Malays J Pathol 2024; 46(1): 119 – 133

Abstracts of the 4th MK FMHS International Conference: Harnessing the Powers of Cell Therapy: Advances from Bench to Bedside, organised by the M. Kandiah Faculty of Medicine & Health Sciences, Universiti Tunku Abdul Rahman on 18-19 September 2023

Dr Wu Lien-Teh Memorial Lecture

The *In-vitro* Circle of Life - Using Embryonic Stem Cells to Build Human Reproductive Organoids

Kehkooi Kee[1,2]
1 Center for Stem Cell Biology and Regenerative Medicine, Department of Basic Medical Sciences, School of Medicine, Tsinghua University, Beijing, China; 2 The State Key Laboratory for Complex, Severe, and Rare Diseases; SXMU-Tsinghua Collaborative Innovation Center for Frontier Medicine; School of Medicine, Tsinghua University, Beijing, China

Germ cells perform many unique and fascinating mechanisms which are vastly different from somatic cells. Understanding mechanisms of human germ cell development is important for building basic knowledge and clinical treatments of infertility, genetic diseases, and tumourgenesis in human reproductive systems. However, genetic and molecular studies of human germ cell development are limited by the ethical and technical constraints to obtain the desired cell type and cell number to conduct molecular and cellular experiments. Realising that differentiating human embryonic stem cells (hESCs) to germ cell will provide a novel platform for studying human germ cell development and developing treatment reproductive medicine, we have developed several *in vitro* differentiation systems to study human germ cells at different developmental stages, including primordial germ cells (PGCs), oocytes and spermatids. Strategy and methodology of building the *in vitro* differentiation systems will be described. The differentiated systems have been utilised to investigate molecular mechanisms of human PGC development, causative effect of infertility mutations, and studying the microgravity of spaceflight on germ cell development.

Plenary Lecture

Cell Therapeutics: New Treatment Paradigms for Blood Disorders

John EJ Rasko, AO[1,2,3]
1 President 2018-20, International Society for Cell & Gene Therapy; 2 Professor, Sydney Medical School, University of Sydney; Head, Gene and Stem Cell Therapy Program, Centenary Institute; 3 Head of Department, Cell & Molecular Therapies, Royal Prince Alfred Hospital, Sydney, Australia

Advanced therapeutic medicinal products based on gene and stem cell therapies are increasingly being approved throughout the world. Immunotherapies including checkpoint inhibitors and CAR-T cells have captured the attention of many scientists, physicians and cancer sufferers. The convergence of substantial incremental technical advances towards combined cell and gene therapy has led to improved clinical outcomes in immune deficiencies, haemoglobinopathies, immunotherapies and other inherited diseases.

In the regenerative medicine field to be detailed in this talk, there is a pressing need to standardize cell manufacturing protocols for widespread clinical assessment and implementation. Strict compliance with government regulation and oversight is essential to maintain the safety of all therapeutic products. In 2020 we reported the first ever completed trial of iPSC-derived Mesenchymal stromal cells in Steroid-Resistant Acute GvHD and in now report the two year follow up. MSCs have been widely investigated as a treatment for graft versus host disease (GvHD), but with mixed results. Factors such as MSC donor variability and the effects of prolonged culture expansion may contribute to inconsistent or disappointing outcomes. The novel Cymerus™ manufacturing process facilitates virtually limitless production of well-defined and consistent MSCs from a single human iPSC bank, using clonogenic progenitor-based technology. This avoids both inter-donor variability, batch-to-batch variation and the need for prolonged *in vitro* expansion of MSCs.

In the area of *ex vivo* gene therapies, we have been the first site in the southern hemisphere pursuing lentivirus modified autologous haemopoietic stem/progenitor transplantation for transfusion dependent beta-thalassaemia (TDT) – the commonest human genetic blood disease. Although advances in red blood cell transfusion and iron chelation have improved the prognosis of patients with TDT, allogeneic haemopoietic stem cell transplantation has been the only curative therapy. Since 2011, we have been a foundation site with BlueBird Bio evaluating LentiGlobin gene therapy in patients with TDT. We have reported results from the completed phase 1/2 Northstar and phase 3 Northstar-2 studies, including 7-Year Post-Infusion Follow-up. From mid-2019 to 2022, Zynteglo was available for the treatment of patients 12 years and older with TDT as approved by the European Medicines Association and the US FDA approved this in 2023. Zynteglo (betibeglogene autotemcel), represents the first cell-based gene therapy for the treatment of adult and paediatric patients with beta-thalassemia who require regular red blood cell transfusions.

However, in parallel with objectively proven therapies, 'stem cell tourism' has become a billion-dollar industry with

increasing examples of false claims. These data should be of immediate concern to governments and ethicist being lobbied to amend laws governing the manufacture, distribution and clinical use of human cell-based medical products. Unregulated, untested or unsafe stem cell 'therapies' place the field at a difficult crossroad. Blurring the lines that distinguish evidence-based cell therapies from those that are not remains a fundamental public health concern.

The implementation of cell and gene technologies in the clinical setting have already provided enormous benefits to human health. Vigilant careful planning, ongoing research and longer-term data are needed to overcome current limitations across many therapeutic areas.

Pelareorep as an Enabling Technology for Both Chemotherapies and I–O Therapies, Including Checkpoint Inhibitors and Chimeric Antigen Receptors (CAR T) Therapy

Houra Loghmani[1], Richard Trauger, Matt Coffey[1], Richard Vile, Thomas Heineman
1 Oncolytics Biotech Inc. Calgary, Canada; 2 Oncolytics Biotech Inc. San Diego, USA; 3 Department of Molecular Medicine, Mayo Clinic, Rochester, USA

Pelareorep (pela) is a naturally occurring oncolytic reovirus, which has a double-stranded RNA (dsRNA) genome that selectively infects transformed cells. It promotes anti-tumour responses through the dual mechanisms of direct cell lysis and stimulation of tumour-directed innate and adaptive immune responses. The combination of productive infection leading to cell lysis and the introduction of dsRNA into tumour cells promotes the recruitment and activation of anti-tumour immune cells and remodels the tumour microenvironment through enhanced cytokine and chemokine expression, which in turn reverses the immunosuppressive environment of cold tumours. Pela is uniquely suited for clinical applications. It is not genetically modified and, therefore, can be administered in a chemotherapy suite without special precautions. In addition, it is transported to tumours bound to immune cells allowing it to evade neutralization in the blood, which permits intravenous administration in contrast to most other oncolytic viruses that must be given intratumorally. Prior studies support the clinical benefit of pela. In the phase 2 IND-213 trial, treatment with pela + paclitaxel resulted in a near doubling of overall survival in HR+/HER2- metastatic breast cancer patients compared to standard-of-care paclitaxel monotherapy. In the AWARE-1 window of opportunity trial, in which newly diagnosed breast cancer patients were treated with letrozole + pela +/- atezolizumab, pela activated T cells and enhanced their infiltration into tumours. In addition, pela increased the expression of PD-L1 by activating the interferon gamma signalling pathway, thus priming the tumour for checkpoint blockade therapy. Synergy with checkpoint blockade therapy was also observed in the GOBLET trial in first-line metastatic pancreatic cancer patients with a near tripling of the overall response rate for the pela and atezolizumab + chemotherapy arm compared to historical controls. Because of its ability to bind T cells that traffic to the tumour, another potential application of pela is as a partner in chimeric antigen receptor T cell (CAR T) therapy for solid tumours. In pre-clinical tumour models, pela-loaded CAR Ts exhibited enhanced expansion, longer persistence, and a more prominent memory phenotype. These expanded functions resulted, in part, from the expansion of dual-specific CAR Ts that are specific to both the CAR target as well as to pela-specific epitopes. This, in turn, allowed them to expand after a single IV pela boost. Accordingly, the combination of pela and CAR Ts has shown better efficacy than either pela or CAR T treatment alone in several pre-clinical tumour models, including pancreatic cancer, glioma and melanoma and warrants clinical investigation.

Symposium

Newcastle Disease Virus as a Therapeutic Vaccine Candidate Against Cancer

Khatijah Yusoff, FASc
National Institutes of Biotechnology Malaysia

The Newcastle disease virus (NDV) is an avian paramyxovirus which has a non-segmented negative stranded RNA genome. The virus infects poultry inducing several levels of pathogenicity. Interestingly, NDV does not pose any threat to humans in terms of pathogenicity, though it might trigger mild conjunctivitis and flu-like symptoms. Nevertheless, the virus has demonstrated a remarkable ability to selectively target and destroy human cancer cells, making it a highly promising therapeutic vaccine candidate for oncovirotherapy. This intrinsic feature of selectively lysing cancer cells with a high degree of specificity and sensitivity, leaving normal cells unharmed provides a strategic avenue to harness NDV's potential for cancer treatment. Multiple clinical trials attest to this potential. The mechanisms underlying its oncolytic prowess encompass two pathways: direct selective infection and killing of tumour cells, as well as indirectly through induction of specific host immune response acting against the tumour tissue. By manipulating the viral genome through reverse genetics, NDV can orchestrate the recruitment of immune cells towards cancer cells, thus enhancing the effectiveness of oncolysis. This dynamic development heralds an exciting and challenging frontier in cancer therapy and opens up new possibilities for leveraging the virus's potential to combat cancer while inflicting minimal harm to healthy cells. This innovative approach holds great promise for advancing cancer treatment and offers hope for improved therapeutic outcomes.

