 articles were included in this review. The worldwide data were predominantly from European countries (9 articles) and United States of America (6 articles). Three qualitative study designs were included, and the rest of the included studies were mainly quantitative studies using questionnaires. All articles met the criterion of the JBI critical appraisal. Barriers and challenges particularly nausea and delayed CINV, failure to adhere to the antiemetic guideline, as well as misconception on CINV and its prevention were among the barriers and challenges reported in the management of CINV. Conclusion: Planning and implementing interventions to address the barriers and challenges identified may improve the quality of CINV management. Keywords: Chemotherapy-induced nausea vomiting, Barriers, Challenges, Systematic review
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PTM04
Cloning of KRAS Oncogene-Targeting Single-Guide RNA in a CRISPR/CAS9 System: A First Step in Generating a Non-Small Lung Cancer Cell Line with KRAS and EGFR Double Mutation
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Introduction: The concomitant presence of EGFR and KRAS mutations in non-small cell lung cancer (NSCLC) patients is relatively rare and their occurrence is believed to be mutually exclusive. However, with advancing detection technologies such as liquid biopsy and next-generation sequencing, approximately 10% of patients with dual concomitant mutations are detected, and this can affect the administration of either EGFR or KRAS tyrosine kinase inhibitors. In order to address this issue, we aimed at generating an isogenic cell line with a dual mutation of EGFR and KRAS. The bacterial type II clustered regularly interspaced short palindromic repeat (CRISPR) and CRISPR-associated (Cas) protein systems were being used to introduce a KRAS G12C mutation into a KRAS wildtype, EGFR-mutant T790M lung cancer cell line and it started with cloning the KRAS-specific single-guide RNA (sgRNA) into an expression plasmid, PX330. Methods: Briefly, to clone the KRAS-targeting sgRNA into PX330, the plasmid was digested with BbsI restriction enzyme. The top and bottom sgRNA sequences were annealed and phosphorylated with antarctic phosphatase enzyme. They were ligated with the open-end PX330 with T4 PNK enzyme and T4 Ligase to create a complete closed plasmid, followed by transformation into the chemically competent DH5a Escherichia. coli (E.coli) strain.
Following picking of the E. coli colonies, the plasmid was extracted and sent for Sanger sequencing to check for the presence of ligated sgRNA. **Results:** The KRAS-targeting sgRNA was successfully phosphorylated and ligated into an expression plasmid. In addition, it was successfully transformed into a bacterial host for cloning. The sequencing results showed that the top and bottom sequences of KRAS-targeting sgRNA were successfully cloned into PX330 expression plasmid. **Conclusion:** The present study showed that the molecular cloning of KRAS-targeting sgRNA was a success and completed the first step in generating a dual mutation isogenic cell line.

**Keywords:** Bacterial transformation, CRISPR-Cas9, KRAS G12C

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**PTM05**

**Identification of the Gene Mutation Profile of Obese-related Colorectal Cancer via TruSight Tumor 15 Analysis**

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**Introduction:** One of the most common risk factors for colorectal cancer (CRC) is obesity. Obese people are more prone to acquire CRC than non-obese people. However, it is unknown how obesity connects to CRC. CRC progression, on the other hand, has been attributed to solid tumor gene mutations such as KRAS, NRAS, BRAF, PIK3CA, AKT1, ERBB2, EGFR, and others. However, limited studies have connected such gene alterations to obesity-related CRC among Malaysians. The Illumina targeted gene panel TruSight Tumor 15 (TS15) determines 15 typical solid tumour genes using next-generation sequencing (NGS) technology. The purpose of this study is to identify the gene mutation profile of obese-related CRC using TS15 analysis. **Methods:** DNA was isolated from 12 formalin-fixed, paraffin-embedded (FFPE) tumor tissue samples from CRC patients. The DNAs were then sequenced using the NGS Illumina Miniseq platform after library preparation with a targeted panel TS15. BaseSpace Variant Interpreter was used for variant annotation. **Results:** This study identified an occurrence of mutations in TP53, KRAS, PIK3CA, ERBB2 and EGFR. TP53 demonstrated the highest frequency of gene mutation (75.0%, 9/12), followed by KRAS (66.7%, 8/12) and PIK3CA (25.0%,3/12). 11 variants were detected in TP53, 6 variants in KRAS, and 2 variants in PIK3CA. EGFR amplification was found in 41.7% (5/12) of CRC cases, whereas ERBB2 amplification was seen in 8.3% (1/12) of CRC cases. **Conclusion:** Overall, the findings reported here demonstrate that TS15-based targeted NGS on FFPE DNA offer a promising approach for detecting genetic mutations that might be employed in the future to diagnose CRC. To better understand the gene mutation profiles of obese-related colorectal cancer, the current findings will be linked to BMI categories and levels of apoB protein expression in the future.

