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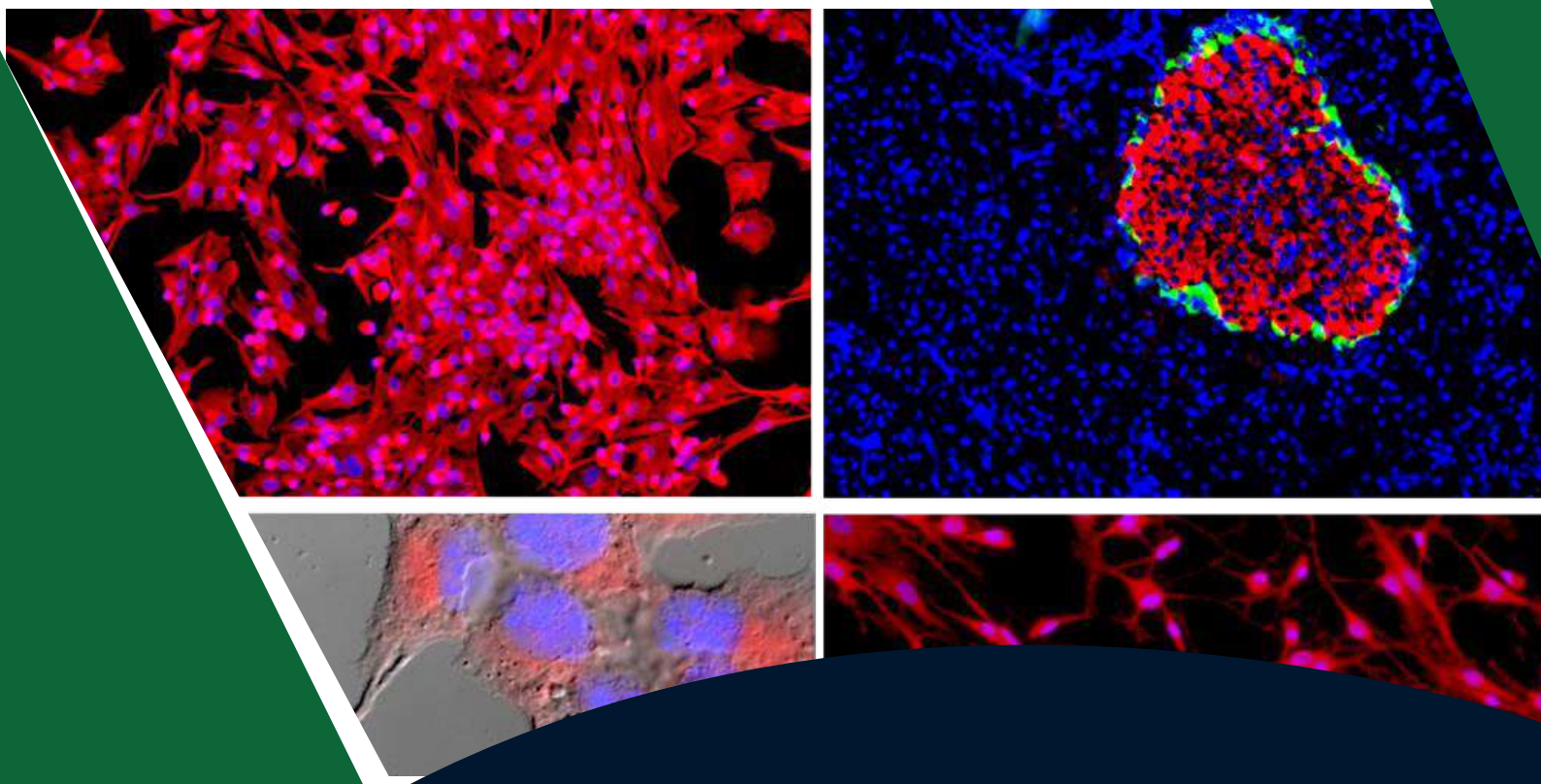