Vaccine for Head and Neck Cancers

Kue Peng Lim[1*,] Syafinaz Zainal[1], Natasha Zulaziz[1], Chai Phei Gan[1], San Jiun Chai[1], Chuan Wang[2], Christian Ottensmeier[2,3], Emma King[2], Gareth Thomas[2], Natalia Savelyeva[2,3] and Sok Ching Cheong[1]
1 Cancer Research Malaysia, 47500 Subang Jaya, Malaysia; 2 Cancer Sciences, University of Southampton, UK; 3 Head and Neck Centre, Institute of Systems, Molecular and Integrative Biology, University of Liverpool, UK
*Corresponding author: kuepeng.lim@cancerresearch.my

The clinical benefit of immunotherapies relies heavily on the ability of T cell to identify antigens presented by tumour cells. As checkpoint blockade therapy has limited efficacy, tumour antigens have the potential to be exploited as a complementary treatment to this therapy. Our team identified MAGED4B and FJX1 to be overexpressed in head and neck cancers and promote tumour growth. Subsequent studies confirmed the immunogenic nature of these two tumour-associated antigens (TAAs) by demonstrating the presence of inherent antigen-specific T cells and the ability of these antigens to stimulate T cell expansion *ex vivo*. Full length DNA of these two TAAs was used to develop vaccine in the form of DNA plasmid. Encouragingly, this novel approach shown to be efficacious in controlling tumour growth *in vivo* and tumour inhibition is further enhanced when the cancer vaccine is used in combination with checkpoint blockade therapy. A bioinformatics study suggested our vaccine works to stimulate antigen presentation and hence augment T cell responses. This observation was confirmed when cancer vaccine-trained T cells successfully restored the antigen presentation in nasopharyngeal cancer cell lines that have compromised antigen presentation. As checkpoint blockade therapy works by re-invigorating CD8 T cells, the ability to restore antigen presentation can complement its efficacy, especially in patients who have downregulated antigen presentation.

Engineering Second, Third, and Next Generation CAR-T Cells

Vita Golubovskaya[1,*], John Sienkiewicz[1], Alan Zhang[1], Jinying Sun[1], Yanwei Huang[1], Liang Hu[1], Hua Zhou[1], Hizkia Harto[1], Shirley Xu[1], Robert Berahovich[1], Walter Bodmer[2] & Lijun Wu[1,3]
1 Promab Biotechnologies, 2600 Hilltop Drive, Richmond, CA 94806; 2 Cancer & Immunogenetics Laboratory Weatherall Institute of Molecular Medicine, John Radcliffe Hospital Oxford, UK, OX3 9DS; 3 Forevertek Biotechnology, Janshan Road, Changsha Hi-tech Industrial Development Zone, Changsha, Hunan 410205, China

Chimeric antigen receptor (CAR) T cell therapies such as CD19-CAR-T cells and BCMA-CAR-T cells have recently been approved by the FDA to treat lymphoma and multiple melanoma. Novel CAR-T cell therapies need to be developed to target resistant or recurrent haematological cancers as well as solid tumours. Novel second, third generation and next-generation CAR-T cells were developed and functionally validated, called bispecific CAR-T cells, that simultaneously targeted two tumour antigens such as CD19-CD37 and CS1-BCMA. In addition, next-generation Her-2-CAR-T cells which secreted GM-CSF or CCL-2 cytokines effectively blocked SKOV-3 ovarian tumour growth *in vivo*. In addition, several T cells engaging bispecific antibodies were designed that effectively killed solid tumours. Recently, a CAR mRNA-LNP platform has been developed to create functional CAR-NK cells. Moreover, mRNA-LNPs encoding an EpCAM-CD3 hFc bispecific antibody were also delivered intratumorally, effectively blocking colon tumour xenograft growth in mice. These novel CAR-T cell therapies, and mRNA-LNP applications for developing CAR-NK cells and bispecific antibodies can be used for future clinical trials.

DC Vaccines for Solid Tumours

Herbert Schwarz
Yong Loo Lin School of Medicine, National University of Singapore

Cancer immunotherapy has finally reached a stage where it is able to prolong patient survival. Immune checkpoint inhibitors and chimeric antigen receptors can be very successful in the treatment of haematological malignancies but their efficacy for solid cancers is limited. Cancer vaccination offers the possibility to induce effective immune responses also against solid cancers. This talk will give an overview on the different available cancer vaccination methods. We developed a cancer vaccine based on a new type of dendritic cell (DC), that are being generated by a CD137 ligand (CD137L) agonist, and that display enhanced potency. In a phase I study, 12 nasopharyngeal carcinoma (NPC) patients were administered CD137L-DC that were pulsed with Epstein-Barr virus (EBV) antigens. Treatment was well tolerated. One partial response (PR) was obtained, and 4 patients are still benefitting from a progression free survival (PFS) of currently 4 years. Patients with clinical benefit had lower plasma EBV DNA levels, and a reduction after vaccination, indicating that this vaccine induces an anti-EBV and anti-NPC immune response, and warranting further studies.

MSCs in GVHD Management

Sze-Piaw Chin[1,2]
1 Cytopeutics Sdn. Bhd., Cyberjaya, Selangor, Malaysia; 2 M. Kandiah Faculty of Medicine & Health Sciences, Universiti Tunku Abdul Rahman, Malaysia

Mesenchymal stem cells (MSCs) have attracted attention for their immunomodulation property that is achieved through the release of various mediators in response to injury with subsequent tissue regeneration. We have demonstrated the therapeutic potential of Cytopeutics® MSCs in various ischemic and inflammatory disorders such as ischemic stroke, diabetes and acute graft-versus-host disease (aGVHD). aGVHD is a devastating complication of bone marrow transplant for lymphoma and leukaemia with up to 50% 1-year mortality. Survival appears to be correlated with promptness and completeness of response to initial therapy. In 2016, Japan had approved the use of allogenic mesenchymal stem cell for children with aGVHD refractory to steroid treatment. MSC therapy improved overall response but mortality rate was still high. In 2018 Cytopeutics along with senior haematologists in Malaysia decided to explore the upfront use of Cytopeutics® MSCs in combination with standard treatment instead. The study was approved by the MREC and NCERT. We obtained the precedent CTX from the NPRA under the new enforcement of CGTP regulations. The study was jointly sponsored by the MOSTI Smartfund. This ambitious double blind randomised placebo-controlled phase I-II clinical trial began at MOH Ampang Hospital and later extended to Sunway Medical Centre, Hospital Universiti Kebangsaan Malaysia and Hospital Sultanah Aminah Johor. The lecture today will provide the interim results of the study which demonstrated faster and sustained complete response with better overall, relapse-free and disease-free survival in aGVHD patients with the upfront use of Cytopeutics MSCs.

BM-MSCs in Stroke Management

Law Zhe Kang
Faculty of Medicine, Universiti Kebangsaan Malaysia

Mesenchymal stem cells had been considered a promising treatment for patients with ischaemic stroke. We conducted a phase 2, single-centre, assessor-blinded randomised controlled trial to investigate the safety and efficacy of intravenous autologous bone marrow-derived MSCs (BMMSCs) in patients with subacute middle cerebral artery (MCA) infarct, which is now published in Cytotherapy. We recruited 17 patients with severe ischemic stroke involving the MCA territory within 2 months of stroke onset. Using permuted block randomisation, 9 patients were assigned to receive 2 million BMMSCs per kilogram of body weight (treatment group) and 8 standard medical care (control group). All patients were severely disabled following their MCA infarct (median mRS = 4.0 [4.0 5.0], BI = 5.0 [5.0 25.0], NIHSS = 16.0 [11.5 21.0]). The baseline infarct volume on the MRI was larger in the treatment group (median, 71.7 [30.5 101.7] mL versus 26.7 [12.9 75.3] mL, P = 0.10). There were no between-group differences in median NIHSS score (7.0 versus 6.0), mRS (2.0 versus 3.0) or BI (95.0 versus 67.5) at 12 months. At 12 months, there was significant improvement in absolute change in median infarct volume, but not in total infarct volume, from baseline in the treatment group (P = 0.027). No treatment-related adverse effects occurred in the BMMSC group. In conclusion, intravenous infusion of BMMSCs in patients with subacute MCA infarct was safe and well tolerated. Although there was no neurological recovery or functional outcome improvement at 12 months, there was an improvement in the absolute change in median infarct volume in the treatment group. In this talk, other trials of stem cell therapies in ischaemic stroke will also be discussed. The design of an ongoing randomised controlled trial on allogenic MSCs for ischaemic stroke will also be shared.

Breathing New Life: Exploring Extracellular Vesicles as Therapeutic Agents in Respiratory Diseases

Badrul Hisham Yahaya*, Lian Jie, Noridzzaida Ridzuan, Nur Shuhaidatul Sarmiza Abdul Halim, Egi Kardia, Syahidatulamali Che Shaffi
Lung Stem Cell and Gene Therapy Group, Department of Biomedical Sciences, Advanced Medical and Dental Institute (IPPT), SAINS@BERTAM, Universiti Sains Malaysia, Penang, Malaysia
*Corresponding author: badrul@usm.my

Over the past decade, there has been an industrial expansion, and patient interest surrounding stem cell-based interventions. This heightened attention has also given rise to a growing number of direct-to-consumer enterprises offering stem cell "therapies" across various medical conditions, often with limited empirical evidence substantiating their safety and efficacy. Concurrently, the utilisation of stem cell secretomes and/or extracellular vesicles (EVs) as a viable alternative to stem cell transplantation has gained prominence within the realm of regenerative medicine. Currently, multiple clinical trials are underway to evaluate the safety and effectiveness of these agents. Nevertheless, this burgeoning field has not been immune to opportunistic businesses and private clinics, which are capitalising on the trend by offering secretome/extracellular-based interventions despite the paucity of supporting data. One specific area where stem cell-based approaches are receiving considerable attention is chronic obstructive pulmonary disease (COPD). Secretomes and EVs, released by a variety of cell types, play a pivotal role in paracrine and extracellular communication. Recent breakthroughs in this field have brought to light the therapeutic potential of stem cell-derived EVs, demonstrating their comparability to the parent cells in terms of efficacy. This study presents a novel exploration into the molecular mechanisms through which extracellular vesicles, derived from mesenchymal stem cells (EV-MSCs), enhance pulmonary inflammatory injury. Our findings indicate that MSC-derived EVs exhibit a remarkable capacity to mitigate inflammation induced by COPD. These discoveries open the

door to a promising avenue of research, suggesting that EVs could serve as a novel cell-free therapeutic approach for the treatment of respiratory diseases. This research not only contributes to our understanding of the intricate interplay between stem cell-derived EVs and respiratory disease but also underscores the potential of EV-based therapies as a groundbreaking advancement in regenerative medicine. As we delve deeper into this uncharted territory, it becomes increasingly evident that the utilisation of EVs holds immense promise, offering a new dimension in our quest to alleviate the burden of respiratory diseases and potentially revolutionise treatment strategies for various other medical conditions.