**Keywords:** Colorectal cancer, FFPE, DNA, Gene mutations, Targeted next-generation sequencing

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**PTM06**

**Profiling of Autoantibodies as Biomarkers in Glioblastoma Multiforme (GBM) Patients**

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**Introduction:** Autoantibodies are circulating antibodies generated against self-antigens. In cancer, certain autoantibodies are produced based on cancer antigens that are present on the cancer cells. These antibodies have the potential to become minimally invasive diagnostic and prognostic biomarkers in cancer. In this study, we aim to profile the presence of autoantibodies in the sera of glioblastoma multiforme (GBM) patients. **Methods:** We obtained 20 serum of ten GBM patients and ten healthy participants from UMBI’s Biobank. We then subjected the profiling of autoantibodies using the i-OME antibody array by Sengenics Malaysia per the manufacturer’s instructions. The slides were scanned using the Agilent Scanner (USA) and the image data was extracted. Data analysis was performed using the Loess normalization and fold change analysis. **Results:** After the normalization, we performed a cut-off analysis at >1.5 or < -1.5 fold change. With these criteria, we obtained 20 upregulated and 2 downregulated autoantibodies. The topmost upregulated autoantibodies include PDCL3, CRYAB, ENO2, HSP90AA1 and CT47A1. Whereas the two downregulated autoantibodies are ASNA1 and FIP1L1. **Conclusion:** Based on our analysis, certain antibodies, such as HSP90AA1 and KRT19 have the potential to become minimally invasive biomarkers for GBM as shown by the ROC analysis.

**Keywords:** Tumor antigens, Brain cancer, Biomarker, Liquid biopsy

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**PTM07**

**The Hypoxia Model using Dimethyloxalylglycine (DMOG) Promoted the Migration and Invasion of HCT116 Colon Cancer Cells**

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Introduction: Hypoxic microenvironments are one of the primary causes of metastasis. Hypoxia inducible factor-1 alpha (HIF-1α) protein levels increase in hypoxic environments and may give rise to vascularization, cytoskeletal reorganization, and epithelial-to-mesenchymal transformation (EMT) of the cancer cells. Many studies evaluate the effect of hypoxia in the laboratory using hypoxic workstations, hypoxia chambers or hypoxic incubators. Alternatively, hypoxic conditions can be induced using dimethyloxalylglycine (DMOG), as the hypoxia mimetic agent in a cell culture model. Therefore, this study aims to determine the effects of hypoxia induced by DMOG on colon cancer metastasis, particularly in terms of cell migration and invasion.  

Methodology: To characterise the molecular background of the hypoxia response, total proteins were isolated from HCT116 colon cancer cells after 6, 24, and 48 hours of hypoxia induction with 1 mM DMOG. Using western blot, the expression of HIF-1α proteins was measured at each time point. The HCT116 cells were then subjected to wound healing assays and transwell invasion assays to investigate the effects of hypoxia on cell migration and invasion capacity. Images and data were statistically analysed using ImageJ and GraphPad Prism software version 9.0.1.  

Results: The expression of HIF-1α protein increases after 6 hours of DMOG induction and remains within 24 hours. The migration percentage of hypoxic cells is significantly increased compared to normoxic cells at the 6 hour (p<0.005) and 24 hour (p<0.05) time points. The transwell invasion assay demonstrated that, within 24 hours, hypoxic cells had significantly higher invasive capabilities (p<0.001) than normoxic cells. Thus, the increase in migration and invasion of the cells is similar to the increase in HIF1-α expression at 6 and 24 hours.  

Conclusion: The current data suggest that DMOG induction induces HIF-1α expression, which results in an increase in cell migration and invasiveness in colon cancer cells. Therefore, this model can be used to evaluate the effects of hypoxia on cells in combination with gene knockdown or drug treatment studies.  

Keywords: Colon cancer, Hypoxia, HIF-1α, Migration, Invasion  

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PTM08

46,XY Female Presenting With Dysgerminoma, Gonadoblastoma And Serous Cystadenoma With Androgen Receptor Mutation: A Swyer Syndrome Coexisting With Androgen Insensitivity Syndrome

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Introduction: Swyer syndrome is characterized by female phenotype, complete gonadal dysgenesis with the existence of Mullerian structures specifically the uterus, fallopian tube, and vagina, meanwhile individual with androgen insensitivity syndrome (AIS) demonstrate Wolffian structures internally and presence of male gonads. Both syndromes fall under 46,XY disorders of sex development (DSD) and are at risk of developing gonadoblastoma. The coexistence of Swyer syndrome and AIS occurring simultaneously is an extremely rare occasion, hence we present this case.  

Case report: 19-years-old, single, and nulliparous female presented with secondary amenorrhea at the age of 16-years-old. She attained menarche at the age of 12-years-old. She had one sister who had primary amenorrhea and passed away at the age of 22-years-old due to dysgerminoma. Clinically she is tall with height of 173 cm, normal female secondary sexual characteristics. Hormonal level demonstrated gonadal insufficiency with markedly elevated FSH and LH, and low estradiol and normal testosterone. MRI pelvis revealed small uterus with presence of gonad at right adnexal region. Karyotype confirmed as 46,XY in two occasions. SRY gene were detected using Fluorescence In Situ Hybridisation (FISH) method. Androgen mutation analysis revealed mutation in exon 1 [NG_009014.2:g.6286_6288GC dup(GCA)]. Laparoscopic gonadectomy were performed and the right gonad was confirmed as dysgerminoma with gonadoblastoma and serous cystadenoma.  