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Repurposing Pirfenidone and Metformin as Antifibrotic Agents in Oral Submucous Fibrosis: Molecular, Cellular, and Clinical Perspectives

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Oral Submucous Fibrosis (OSF) is a chronic, progressive, and potentially malignant disorder characterized by fibroelastic changes in the oral mucosa and excessive extracellular matrix deposition. Despite multiple therapeutic strategies, reversal of fibrosis remains limited. This study aimed to explore the repurposing potential of antifibrotic agents, Metformin and Pirfenidone, using OSF-derived fibroblast cell lines alongside a parallel clinical trial. OSF fibroblast cell lines were cultured and treated with Pirfenidone and Metformin. Cytotoxicity was assessed using the MTT assay to determine cell viability and IC_{50} values. Microscopic morphological analysis was performed to observe structural and cellular alterations following treatment. The scratch (wound healing) assay was utilized to evaluate the anti-migratory potential of both drugs. Quantitative real-time PCR (qPCR) was conducted to assess the expression of key fibrotic and antifibrotic markers, including *TGF-β1, α-SMA, COL1A2, COL1A3, CTGF, MMP-2, Fibronectin, E-cadherin, N-cadherin,* and *β-catenin*. Both drugs demonstrated dose-dependent cytotoxicity and significant inhibition of fibroblast proliferation and migration. Microscopic analysis revealed cell shrinkage, reduced confluence, and loss of fibroblastic morphology in treated cultures. qPCR results indicated downregulation of profibrotic genes (*TGF-β1, α-SMA, COL1A2, CTGF,* and *Fibronectin*) and upregulation of epithelial marker *E-cadherin*, with a concomitant decrease in *N-cadherin* and *β-catenin* expression. Pirfenidone showed stronger inhibition of migration, while Metformin exhibited more pronounced modulation of gene expression at sub-cytotoxic concentrations. These findings suggest the potential therapeutic value of these agents in modulating the fibrotic pathways of OSF. In the clinical phase, OSF patients were randomized into two treatment groups: Group A received Metformin (500 mg twice daily), and Group B received Pirfenidone (200 mg twice daily) for eight weeks alongside standard therapy. Clinical evaluation revealed improvement in burning sensation, mouth opening, and mucosal suppleness, with both groups showing statistically significant symptomatic relief. These findings highlight the translational potential of Metformin and Pirfenidone as adjunct antifibrotic therapies for Oral Submucous Fibrosis, with Metformin offering superior tolerability and Pirfenidone demonstrating stronger early clinical response—supporting their future evaluation in larger, long-term clinical trials.

MM-002

Hesperidin and its PLGA-nanoformulation mitigate rhabdomyolysis-induced acute kidney injury *via* attenuation of oxidative stress and inflammation in BALB/c mice

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Background and Objectives: Preservation of renal morphology and function is essential in rhabdomyolysis-induced acute kidney injury (RIAKI). Hesperidin (HSP), a natural bioflavonoid, seems promising for nephroprotection. We evaluated and compared nephroprotective effects of HSP and its polylactic-co-glycolic acid (HSP-PLGA) nanoformulation RIAKI. **Methods:** HSP-PLGA was developed by nanoprecipitation method and validated by zeta potential study, Fourier-transform infrared analysis and scanning-electron microscopy. BALB-c mice treated with intraperitoneal HSP-200mg/kg or HSP-PLGA-20mg/kg for 5 days, were given intramuscular glycerol on day 5 and dissected next day. Serum lactate dehydrogenase (LDH), creatine kinase (CK), creatinine, and urea levels were determined. Tissue lipid peroxidation (LP), superoxide dismutase (SOD), reduced glutathione (GSH), and nitric oxide (NO) levels were determined. H&E, PAS, and immunohistochemistry for hemeoxygenase-1, tumor necrosis factor-α (TNF-α), and inducible nitric oxide synthase (iNOS) were done. ELISA was performed to determine tissue levels of interleukin-6, interleukin-10, and interferon-γ. The nanoeffect of HSP-PLGA vs HSP was calculated. **Results:** Significant increase in serum CK, LDH, urea, and creatinine validated the model. Urea and creatinine were significantly reduced in treatment groups. Treatment enhanced antioxidant defense by lowering LP and NO, simultaneously increasing SOD, GSH and iNOS. It also reduced inflammation by suppressing TNF-α, interleukin-6, and interferon-γ, and increasing interleukin-10. Expression of the protective enzyme hemeoxygenase-1 was enhanced with treatment, which histologically translated into reduced hyaline cast formation and tubular necrosis. Calculation of nanoeffect revealed the superiority of HSP-PLGA. **Conclusion:** HSP and HSP-PLGA preserve renal tubular morphology by decreasing oxidative stress and inflammation, thus conserving renal function. HSP-PLGA enhances these nephroprotective effects.