New Applications of Cord Blood

Zalina Mahmood
National Blood Centre, Kuala Lumpur, Malaysia

Umbilical Cord Blood (UCB) contains relatively heterogeneous cell populations including haematopoetic stem cells (HSC), mesenchymal stem cells (MSCs), multipotent adult progenitor cells, unrestricted somatic stem cells, endothelial progenitor cells and immature immune cells. These cells are capable of giving rise to hematopoietic, epithelial, endothelial, neural and other tissues. Thus, it has a potential to treat a wide variety of diseases including cardiovascular, ophthalmic, orthopaedic, neurological and endocrine diseases. Among all, the most commonly studied was neurological disorders such as cerebral palsy (CP), hypoxic ischaemic encephalopathy (HIE) and autism spectrum disorder (ASD). Other disorders include diabetes mellitus, cardiac and vascular diseases as well as hepatic diseases. Even though many preclinical and initial clinical (safety and feasibility) studies are quite convincing, the lines of investigation are still in the early stages as evidenced by the fact that the majority of the clinical studies are Phase 1 or combined Phase 1/2.

Generation of Red Cells from iPSC and Erythroid Cell Lines

Becky Griffiths
Australian Red Cross, Australia

Fully functional cultured red blood cells (cRBCs) can be grown in the laboratory from haematopoietic stem cells (HSCs) isolated from adult peripheral blood, cord blood or bone marrow. There are numerous advantages of cRBCs over donated RBCs: (1) Greater transfusion efficacy due to increased lifespan in comparison to donor RBCs, (2) Reduced immunisation risk for those who have rare blood group antigens or are multi-transfused, (3) Minimisation of infection risk and (4) Constant availability due to stem cell banks. However, primary HSCs have a finite proliferative capability and are technically challenging to genetically manipulate. Alternative stem cell sources for RBC production which are both sustainable and genetically malleable such as iPSC and immortalised erythroid cell lines have been developed and continue to be explored yet fail to recapitulate erythropoiesis as well as primary derived HSC stem cell sources. The benefits and challenges of iPSC and erythroid cell line derived RBCs will be considered and discussed.

iPSC Banking from Cord Blood Sources

Ngaire Elwood[1,2,3], Keren Abberton[1,2], Junann Whish-Wilson[1,2], Kawa Choi[1,2], Denise Tsoutras[1,2], Trish McDonald[1,2], Pei Tian[1,2]
1 BMDI Cord Blood Bank, Melbourne, Australia; 2 Murdoch Children's Research Institute, Melbourne, Australia; 3 Department of Paediatrics, University of Melbourne, Melbourne, Australia

Induced pluripotent stem cells (iPSCs) can be differentiated into any cell in the body. Banked cord blood (CB) is an ideal source of starting material for the creation of iPSCs, with many advantages not found in other cell sources. An iPSC bank derived from CB selected on the basis of human leukocyte antigens (HLA) homozygosity could form the basis of future cell-based therapies. The BMDI Cord Blood Bank (CBB) in Melbourne is a government-funded public cord blood bank. The bank holds a Good Manufacturing Practice (GMP) cell-manufacturing licence from the Therapeutic Goods Administration (TGA) and is internationally accredited by the Foundation for the Accreditation of Cellular Therapy (FACT). Our aim is to create and manufacture a bank of GMP-compliant CB-derived iPSCs of HLA homozygous haplotypes for potential therapeutic use. We have developed a process for reconsent of CB donors to use a portion of their banked CB to create iPSC lines for potential new cellular therapies (https://doi.org/10.1093/stcltm/szac060). We have developed methodology to create "GMP-like" iPSCs from banked CB (https://www.frontiersin.org/articles/10.3389/fcell.2022.835321/full). This technology has now been transitioned from the research lab to be GMP-compliant, mimicking the Quality Systems in place for the CBB. We have established an Institutional Biosafety Committee (IBC)-approved PC2 laboratory co-located within the GMP-compliant CBB facility. In addition to the physical space, we have developed a Quality Systems framework, with processes in-line with the FACT Common Standards for Cellular Therapy, leveraging off those in place for the CBB. A full mock run has been completed to test and validate the process in its entirety, resulting in the creation of new CB-derived "GMP-mock" iPSC lines. We are now in the process of creating our first fully GMP-compliant HLA homozygous CB-derived iPSC line. Collaborations have been established for pre-clinical studies to use our CB-derived iPSC lines for therapies directed towards retinal and neurological repair, NK and CAR-NK immunotherapies. Proof of principle has been established for a GMP-compliant CB-derived iPSC bank, co-located and leveraging the BMDI CBB. Production of iPSC lines for potential clinical use extends the utility of the public CBB inventory, and value-adds to the altruistic donations of those who donate this precious resource.

Exploring the Impact of T-Cell Exhaustion on CAR-T Cell Therapy

Leow Chiuan Yee
School of Pharmaceutical Sciences, Universiti Sains Malaysia, Pulau Pinang, Malaysia

Tumour immunotherapy has emerged as a promising therapeutic approach, with cytotoxic T cells playing a pivotal role in combating cancer. Among these, chimeric antigen receptor T-cell (CAR-T-cell) therapy has revolutionised haematological cancer treatment. Despite the success of CAR-T-cell therapy in haematological cancers, the exhaustion of T cells, particularly in solid tumours, presents a challenge. The interplay between "stimulatory" and "suppressive" signals regulating immune responses is disrupted in cancer and pathogenic invasion, with the programmed-cell-death-1 (PD-1) receptor and its ligands (PD-L1 and PD-L2) contributing to immune suppression. Intriguingly, PD-L2's interaction with galectin-9 (GAL9) unfolds as a key regulatory mechanism. GAL9's elevated expression on activated immune cells forms lattice-raft structures, fostering dense immune molecule clustering. Innovative approaches utilising multimeric PD-L2 protein and an anti-GAL9 antibody stabilise the lattice-raft, enhancing co-stimulatory molecule expression, TNF secretion, and reducing inhibitory molecules. The anti-GAL9 antibody demonstrates promise *in vitro* and in pre-clinical models, holding potential as a multifaceted immunotherapeutic strategy to counter T-cell exhaustion and reinforce CAR-T-cell therapy outcomes for solid tumours.

Cytokine-Induced Killer (CIK) Cells and Dendritic Cell (DC) vaccines Have Emerged as Promising Tools in Cancer Immunotherapy

Ho Gwo Fuang
Faculty of Medicine, University Malaya, Malaysia

Cytokine-Induced Killer (CIK) cells exhibit MHC-unrestricted killing. CIK cells have a unique ability to target and eliminate tumour cells without requiring direct recognition of specific MHC-peptide complexes, due to the expression of both NK (natural killer) cell receptors and T cell receptors on CIK cells, enabling them to recognize stress-induced molecules or other factors on the surface of tumour cells. Because MHC-unrestricted killing does not rely on the presence of specific MHC-peptide complexes, it offers potential advantages in cancer immunotherapy, as it can target a broader range of tumour cells and might bypass some immune evasion mechanisms employed by tumours. Dendritic cells, the sentinels of the immune system, play a pivotal role in orchestrating immune responses. DC vaccines involve loading these cells with tumour antigens to enhance antigen presentation and T-cell activation, thereby priming a targeted anti-tumour response. Combining CIK cells with DC vaccines synergistically amplifies the immune cascade, bolstering both innate and adaptive immunity against malignancies. The dynamic interplay between CIK cells and DC vaccines may generate synergistic effect in cancer immunotherapy. By harnessing the inherent strengths of both approaches, a better anti-cancer immune response can be achieved, paving the way for more effective and tailored therapeutic strategies in oncology.

Managing CAR-T Complications

Michaela Seng
Department of Paediatric Haematology/Oncology, KK Women's and Children's Hospital, Singapore

Recognising and managing toxicities encountered by patients following CAR-T cell therapy are critical to successful patient outcomes. The lecture will provide an overview of CAR-T associated adverse events, and the set up required in a paediatric CAR-T programme. Using case studies primarily in paediatrics, we will discuss the medical management and interventions employed to address these complications, emphasising the importance of vigilant monitoring and prompt intervention to optimise patient outcomes. Through this lecture, healthcare professionals will gain valuable insights into the nuances of post-CAR-T complications and their management. The presentation aims to foster meaningful discussions and knowledge exchange among attendees, empowering them to provide high-quality, patient-centred care in the realm of cell and gene therapies.

Cell Therapy: Roles and Functions of Nurses in Navigating the Patient Through the Treatment Process

Choong Jye Yi
Cancer Centre, Sunway Medical Centre, Kuala Lumpur, Malaysia

Cell therapy is a rapidly evolving treatment modality that offers a new hope for patients with a variety of diseases and conditions. As cell therapy becomes increasingly available to patients, nurses play a critical role in guiding patients through the treatment process. The roles and functions of nurses are navigating patients through the cell therapy treatment process, including patient education, assessment, and coordination of care. There are many unique challenges and opportunities presented by cell therapy, including issues related to patient selection, adverse events, and long-term follow-up. Patient education in the context of cell therapy, including the need to inform patients about the potential benefits and risks of treatments as well as the need for close monitoring and follow-up care, is important. Nurses plays a critical role in facilitating communication and collaboration among members of the healthcare team, including physicians, pharmacists, and other healthcare providers. In addition, nurses also need to address the ethical and legal considerations that arise in the context of cell therapy, including issues related to informed consent, privacy, and confidentiality. Ultimately, nurses play a critical

role in the successful implementation of cell therapy. By providing patients with high-quality care and support throughout the cell therapy treatment process, nurses can help optimize patient outcomes and improve the overall quality of care for patients undergoing this promising treatment modality.