Discussion: To the best of our knowledge this is the first case of coexistence of Swyer and AIS syndrome reported. This case demonstrates to us how crucial the role of karyotype analysis in a situation of a young female with amenorrhea which can give us diagnostic clue and further targeted management. This case may raise a suspicion of possibility of familial or inherited condition which need further evaluation. DSD should be co-managed by multidisciplinary team.  

Keywords: Swyer syndrome, Androgen insensitivity syndrome, 46,XY female, Dysgerminoma, Gonadoblastom  

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PTM09

ADAR1 Dependency in Oral Squamous Cell Carcinoma

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Introduction: Our recent genome-wide CRISPR knockout screen revealed that the Adenosine deaminase acting on RNA-1 (ADAR1) gene is essential for the survival of a majority of oral squamous cell carcinoma (OSCC) cell lines. Given that deleting ADAR1 caused severe lethality in OSCC, targeting ADAR1 could have therapeutic benefits. ADAR1 catalyzes Adenosine-to-Inosine RNA editing, and suppresses dsRNA sensing-triggered cell death. ADAR1 has two isoforms, the constitutively expressed P110 and the interferon-inducible P150. It is also an interferon-stimulated gene (ISG) that is highly expressed in cancers including OSCC. In this study, we aimed to determine the molecular mechanisms underlying ADAR1 dependency which may afford an opportunity to develop better treatment strategies for OSCC. Methods: ADAR1 dependency is validated by competitive co-culture assay, apoptosis and colony formation assay using single-guide RNA (sgRNA) knockout in OSCC cell lines (ORL-48, ORL-214, ORL-174, ORL-195, SCC9 and BICR10). Changes in protein expression upon gene(s) knockout are determined by western blotting. Results: We confirmed that ADAR1 depletion significantly increased apoptosis by flow cytometry and inhibited colony formation in selected cell lines. For cell lines that are ADAR1-less dependent (BICR10 and ORL-174), IFN-β treatment increased the expression of ADAR1 and other ISGs, sensitizing these cells to cell death. Notably, overexpression of ADAR1-P150 but not ADAR1-P110, significantly rescued cell lethality in ADAR1 depleted cells suggesting that the ADAR1-P150 is important in the survival of OSCC. We also showed that cell death is mediated by dsRNA sensors protein kinase R (PKR) and melanoma differentiation-associated protein 5 (MDA5) whereby knocking-out either one of these genes did not reverse cell lethality, but co-deleting both genes partially rescued cell death in ADAR1-dependent cell lines. Conclusion: The present findings revealed that the interferon-inducible P150 of ADAR1 is essential for OSCC survival. Activation of the dsRNA-sensing pathways such as PKR and MDA5 underlies this dependency.

Keywords: ADAR1 dependency, dsRNA sensing, CRISPR knockout, Interferon-stimulated gene, OSCC

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PTM10

Induction of in vitro Cytotoxicity in High-Risk Oral Leukoplakia Using A Cancer Vaccine

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Introduction: Patients with oral leukoplakia diagnosed with moderate-severe oral epithelial dysplasia (OED) have an increased risk of developing oral cancer. However, chemoprevention agents targeting epithelial cells are ineffective in preventing malignant transformation and disease recurrence. Emerging evidence indicates that host immunity can impact premalignant disease progression, but the immune profile of oral leukoplakia has not been studied. In this study, we characterized the immune profile of high-risk oral leukoplakia with a long-term aim of identifying immunotherapeutic strategies to intercept cancer development. Methods: The immune profile of moderate-severe OED was determined by transcriptomic analysis of 125 immune signatures reported in cancer. To determine the utility of a cancer vaccine targeting MAGED4B, we evaluated MAGED4B expression by immunohistochemistry on oral leukoplakia tissues. Then, in-vitro T cell-based immunogenicity studies were performed using patients’ blood samples to evaluate antigen-specific immune responses. Results: Immune profiling demonstrated the induction of both immune surveillance and immune suppression mechanisms in moderate-severe OED. Notably, three distinct immune subtypes were identified: (1) immune cytotoxic; (2) non-cytotoxic; and (3) non-immune reactive. Patients progressed to cancer appear to lack cytotoxic T cells responses, suggesting that restoring T cell immunity via cancer vaccine may intercept malignant development. Next, we evaluated the feasibility of activating patients’ immune responses using a cancer vaccine targeting MAGED4B. We demonstrated that moderate-severe OED significantly over-expressed MAGED4B. Our T cell-based immunogenicity studies showed that
MAGED4B-specific CD8+ T cells could be expanded in-vitro and become activated as indicated by increased interferon gamma secretion and CD38 expression. Importantly, these CD8+ T cells demonstrated antigen-specific killing of tumour cells expressing MAGED4B. **Conclusion:** Our series of studies demonstrated that discrete immune responses are present in high-risk leukoplakia, and antigen-specific immune response can be harnessed to induce cytotoxic responses in these lesions.

**Keywords:** Oral leukoplakia, Immune profile, Oral epithelial dysplasia, Cancer vaccine