Stem Cell Transplantation Treatment: Specific Population with Financial Implication

Seery Zaliza Azura binti Zaider
Hospital Canselor Tuanku Muhriz - UKM Medical Centre, Kuala Lumpur, Malaysia

Haematopoietic stem cell transplantation (HSCT) is a high intensity procedure with the intention of curing acute leukaemia, high grade or relapsed lymphoma, multiple myeloma and benign haematological diseases such as thalassaemia in both paediatric and adult patients. It is categorised according to the source of stem cells: autologous (patient's own stem cells), allogeneic (related or unrelated HLA-matched donor, related and unrelated HLA-mismatch donor) or haploidentical family donors. Since the first procedure in 1987, HSCT services are now widely offered in thirteen Malaysian healthcare providers, i.e., government subsidised public hospitals and university hospitals with partial funding from the Ministry of Education and private institutions. HSCT procedures involve a complex coordination between clinicians, diagnostic laboratory, stem cell laboratory, stem cell registry and pharmacy. In addition, financial hardships incurred to patients often becomes a limiting factor for a timely transplant. In cases of allogeneic unrelated donors, the cost of HLA typing and stem cell procurement are individually funded although the actual transplant itself is heavily subsidised, especially in government institutions. The government's HSCT fund assistance offers care package inclusive of the first 100 days, however this often excludes the costs of non-formulary drugs, including immunoglobulin and new generation antifungal or antiviral prophylaxis. Most patients often required time off from work upon the initial diagnosis of cancer. In addition, the majority of patients have already exhausted a large amount of savings and / or insurance at initial disease work-up investigations and during hospitalisations for induction, consolidation, or re-induction chemotherapies. In view of segregation of facilities between the three major healthcare sector providers in Malaysia, access to HSCT services and subsequent patients' eligibility for funding assistance are subjected to a thorough socio-economic evaluation by the stem cell coordinator and hospital social worker. In general, the cost of HSCT in public institution is estimated at RM 50,000 (USD 15,000) while the cost is higher at RM200,000 (USD 50,000) in private institutions. Financial hardships experienced by HSCT patients, especially those with a lower socio-economic status, those with no regular income, and those with limited to no insurance coverage, have unfavourable impacts on their long-term survival outcomes. They are less likely to adhere to post-transplantation treatment regimens due to difficulty paying hospital bills or to be less compliant with outpatient appointment schedules due to travel costs. Unfortunately, there is no easy and immediate solution to address this issue. However, current efforts from both government agencies and non-governmental organisations may be improved further by rising awareness and health education in the community. Innovative financial hardship screening tools and subsequent models for access to government assistance must be improvised to better support those high-risk patients so that they are not discouraged from having access to this potentially life-saving procedure.

MSC and Cartilage Tissue-derived Extracellular Vesicles to Treat Osteoarthritis

Chiew Yong Ng[1], Min Hwei Ng[1], Ying Yang[2], Jhi Biau Foo[3], Chee Wun How[4], Kien Hui Chua[5], Nor Hamdan bin Mohamad Yahaya[6], Jia Xian Law[1]
1 Centre for Tissue Engineering and Regenerative Medicine, Faculty of Medicine, National University of Malaysia, Kuala Lumpur, Malaysia; 2 School of Pharmacy and Bioengineering, Keele University, Stoke-on-Trent, United Kingdom; 3 School of Pharmacy, Faculty of Health and Medical Sciences, Taylor's University, Kuala Lumpur, Malaysia; 4 School of Pharmacy, Monash University Malaysia, Kuala Lumpur, Malaysia; 5 Department of Physiology, Faculty of Medicine, National University of Malaysia, Kuala Lumpur, Malaysia; 6 Department of Orthopaedic and Traumatology, Faculty of Medicine, National University of Malaysia, Kuala Lumpur, Malaysia

Extracellular vesicles (EVs) are membrane-bound vesicles secreted by cells. EVs are rich in biological molecules, including nucleic acids, proteins and lipids, that are known to promote tissue regeneration, including the cartilage tissue. As the content of the EVs varies according to its parent cells, it is important to compare the functionality and efficacy of different sources of EVs in supporting cartilage repair in order to identify the optimal source of EVs for the treatment of cartilage injury. In this study, experiments were performed to compare the efficacy of human umbilical cord mesenchymal stem cell (UC-MSC)-derived EVs (MSC-EVs) and human cartilage tissue-derived EVs (cartilage-EVs) in promoting cartilage regeneration. EVs were collected from passage five UCMSCs and partially digested cartilage tissue using the ultrafiltration and tangential flow filtration methods. The isolated EVs were characterised using the nanoparticle tracking analysis, bicinchoninic acid assay and Western blot in accordance to the MISEV2018 recommendation. Then, the effects of EVs on chondrocyte viability, proliferation, migration and extracellular matrix (ECM) gene expression were analysed. Results showed that the size of MSC-EVs and cartilage-EVs were 85.1 ± 1.4 nm and 95.8 ± 0.6 nm, respectively. The MSC-EVs were positive for CD63 and HSP70 as well as negative for GRP94 whilst cartilage-EVs were negative for all these markers. The EVs were readily uptake by the chondrocytes. The MSC-EVs were found to increase the chondrocyte proliferation but did not influence the migration and ECM gene expression. On the other hand, cartilage-EVs increased the gene expression of type II collagen and cartilage oligomeric matrix protein but demonstrated no effect on chondrocyte proliferation and migration. These findings indicated that the functionality of EVs varies according to its cell origin, and this is likely due to differences in the EV's cargo. Thus, it is important to characterise the protein and nucleic acid contents of EVs to understand its functionality and mechanism of action. Based on the findings, it is also postulated that combination of MSC-EVs and cartilage-EVs might be more efficient in promoting cartilage regeneration.

The Use of Platelet-derived Extracellular Vesicles for Musculoskeletal Tissue Regeneration: From Basic Research to Clinical Outcome

Pan Pan Chong[1,*], Nur Hidayah Hassan[1,2], Jonas Fernandez[1], Shani Samuel[1], and Tunku Kamarul[1]
1 National Orthopaedic Centre of Excellence for Research and Learning (NOCERAL), Department of Orthopaedic Surgery, Faculty of Medicine, Universiti Malaya, Kuala Lumpur, Malaysia; 2 Institute of Medical Science Technology, Universiti Kuala Lumpur, Kuala Lumpur, Malaysia
*Corresponding author: pan2chong@gmail.com; panpanchong@ummc.edu.my

The increasing number of musculoskeletal injuries has produced a concurrent stimulus in both the number and the effectiveness of different treatments of these lesions, especially in the search for minimally invasive procedures or adjuvants. It is well known that platelet-rich concentrate (PRC), a high concentration of platelet within a small amount of plasma, is widely used in promoting tissue repair. PRC appears to be very potent in inducing chondrogenic differentiation of human mesenchymal stromal cells, and offers the additional benefit of suppressing chondrocyte hypertrophy, rendering it a promising approach for providing an abundant pool of chondrogenic mesenchymal stromal cells (MSCs) for application in cartilage tissue engineering. Moreover, PRC enhances the reparative effects of MSC in treating focal articular cartilage injuries. Platelet-derived extracellular vesicles (PEV) are believed to work in a similar method as PRP, and further research has led to a better understanding of its mechanism of action in the process of tissue repair. The most apparent difference between PRC and PEV is in its size. PEV was isolated via differential gradient centrifugation. The characterisations of the PEV were performed using a scanning transmission electron microscope (SEM), and nanoparticle tracking analysis (NTA), followed by chondrocyte culture in vitro. The PEV treatment is carried out using the accredited Good Manufacturing Practice (GMP) laboratory at the NOCERAL. Patients with Kellgren-Lawrence grade I or II knee osteoarthritis based on a knee x-ray were enrolled. They were then asked to complete the questionnaire before the treatment and again one month later. A total of 58 osteoarthritis patients were recruited. Seventeen patients were injected with autologous PEV, 20 with hyaluronic acid, and 21 were treated conservatively (control). The KOOS, WOMAC, and SF36v2 questionnaires were filled out before and one month after the treatment. Data obtained were analysed using the SPSS. The activated platelets and vesicles were observed in SEM. Visualisation by electron microscopy revealed that activated platelet released EVs of a typical shape, i.e., irregular round vesicles, membrane-bounded, and no contaminants, could be observed. Moreover, the NTA demonstrated a poly-dispersed population of PEV with a particle size range of 50-500 nm. Two distinct populations of particles with sizes at 100-200 nm correspond to exosomes, and a substantial proportion of larger particles with sizes at 250 to 500 nm fall into the size range of microvesicles. The cell cultured in 10% PEV attained 100% confluence at day 7 of expansion, while only 80% confluence for 5% PEV and 50% confluence for 10% FBS. The overall morphology of growing cells is fibroblastic and identical in size. Cell counting analysis revealed an increase in the chondrocytes that were cultured in 10% PEV supplementation, 9 times and 5.5 times higher than those chondrocytes cultured in 10% FBS and 5% PEV, respectively. For the clinical outcome, PEV can improve the symptoms and lifestyle of a patient with mild or moderate knee osteoarthritis. Although not statistically significant in all subscales, when comparing the differences in scores among the groups, patients treated with PEV showed the most improvement, especially compared to patients in the control groups.

Application of Genomic Editing Technology in Retinal Diseases

Chiou Shih-Hwa
National Yang Ming Chiao Tung University, Taiwan PRC

CRISPR-Cas9 is a potential technology that can edit the genome by removing, adding, or modifying certain sections of a DNA sequence. This technology provides the opportunity for scientists and researchers to manipulate the interested gene. X-linked juvenile retinoschisis (XLRS) is an early-onset retinal degenerative disease that can cause visual impairment and retinal detachment. We have successfully established personalized iPSC-derived retinal organoids and further used the latest CRISPR/Cas9 technology to repair the mutated genes and the "schisis" phenotype in the retinal organoids. This advanced CRISPR-Cas9 gene editing may hold promise in the treatment of inherited diseases and will be extended to clinical application in the near future.

Modelling Heart Disease from Animal to *In Vitro* Models

Jun Jie Tan
Advanced Medical and Dental Institute, Universiti Sains Malaysia, Pulau Pinang, Malaysia

Heart disease remains a significant contributor to high morbidity and mortality, imposing a substantial healthcare burden worldwide. The race to successfully regenerate myocardium lost in ischemic hearts is ongoing, but the methods used to model the disease and mimic the diseased phenotype for regenerative research have heavily relied on animals, ranging from rodents to non-human primates. The emergence of the *in vitro* tissue-engineered heart tissue and the development of cardiac organoids have piqued substantial interest in the research community, offering the potential to reduce animal usage and facilitate personalised testing. However, both animals and the *in vitro* tissue engineered heart tissue or organoid models remain indispensable in research and testing at this stage due to the unique advantages and limitations of each. In general, myocardial regenerative research must consider factors such as post-transplantation cell survival, fate, engraftment, synchronisation with the host myocardium, and immune rejection when interpreting observed cardiac function. These

parameters involve the complex interplay between cardiac haemodynamics and the systemic immune response, which are challenging to replicate *in vitro*. On the other hand, organoids derived from patient-specific induced pluripotent stem cells provide a personalised genetic makeup that comprises individual regulatory proteins or phenotypes. This genetic individuality is crucial for understanding responses and sensitivities to drugs, enabling more personalised and effective drug treatments by considering genetic variations among patients. The cellular composition and maturity, tissue structure and function are keys to developing good resemblance to human heart for such testing. In this lecture, the current use of animals and *in vitro* models, the limitations and as well as the future applications in heart regeneration study will be discussed.

Oral Paper:

Establishment of hiPSCS Derived 3D Lung Organoids as Disease Modelling for Respiratory Diseases

Nalini Devi Verusingam[1,2,3,4], Habeebat Aderonke Mustapha[2,3], Soon-Keng Cheong[1,4], Alan Han-Kiat Ong[1], Shih-Hwa Chiou[2,3,5] & Mong-Lien Wang[3,6]
1 Centre for Stem Cell Research, Faculty of Medicine and Health Sciences, Universiti Tunku Abdul Rahman, Selangor, Malaysia; 2 Institute of Pharmacology, School of Medicine, National Yang Ming Chiao Tung University, Taipei, Taiwan; 3 Department of Medical Research, Taipei Veterans General Hospital, Taipei, Taiwan; 4 Department of Research and Development, Majlis Kanser Nasional (MAKNA), Kuala Lumpur, Malaysia; 5 Genomic Research Center, Academia Sinica, Taipei, Taiwan; 6 Institute of Food Safety and Health Risk Assessment, National Yang-Ming University, Taipei, Taiwan

Introduction: Three-dimensional (3D) lung organoids are a groundbreaking platform to model respiratory diseases, derived from human induced pluripotent stem cells (hiPSCs) through stepwise differentiation. Current therapeutic options for respiratory diseases are limited due to disparities between animal models and human lung physiology. Therefore, relevant lung model systems are needed to develop effective therapies. Recent advances in single-cell transcriptomic analysis have shown that human lung organoids (hLO) from hiPSCs significantly replicate human lung development, making them an ideal model for studying the development of lung-related respiratory diseases. In our study, we focused on the generation of hLO in disease modelling for respiratory diseases. Materials & Methods: HiPSCs were differentiated into definitive endoderm (DE) for 4 days and then into anterior foregut (AF) within 10 days using induction medium. Next, AF cells were further induced to form hLO using organoid medium for 14 days. The hLOs were molecularly characterised for pulmonary-alveolar markers using qRT-PCR, western blot and immunofluorescence (IF) staining. Results: Characterisation results indicated upregulation of SFTPB+, SFTPC+, SOX9+, and NKX2.1+ expression in hLOs, suggesting the presence of alveolar type II cells. Discussion: We successfully generated hLO *in vitro* as evident by phenotypic and genotypic characterisation. HLOs can serve as personalised disease models in clinical settings, enhancing our understanding of respiratory diseases like lung cancer, SARS-CoV-2 lung injury, and idiopathy pulmonary fibrosis (IPF).

Multiple Dosing of Cytopeutics® Human Umbilical Cord Mesenchymal Stem Cells is Safe in BALB/c Mice Toxicity Evaluation

Sze-Piaw Chin[1], Nur Izzati Mansor[1], Natasha Najwa[1], Kong Yong Then[1] & Soon Keng Cheong[2]
1 Cytopeutics Sdn Bhd, Cyberjaya, Selangor, Malaysia; 2 M. Kandiah Faculty of Medicine & Health Sciences, Universiti Tunku Abdul Rahman, Selangor, Malaysia

Introduction: The safety of single dosing of human umbilical cord mesenchymal stem cells (hUCMSCs) infusion has been well documented in various animal models. However, the safety and toxicity of multiple, repeated infusions remain uncertain. Yet certain clinical conditions may require multiple and frequent dosing to achieve the benefits of hUC-MSC. This toxicity study aimed at assessing the safety of Cytopeutics® hUC-MSCs administered at multiple frequencies in healthy BALB/c mice, followed by a 14-day monitoring period. Materials and Methods: Repeated dose toxicity was assessed in 2 groups of healthy BALB/c mice by slow bolus intravenous infusion of Cytopeutics® hUC-MSCs. The first group of mice (n=14) were injected with saline and acted as controls. The second group (n=14) received 5×10^6 cells/kg BW on days 1, 4 and 7. The dose of 5×10^6 cells/kg BW was chosen based upon the findings and efficacy study of Cytopeutics® hUC-MSCs in the Phase I/II clinical trial GVHD Study (NCT03847844). All mice were observed for 14 days to evaluate morbidity and mortality, clinical signs and clinical chemistry. At the end of the assessment period, all mice were euthanised for gross necropsy and histopathology analysis of all organs. Results: The findings showed that multiple infusions of Cytopeutics® hUC-MSCs at 5×10^6 cells/kg BW were safe and well-tolerated in all mice. No morbidity, mortality or significant changes in clinical signs or clinical chemistry were reported during the 14-day monitoring period. A minimal 16% increment in the spleen weight and increased cellularity of the white pulp in the spleen were observed at the terminal time point in mice treated with Cytopeutics® hUC-MSCs when compared to the control group. All other organs were normal. Discussion: All mice were not subjected to any fatalities or unusual clinical signs during the monitoring period. The administration of Cytopeutics® hUC-MSCs led to a slight increase in spleen weight and number of lymphocytes in the white pulp due to lymphocyte traffic to the spleen as a secondary lymphoid organ (SLO). This correlates with other research and suggests a physiological reaction to MSC administration. MSCs migrate to SLO and the interaction of MSCs with the spleen may account for the therapeutic immunomodulatory action on lymphocyte proliferation and activity. In conclusion, multiple infusions of Cytopeutics® hUC-MSCs at 5×10^6 cells/kg BW on days 1, 4 and 7 were safe and did not cause any adverse effects on morbidity, mortality, clinical signs or clinical chemistry in BALB/c mice.

Analysis of Secretome Profile in Umbilical Cord-derived Mesenchymal Stromal Cells Co-cultured with Senescent Normal Human Dermal Fibroblast

Soke Sun Lee[1,4,5], Soon Keng Cheong[1,3,4,5] & Hoon Koon Teoh[1,2,4*]
1 Postgraduate Laboratories, M. Kandiah Faculty of Medicine and Health Sciences Universiti Tunku Abdul Rahman (UTAR), Sg Long, Selangor 43000, Malaysia; 2 Department of Pre-clinical Sciences, M. Kandiah Faculty of Medicine and Health Sciences Universiti Tunku Abdul Rahman (UTAR), Sg Long, Selangor 43000, Malaysia; 3 Department of Medicine, M. Kandiah Faculty of Medicine and Health Sciences Universiti Tunku Abdul Rahman (UTAR), Sg Long, Selangor 43000, Malaysia; 4 Centre for Stem Cell Research, M. Kandiah Faculty of Medicine and Health Sciences, Universiti Tunku Abdul Rahman (UTAR), Sg Long, Selangor 43000, Malaysia; 5 Department of Research & Development, National Cancer Council (MAKNA)
* Corresponding author: teohhk@utar.edu.my

Introduction: Cellular aging, also known as a state of irreversible growth arrest, is characterised by the gradual and irreversible loss of proliferative potential and functional capacity of cells. Fibroblasts are the most widely used model in the study of oxidative stress-induced cellular senescence and replicative cellular senescence. Mesenchymal stromal cells (MSC) are multipotent cells that can be derived from different organs and tissues and possess the ability to expand *ex vivo* and differentiate into various mesoderm-type cells. In previous studies, MSC has been shown to exert therapeutic functions through two mechanisms: differentiation and paracrine signalling. MSC can secrete bioactive factors in the culture medium to regulate local cellular responses through paracrine signalling. Thus, in our study, we evaluated the secretome profile of umbilical cord-derived mesenchymal stromal cells (UC-MSC) after exposure to senescent fibroblasts. Materials and Methods: Normal human dermal fibroblasts (NHDF) were first treated with 200 μM hydrogen peroxide (H_2O_2) for 2 hours and allowed to recover for 5 and 7 days to develop the senescent model. The characterisation of senescent NHDF was done by a senescence-associated beta-galactosidase assay and measuring the cell proliferation rate. The senescent NHDF was then co-cultured with umbilical cord-derived mesenchymal stromal cells (UC-MSC) using the transwell system for 48 hours. The supernatant was then collected and semi-quantitative analysis of the secretome profile was carried out using chemiluminescence detection. Senescent NHDF were used as the negative control. Results: H_2O_2-treated NHDF showed an increase in beta-galactosidase activity and a decrease in the cell proliferation rate. These findings indicated the successful generation of senescent NHDF. The secretome profile analysis of the supernatant collected from senescent NHDF after co-culture with UC-MSC showed an increase in different cytokines and growth factors which were not seen in the supernatant of untreated senescent NHDF. Among the cytokines and growth factors that were increased were Fibroblast Growth Factor-7, Platelet-derived Growth Factor, Interleukin-8 and C-X-C motif chemokine ligand family. Pathway analysis using Reactome and the KEGG Pathway Database indicated that these proteins were largely involved in cell proliferation. Discussion: Secretome analysis showed the overexpression of proteins involved in cell proliferation in the supernatant of senescent NHDF and UC-MSC. This suggested that MSC may be able to secrete bioactive factors to ameliorate senescence in NHDF by expressing proteins that can activate cell cycle progression. Further investigations are needed to determine the functional effect of these proteins on the senescent NHDF.

Poster Abstract:

Effect of Cytopeutics® hUC-MSCs against Systemic Inflammation and Multiple Organ Injuries

Sze-Piaw Chin[1], Lihui Tai[1], Nurul Ashikin Mohamed Shahrehan[2], Christine Ricky[2], Audrey Fanty anak Jangan[2], Mohd Noor Syuhada Md. Halim[2], Nur Izzati Mansor[1], Natasha Najwa[1], Chui Thean Low[2] & Soon Keng Cheong[3]
1 Cytopeutics Sdn Bhd, Cyberjaya, Selangor, Malaysia; 2 Institute for Medical Research, Setia Alam, Selangor, Malaysia; 3 M. Kandiah Faculty of Medicine & Health Sciences, Universiti Tunku Abdul Rahman, Selangor, Malaysia

Introduction: Graft-versus-host disease (GVHD) is commonly characterised by systemic inflammation and multiple organ injuries. The immunomodulatory effects of human umbilical cord-mesenchymal stem cells (hUC-MSCs) in ameliorating acute systemic inflammation and multi-organ injury due to acute GVHD is unknown. This study aimed to investigate the efficacy of Cytopeutics® hUC-MSCs in reducing LPS-induced systemic inflammation with liver and lung injuries in BALB/c mice model. Materials & Methods: Eighteen mice were randomly allocated into three groups: the healthy group received normal saline; the LPS-only group was induced with 5 mg/kg LPS at 0.1 mL/mouse; and the LPSCytopeutics®-hUC-MSCs group was treated with 18.5×10^6 cells/kg (human equivalent dose of 1.5×10^6 cells/kg) at 24 h post-LPS induction at 0.2 mL/mouse. Tumour necrosis factor-alpha (TNF-α), interleukin (IL)-1beta (1β), IL-6, as well as aspartate aminotransferase (AST) and alanine amino transferase (ALT) were analysed by ELISA at 24 h and day 7-post treatment. At the end of the study, all mice were euthanised by cervical dislocation and target organs were subjected to necropsy and histopathological examination. Results: LPS at 5 mg/kg for 24 h induced extensive liver and lung injury, as evidenced by H&E staining. After treatment with hUC-MSCs, the liver injury score on day 7 was reduced to 0.40 ± 0.55 compared to the LPS-only group (1.33 ± 0.58). The observation was consistent with the reduction in AST (272.7 ± 6.1 U/L vs 398.3 ± 206.0 U/L) and ALT (57.7 ± 7.0 U/L vs 65.7 ± 18.2 U/L) levels in the hUC-MSCs group in comparison with the LPS-only group. Likewise, in the lung, the mean score of infiltrated inflammatory cells on day 7 was markedly higher in both healthy and LPS-only groups (2.33 ± 0.16), whereas the score decreased to 1.80 ± 1.30 in the hUC-MSCs treatment group. Furthermore, hUC-MSCs significantly reduced the levels of TNF-α ($p = 0.0221$) and IL-1β ($p = 0.0419$) at 24 h. IL-6 level was also reduced ($p > 0.05$). The levels of these cytokines returned to near-normal levels on day 7. Collectively, hUC-MSCs have demonstrated the capability to attenuate systemic inflammation and alleviate the severity of liver and lung injury induced by LPS. Discussion: Treatment of 18.5 × 106 cells/kg hUC-MSCs in BALB/c mice following LPS injection led to an early resolution of acute inflammation

and a subsequent reduction in lung and liver injuries. These findings provide the rationale for using hUC-MSC to improve the clinical signs of acute GVHD.

Immunotherapy in Cervical Cancer Treatment

Divyangana Kiran Vartak*, Than Htay Tin & Myo Oo
Department of Medicine, M. Kandiah Faculty of Medicine and Health Sciences, University Tunku Abdul Rahman, CTC Ampang Selangor, Malaysia
*Corresponding author: kiranv@utar.edu.my

Introduction: Cervical cancer ranks as the 4th most common cancer among women worldwide. It is mainly caused by human papillomavirus (HPV) types 16 and 18, which account for nearly 50% of high-grade cervical pre-cancers. Material & Methods: The poster includes information on cervical cancer, prevention with vaccination and staging, an overview, current management and the role of immunotherapy in treating cervical cancer. Data and information were collected from important websites and journals, including PubMed, Journal of Cancer, International Reviews on Immunology, and Journal of Gynaecological Cancer. Results: Preventing HPV infection through vaccination, screening and treating pre-cancerous lesions can effectively reduce the risk of cervical cancer and has the potential to prevent more than 90% of HPV-related cancers. However, for women who develop cervical cancer, the traditional treatments of surgery, chemotherapy and radiotherapy have limitations and complications. Discussion: Immunotherapy, a novel treatment that harnesses the body's immune system to fight cancer, offers hope to those who have not responded well to traditional treatments or have experienced significant side effects. Immunotherapy, either alone or in combination with other systemic therapies, may provide significant benefits and improve the quality of life for women with advanced, recurrent or metastatic cervical cancer.

Fructose-Streptozotocin-Induced Diabetes: A Severe Rat Diabetic Model

Rania Ibrahim[1], Lim Yang Mooi[1], Ng Wen Jie[2], Lee Siew Keah[1]
1 Department of Pre-clinical Sciences, M. Kandiah Faculty of Medicine and Health Sciences, Universiti Tunku Abdul Rahman, Selangor, Malaysia; 2 Department of Allied Health Sciences, Faculty of Sciences, Universiti Tunku Abdul Rahman, Kampar, Malaysia

Introduction: Notorious for its high prevalence, diabetes mellitus (DM) has been a formidable rival for the healthcare system and a leading cause of mortality globally. In its most threatening form, the severe disorder manifests as uncontrollable and high fasting blood glucose (FBG) levels (> 22 mmol/L) associated with pancreatic beta cell decompensation and reduced volume. Without appropriate therapeutic advances, its prevalence and mortality rate are predicted to increase exponentially. This necessitates the development of a reliable model for the severe form of the disease. To advance diabetes research, there is a need for a cost-effective and an easily maintained animal model that corresponds to the severe stage of the disease as there is a scarcity of inexpensive ones exhibiting uncontrollable hyperglycaemia. Our aim is to corroborate the establishment of a robust and accessible rat model of severe diabetes. Materials & Methods: 27 Sprague Dawley male rats were divided into a diabetic (DG) (n=21) and a normal control group (NC) (n=6). NC received regular drinking water while the DG received 10% fructose water ad libitum for 14 days. On the 15th day, DG received a single intraperitoneal injection of streptozotocin (40 mg/kg body weight) dissolved in citrate buffer (0.1M). NC received citrate buffer only. A week later, animals were fasted for 6 hours and their FBG levels were measured. To confirm the establishment of a severe diabetes model, a group of rats from DG (n=7) were given metformin (DM) dissolved in reverse osmosis water orally (300 mg/kg body weight) for 28 days, while a diabetic control group (DC) (n=7) and NC received the vehicle only. Body weight, and food and water intake were measured daily. Cage-side observation was conducted and FBG levels were monitored weekly. Results: The induction had a success rate of 94%. A week after the induction, the DG had a mean FBG of 25.4 mmol/L, severe weight loss, fatigue, polyuria, polyphagia, and polydipsia compared to the normal control ($p<0.0001$). Over the 28 days, all the signs persisted even in the metformin-receiving group. The mortality rate was 28.6% in the DC group, and 14.3% in the DM group. Discussion: Fructose drinking and a single dose of streptozotocin can induce severe diabetes characterised mainly by persistent hyperglycaemia that is uncontrollable despite administration of metformin. This animal model provides an accessible tool for studying unmanageable hyperglycaemia and evaluating potential therapeutic interventions.

Cardiac Complications Post-COVID-19 Vaccination – An Interim Report of a Systematic Review

Kai Wei Lee[1,2,3], Sook Fan Yap[1,2*] & Yun Fong Ngeow[1,2]
1 Department of Pre-clinical Sciences, M. Kandiah Faculty of Medicine and Health Sciences, Universiti Tunku Abdul Rahman, Kajang, Selangor, Malaysia; 2 Centre for Research on Communicable Diseases, Universiti Tunku Abdul Rahman, Kajang, Malaysia; 3 Department of Medical Microbiology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia
*Corresponding author: yapsf@utar.edu.my

Introduction: Cardiac complications following COVID-19 vaccination, the most widely recognised of which is myocarditis, are rare occurrences. This systematic review aimed to identify reported cardiac complications post-vaccination and determine factors associated with undesirable outcomes. Materials & Methods: The review was registered with PROSPERO

(CRD42022310861) and followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses guidelines. Three electronic databases were searched using specific strategies. Inclusion criteria involved adult cases aged 18-65 years with detailed descriptions of cardiac complications, while cases with a history of COVID-19 infection were excluded. Univariate and multivariate analyses were performed to identify associations between undesirable outcomes and various demographic, lifestyle (such as smoking, drug use, and alcohol consumption), underlying illness, and vaccine-related factors (vaccine type and number of vaccine doses). Results: 906 articles were initially found; 106 articles comprising data from 178 patients were identified for analysis after content evaluation. The study found that the most commonly observed complications were inflammatory heart conditions, accounting for 89.9% of all reported cardiac complications. Other cardiac complications were uncommon and included, in decreasing order, ischemic heart disease (3.9%), cardiomyopathy (3.9%), cardiac arrhythmias (1.7%), and myocardial injury (0.6%). Patients with myocarditis accounted for a large proportion (n=123, 69.1%) of the inflammatory cardiac conditions, the majority of whom were males who comprised 83.3% of all myocarditis cases. Overall, the myocarditis patients were relatively young with an average age of 30.8±11.6 years (mean ± SD). The clinical course was uneventful in most cases (n=107, 77.5%). However, 19 patients (13.86%) had an acute disease course and four cases were fatal (2.9%); information for eight cases (5.8%) was insufficient to determine the disease course or complications. Univariate analysis revealed that undesirable outcomes of inflammatory cardiac complications were associated with female gender. Discussion & Conclusion: The findings confirm that myocarditis is the most frequent cardiac complication following COVID-19 vaccination. An unexpected observation was the association between female gender and a higher risk of undesirable outcomes of myocarditis in the background of the male predominance of this condition. This finding requires further validation and if validated, fundamental investigations to uncover its pathophysiological basis. This interim review report highlights inflammatory disorders, particularly myocarditis, as the most common cardiac complication post-COVID-19 vaccination, with the majority being uncomplicated. Further research is necessary to enhance our understanding and prevent these complications.

Approaching Patients Presented with Bone Tumours

Wisam Al-Obaidy[1], Naghem Abed[1], Win Min Thein[2]
1 M. Kandiah Faculty of Medicine & Health Sciences, Universiti Tunku Abdul Rahman, Sungai Long Campus, Jalan Sungai Long, Bandar Sungai Long, Cheras, 43000, Kajang, Selangor–Malaysia; 2 Taylor's University, Jalan Taylors, 47500 Subang Jaya, Selangor

Introduction: Due to their diversity and extensive details, bone tumours are one of the most difficult orthopaedic topics for both undergraduate students and doctors. All the references present this subject thoroughly and based on the type. The goal is to determine the best course of action rather than to identify the specific type of bone tumours. Materials and Methods: Via an algorithm system, this poster demonstrates the approach of those patients depending on their clinical picture and initial plain X-ray. The clinical picture of benign bone tumour patients is milder than malignant tumours. On the other hand, the radiological signs of bone tumours can fairly distinguish between benign and malignant bone tumours. The presence of well-defined borders, a narrow zone of transition, bony expansion, and trabeculations without periosteal reaction or soft-tissue extension are imaging indicators that the tumour is benign as opposed to malignant. This algorithm system uses Enneking`s classification of benign bone tumours (Latent, Active, and Aggressive) for categorising the patients into three classes. Patients who presented with aggressive benign bone tumour features must be considered malignant until proven otherwise. Recommendations: We recommend using this guideline as a simple framework for clinicians and students in addressing the complexities of managing bone tumours.

Genetically Engineered Human Umbilical Cord-derived Mesenchymal Stromal Cells Expressing Human Interleukin-12 Inhibit Growth of Lung Adenocarcinoma Cells *In Vitro*

Jiunn Jye Goh[1,2,4], Hoon Koon Teoh[1,4], Hooi Tin Ong[1,4], Bee Sun Lee[3,4] & Soon Keng Cheong[3,4]
1 Department of Pre-clinical Sciences, M. Kandiah Faculty of Medicine and Health Sciences, Universiti Tunku Abdul Rahman, Selangor, Malaysia; 2 Department of Research and Development, Cryocord Sdn. Bhd, Selangor, Malaysia; 3 Department of Medicine, M. Kandiah Faculty of Medicine and Health Sciences, Universiti Tunku Abdul Rahman, Selangor, Malaysia; 4 Centre for Stem Cell Research, Universiti Tunku Abdul Rahman, Selangor, Malaysia

Introduction: Interleukin-12 (IL-12) is a crucial immunomodulatory cytokine known for its antitumour effects. Nonetheless, the systemic administration of IL-12 at therapeutic dosage leads to serious toxicity in cancer patients due to the induction of an extremely high systemic level of interferon-γ. Mesenchymal stromal cells are promising cellular vehicles for cancer therapy. They are highly amenable to transduction by viral vectors to express and deliver exogenous proteins to tumour sites due to their tumour-homing ability. In this study, we genetically engineered human umbilical cord-derived mesenchymal stromal cells (hUCMSC) using an adenoviral vector to express hIL-12 and examined their effect on lung adenocarcinoma cells. Materials and Methods: The hIL-12 gene was first cloned into linearised pAdenoX-ZsGreen1 using Adeno-XTMAdenoviral System 3. The linearised recombinant adenoviral plasmid was then packaged into a recombinant adenovirus using HEK293 cells and further amplified and purified. Viral titers were determined and multiplicity of infection (MOI) 10 was selected to infect hUCMSC in generation of hUCMSC expressing hIL-12 (hUCMSC-IL12). The hUCMSC-12 ($1x10^4$ cells/well) were co-cultured with H1975 lung adenocarcinoma cells ($1x10^3$/well) in a 24-well transwell system for 5 days. Cell viability of H1975 was assessed using the CCK-8 assay, with untransduced hUCMSC serving as a negative control. A similar co-culture assay was repeated again using MRC-5 human lung fibroblast cells. The supernatant in the co-culture assay was collected

for quantification of hIL-12 levels using ELISA. Results: On the fifth day of co-culture with hUCMSC-IL12, H1975 cell viability significantly reduced to 66.8%. In contrast, H1975 co-cultured with untransduced hUCMSCs did not result in any significant difference in cell viability (91.6%). Similarly, the viability of MRC-5 human lung fibroblast cells was also not affected after 5 days of co-culture with hUCMSC-IL12 (114.0%). Lastly, the hIL-12 protein expressed by hUCMSC-IL12 increased from 1.2µg/ml on day 3 to 2.2µg/ml on day 5. Discussion & Conclusion: Based on our results, hUCMSC-IL12 exhibited a growth inhibition effect on lung adenocarcinoma cells without adversely affecting the viability of normal human lung fibroblast cells. Hence, genetically engineered hUCMSC expressing hIL-12 using the adenoviral vector can be potentially utilised as cellular vehicles in cancer therapy to overcome the systemic toxicity of IL-12.

Social Demographics and Vaccine-Related Perceptions on the Intention for COVID-19 Vaccine Booster among the Elderly Residing in Long-Term Care (LTC) Homes in Klang Valley

Natalie Yong Xin Wong[1], Sook Fan Yap[1,2], Peak Yean Choo[1,3], Myo Oo[1,3], Eugine Foo[1], Kah Yoong Lee[1], Yue Chen Goi[1], Bryan Choon Aun Ooi[1] & Hooi Tin Ong[1,3]
1 Department of Pre-clinical Sciences, M. Kandiah Faculty of Medicine and Health Sciences, Universiti Tunku Abdul Rahman, Selangor, Malaysia; 2 Centre for Research in Communicable Diseases, Universiti Tunku Abdul Rahman, Selangor, Malaysia; 3 Centre for Cancer Research, Universiti Tunku Abdul Rahman, Selangor, Malaysia

Introduction: COVID-19 vaccine booster is an effective measure to boost the declining immunity of vaccine recipients, thereby protecting these recipients from severe disease. The elderly comprises a target group for boosters due to their weakened immune systems and higher risk of underlying chronic diseases. Despite this, only 5.8% of the elderly in Malaysia have taken the second booster as of 13th October 2022. Hence, this study aimed to identify the prevalence of vaccine booster hesitancy (VBH) and its associated factors among the elderly residing in long-term care (LTC) homes in the Klang Valley. Materials & Methods: A cross-sectional analytical study was conducted from 28th November to 8th December 2022. Universal sampling was employed to select the LTC homes in the Klang Valley as well as the participants. A questionnaire was designed and validated to assess the sociodemographic factors and vaccine-related perceptions on vaccine booster hesitancy. The survey was carried out through face-to-face interviews with 158 LTC home residents aged ≥60. The chi-square test and binary logistic regression were used for data analysis. Results: We observed a high prevalence (42.4%) of VBH among the participants; indeed, 40.5% indicated that they were unlikely/very unlikely to receive an annual booster dose. Among the significant factors positively associated with VBH are female gender (OR: 1.42, 95% CI: 1.01-3.89), history of side effects from past COVID-19 vaccinations (OR: 2.18, 95% CI: 1.14-4.20), fear of side effects following a booster dose (OR: 15.37-16.36), and low trust in vaccines (OR: 5.09, 95% CI: 2.17-11.90), medical experts (OR: 5.47, 95% CI: 2.41-12.40), mass media (OR: 2.31, 95% CI: 1.16-4.59) and the government (OR: 5.78, 95% CI: 2.81-11.92) respectively. Discussion: The results emphasise that targeted health promotion activities are a necessary tool in disseminating reliable sources of information to the public to prevent them from developing unwarranted fears and negative perceptions towards the vaccine booster.

Reprogramming of Double-Hit Diffuse Large B-cell Lymphoma Cells Line into Induced Pluripotent Stem Cells

Xi Zeng Low[1], Alan Han Kiat Ong[1], Soon Keng Cheong[2] & Bee Sun Lee[2]
1 Department of Pre-clinical Sciences, M. Kandiah Faculty of Medicine and Health Sciences, Universiti Tunku Abdul Rahman, Selangor, Malaysia; 2 Department of Medicine, M. Kandiah Faculty of Medicine and Health Sciences, Universiti Tunku Abdul Rahman, Selangor, Malaysia

Introduction: Studies have demonstrated the success of reprogramming cancer cells into induced pluripotent stem cells (iPSCs). However, not all cancer cells are amenable to reprogramming. The underlying mechanisms limiting the reprogramming of cancer cells are largely unknown. This study demonstrated the resistance of the double-hit diffuse large B-cell lymphoma (DH-DLBCL) cell line to cellular reprogramming using Sendai virus (SeV)-mediated gene transduction. Materials & Methods: The CytoTune-iPS 2.0 Sendai Reprogramming Kit was used for the reprogramming. It contains four SeV-based reprogramming vectors: hOCT4, hKOS, hc-Myc and hKlf4, which consist of the four transcription factors for efficient reprogramming. The DH-DLBCL cell line (CRL-3382) was purchased from American Type Culture Collection (ATCC). CRL-3382 was reprogrammed using the kit according to the feeder-dependent protocol. Mouse embryonic fibroblasts (MEF) were used as feeder layers. The transduced cell RNA was extracted using trizol reagent and bleach gel electrophoresis was performed to evaluate the extraction efficacy. The resulting RNA was converted into cDNA using RevertAid First Strand cDNA Synthesis Kit, and PowerUp SYBR Green Master Mix for PCR. PCR was performed with gene-specific primers Sendai SeV, Sendai Sox 2, Sendai Klf 4 and Sendai c-Myc to evaluate the transduction efficacy of CRL-3382. Results: The DH-DLBCL cells continued to proliferate with no iPSC-like colonies observed after 30 days post-transduction. The bleach gel electrophoresis showed the RNA was successfully extracted and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a housekeeping gene, was used as comparison of gene expression data. Evaluation of genes transduction efficacy by PCR showed positive expression of the KOS and Sendai SeV genes. The other c-Myc and Klf4 genes expression was not demonstrated by PCR. Discussion: CRL-3382 has the oncogenes c-Myc and BCL-2. The results indicate that the SeV successfully delivers the KOS-transcription factors into CRL-3382. The detection of Sendai SeV expression in the PCR proved that the virus was successfully transduced into the cells. However, no iPSC-like colonies were formed. Different approaches were attempted to improve the reprogramming efficacy, such as lowering the cell density for transduction and lowering the seeding density. However, all of the attempts failed to yield iPSC-like colonies. Resistance of cancer cells to reprogramming capacity has been reported but the underlying mechanisms limiting its efficiency remain elusive. Previous studies have reported presence

of genes that could be obstacles in cancer cell reprogramming such as EZH1, PRMT6 and MXD1. Further study is needed to evaluate if the two oncogenes c-Myc and BCL-2 are hindering the reprogramming of our cell line.

Generation and Characterisation of an iPS Cell Line Derived from Peripheral Blood Mononuclear Cells of a Di(a+) Blood Donor for the Purpose of Producing Antibody-screening Red Cells

Han Yao Teo, Hoon Koon Teoh, Veera Sekaran Nadarajan
Department of Pre-clinical Sciences, M. Kandiah Faculty of Medicine and Health Sciences, Universiti Tunku Abdul Rahman, Selangor, Malaysia

Introduction: Red blood cells (RBC) express various antigens that can elicit a humoral immune response. Antibodies against the RBC antigens can cause haemolytic transfusion reactions and haemolytic disease of the foetus and newborn. Antibody screening (ABS) for these RBC antigens is therefore essential during pretransfusion testing and antenatal screening. ABS is usually performed using red cell panels that are developed from red cells procured from selected donors with the specific antigen combination. This approach lacks sustainability as it can be challenging to recruit donors with the desired antigen combination, especially within the Asian population whereby the spectrum of clinically significant antibodies is wider. For example, anti-Mia and anti-Dia are antibodies that are found commonly among Asians but rare among the Caucasian population. Thus, the use of induced pluripotent stem cells (iPSC) to generate RBCs expressing these antigens is proposed to circumvent this issue as iPSC can provide a consistent source of erythrocytes. Materials & Methods: Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation from a blood donor expressing Dia. The cells were then culture-expanded to form erythroid precursors in an erythroid expansion medium, and subsequently reprogrammed into iPSC using the Epi5 Episomal Reprogramming Kit. Pluripotency was characterised by immunofluorescence staining against Oct4, Sox2, Nanog and Tra-1-81. Embryoid body (EB) formation was performed to assess the trilineage differentiation potential. Quantitative RT-PCR was performed on the EB and iPSC to detect markers of the three germ layers and pluripotency markers, respectively. The H9 human embryonic stem cell line was used as a positive control for the assays. Results: The iPSC colonies were observed on day 20 post-transfection. The colonies were positive on immunofluorescence staining for all the pluripotency markers studied. The generated iPSCs expressed the pluripotent genes as confirmed on qRT-PCR, with 0.30-fold for Oct4; 0.12-fold for Sox2; 0.16-fold for Nanog, as compared to the embryonic stem cell line, H9. EBs were shown to form on differentiation day 1. Discussion: We have demonstrated the successful generation of an iPS cell line from erythroid precursors derived from the PBMC of a blood donor expressing the Dia antigen. This iPSC line will potentially serve as a source for consistent production of Dia+ reagent cells for ABS panels.

Role of Physiotherapy in Regenerative medicine and Stem Cell Therapy

Swapneela Jacob, Tarun Amalnerkar
Department of Physiotherapy, M. Kandiah Faculty of Medicine and Health Sciences, Universiti Tunku Abdul Rahman, Selangor, Malaysia

Introduction: Regenerative medicine is an interdisciplinary innovative field of medicine that applies principles of engineering and life sciences to promote tissue regeneration. It includes the injection of stem cells or progenitor cells, immunomodulation therapy and tissue engineering in the injured part. Stem cell therapy promotes the repair response of inflamed, dysfunctional and injured tissue using stem cells and their derivatives. The treatment is designed to heal injuries and reduce pain. It can be used in the treatment of arthritis, cancer, endometriosis, injuries to ligaments, tendons, cartilage, or bone. Physiotherapy deals with restoring body movement and function after injury, illness and disability. Physiotherapy management given to patients undergoing stem cell therapy has been reported to enhance the overall recovery time and quality of life. Physiotherapy treatment strategies include aerobic programming, resistance exercises, and functional training in order to alleviate debilitating symptoms of fatigue and pain, and deconditioning is used along with the therapy. Conditions like cancer, spinal cord injuries and tendon injuries have recovered well with a better patient outcome. Strength training intervention has been shown to enhance early recovery and improve muscle strength and functional ability as well. Materials & Methods: A general review of the latest studies using Google, Pubmed and medical journals highlights the role of physiotherapy in various patients undergoing regenerative medicine and stem cell therapy. Results: Most of the studies support the use of physiotherapy strategies along with the stem cell therapy. Exercise for tendonitis, rotator cuff injury, multiple sclerosis in combination with regenerative therapy has helped patients achieve better clinical outcomes. Discussion & Conclusion: Physiotherapy in combination with regenerative medicine and stem cell therapy can aid in restoring, maintaining and improving mobility, function and wellbeing and relieve the condition faster so that patients can resume an active, pain-free life. Physiotherapy has been shown to play a vital role in the early rehabilitation of patients undergoing stem cell therapy. Though there is no specific modification in the rehab protocol, it still aids in the faster and complete recovery of the patients.

Differentiation of hiPSCs and hESCs into Haematopoietic Stem and Progenitor Cells Thru Haemogenic Endothelia Formation

Yee Ching Lim[1,2], Soon Keng Cheong[1,2] & Pooi Pooi Leong[1]
1 Centre for Stem Cell Research, M. Kandiah Faculty of Medicine and Health Sciences, Universiti Tunku Abdul Rahman, Sungai Long, Selangor 43000, Malaysia; 2 National Cancer Council Malaysia, MAKNA
*Corresponding author: leongpp@utar.edu.my

Introduction: Haematopoietic stem cells (HSCs) are one of the important candidates for cell-based therapy due to their capability of differentiating into functional haematopoietic cells. HSC can be derived from pluripotent stem cells such as embryonic stem cells. Human induced pluripotent stem cells (hiPSCs) have been explored as an alternative cell source for HSC generation as the use of human embryonic stem cells (hESCs) raises ethical controversy. Notably, the formation of intermediate haemogenic endothelia (HE), the specialised endothelial cells with haematopoietic potentials, is a crucial step for haematopoietic differentiation processes. In this study, we aimed to compare the differentiation capacities of hiPSCs and hESCs into HE as well as those of haematopoietic stem and progenitor cells (HSPCs). Materials & Methods: iPSCs derived from human dermal fibroblasts (hNHDF-iPSC) and hESC lines (hUES9), were differentiated using STEMdiff™ Hematopoietic Kit into heterogeneous cultures for 12 days. The identities of the differentiated cells were then characterised with HE-related markers (CD31, CD34, CD144 and CD43) and HSC markers (CD34, CD43 and CD45). Results: Before differentiation, hNHDF-iPSC showed significantly higher expression of TRA-1-81 ($p = 0.04$), SSEA4 ($p = 0.03$) and TRA-1-60 ($p = 0.04$) as compared to hUES9. After 12-day differentiation, the hNHDF-iPSC-derived differentiated adherent cells expressed significantly higher HE-related markers [CD144 ($p < 0.001$) and CD43 ($p = 0.04$), and CD34+CD144+ ($17.0 \pm 0.8\%$ vs $5.2 \pm 0.3\%$, $p < 0.001$)]. A regression test showed that the expression of CD34+CD144+ HE cells was significantly affected by the expression of pluripotency markers [SSEA4 ($p = 0.023$) and TRA-1-60 ($p = 0.024$)]. Interestingly, the HSPC production from hNHDF-iPSC was significantly higher than from hUES9 ($39.2 \pm 3.3\%$ vs $54.0 \pm 1.4\%$, $p = 0.008$). Regression test indicated that the expression of HSPCs may be affected by the CD34+CD144+ population ($p = 0.008$). Immunophenotyping analysis showed HSPCs are heterogeneous cultures consisting of two daughter populations: early haematopoietic progenitor (EHPs) with CD34+CD43+CD45- and haematopoietic stem cells (HSCs) with CD34+CD43+CD45+ expression. No significant differences were found between the production of EHPs ($37.2 \pm 3.2\%$ vs $25.4 \pm 2.7\%$, $p = 0.10$) and HSCs ($16.8 \pm 2.0\%$ vs $13.9 \pm 4.1\%$, $p = 0.97$) from hNHDF-iPSC and hUES9. Discussion: In conclusion, our study showed that hiPSCs possess comparable differentiation capacities as hESCs in deriving HSCs and HSPCs which could be a potential source for cell therapy. The hiPSC, which consisted of a more homogenous population of pluripotent cells, may have greater differentiation capacities to produce functional HE with haematopoietic potential as compared to hUES9.

OCT4 and BMP4 Expressions in Human Adipose-derived Stem Cells from Subcutaneous and Visceral Fat

Abdul Malik Setiawan[1,2], Fairus Ahmad[1], Adila A. Hamid[3], Abdul Kadir Abdul Karim[4], Taty Anna Kamarudin[1]
1 Department of Anatomy, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia; 2 Department of Anatomy, Maulana Malik Ibrahim State Islamic University, Malang 5124, Indonesia; 3 Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia; 4 Department of Obstetrics and Gynaecology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia
*Corresponding author: tatykamarudin@ukm.edu.my

Introduction: Adipose-derived stem cells (ADSC), are mesenchymal stem cells (MSC) found in the vascular stroma of human fat tissues. ADSC have been proposed as a potential alternative for human donated corneal tissue. Octamer-binding transcription factor 4 (OCT4) regulates pluripotency in stem cells including ADSC, while bone morphogenetic protein 4 (BMP4) signalling is essential for the differentiation of stem cells towards ectodermal lineages. In this preliminary study, the expression of BMP4 and OCT4 in primary ADSC from two different adipose tissue depots were assessed. Materials & Methods: Subcutaneous and visceral adipose tissue were harvested from the abdomen of 6 female patients (mean age 31 ± 3 years, range 29-33 years) that underwent caesarean section or laparotomy at the Obstetrics and Gynaecology department Hospital Canselor Tuanku Muhriz. The subcutaneous and visceral adipose tissues were processed and ADSC were isolated. The cells were then seeded at 4000 cells/cm^2 until confluence and expanded up to passage 4 (P4). The expression of BMP4 and OCT4 were assessed at P4 via qPCR and immunohistochemistry and compared to an ADSC cell line as a control group. Results: Generally, the expressions of OCT4 and BMP4 were higher in the primary ADSC from both subcutaneous and visceral locations compared to the control. The expressions of both OCT4 and BMP4 of the visceral and subcutaneous ADSC showed a similar pattern. Majority (67% and 80%) of the visceral ADSC expressed higher OCT4 and BMP4 than the subcutaneous ADSC, respectively. Discussion: These findings suggest a potential correlation between the potential differentiation capacity and the location of the ADSC, which can be explained by the interplay of underlying regulatory pathways between pluripotency and ectodermal differentiation capacity. This could also offer a useful insight in an effective selection of MSC for tissue engineering and cell therapy purposes.