MM-003

The Embryo Enteric Model: Nutritional, Pathophysiological and Therapeutic Implications on Gut

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Background and Objectives: The research on the gastrointestinal tract is largely hampered by the presence of materials in the gut lumen (excessive water, food materials, bacteria, fungi, etc.), which may interfere with outcome of experimental results. This is especially true when studies are related to enteric pathogens and their pathogenesis. Intestine from embryos during development offers several advantages as the intestine is free from food materials, environmental and gut bacteria, and other contaminating organisms. During present studies, we used turkey embryos to see the effects of specific food materials on gut growth, pathogenesis of enteric viral and bacterial pathogens (alone or in combination), and products from immune cells on the intestine. The present studies were carried out to evaluate the response of the gut-to-gut growth promoters, enteric pathogens, and immune cell mediators. **Methods:** Twenty-four-day-old turkey embryos were used during the studies. The embryos were inoculated via the intra-amniotic route using 0.2 ml inoculum. L-glutamine (200 mg/ml) and ethanolamine (500 mM) were injected on days 24, 25, and 26, and the weight of the intestine was determined at day 28 of embryo incubation to determine the effects of nutrients on the intestine. Embryos (24-day-old) were inoculated with an *Enterovirus* alone or in combination with *Campylobacter jejuni*. The weight of the intestine, amount of fluid secretion, and the activity of maltase and intraepithelial lymphocytes were determined at day-28. To determine the effects of immune cell mediators, the concentrated supernatant from Concanavalin-A (Con-A) and Lipopolysaccharide (LPS) stimulated lymphocytes was injected into 24-day-old embryos via the amniotic route. **Results:** Both the L-Glutamine and Ethanolamine resulted in an increase in the weight of the intestine with L-glutamine. There was a significant increase in the amount of intestinal fluid secretion and activity of intestinal intraepithelial lymphocytes by the embryos inoculated with *Campylobacter jejuni* following virus inoculation, when compared with embryos inoculated with virus alone. The activity of brush border maltase was significantly reduced for embryos with dual inoculation. The amount of intestinal fluid secretion was significantly higher for embryos inoculated with Con-A stimulated lymphocytes products. However no response was observed for inoculum from LPS-stimulated lymphocytes. **Conclusion:** This study shows that the gut of the embryo is functionally and immunologically developed, with the studied response parallel to those explicated by the hatched, young turkeys.

MM-004

TMF amplifies glucose-stimulated insulin secretion via the cAMP-PKA signaling Cascade

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Aims/objectives: TMF, a natural bioflavonoid, is reported as an anti-diabetic agent since it possesses the ability to inhibit α-glucosidase activity, cause stimulation of insulin action and secretion, manage ROS, and prevent diabetes complications. TMF was identified as a new insulin secretagogue that enhances glucose-stimulated insulin secretion and seems like a better antidiabetic drug candidate. Here we explored the insulinotropic mechanism(s) of TMF in vitro in mice islets and in-silico. **Methods:** Size-matched pancreatic islets were divided into groups and incubated in the presence or absence of TMF and agonists/antagonists of major insulin signaling pathways. The secreted insulin was measured by ELISA. Molecular docking studies were performed with the key player of insulin secretory pathways. **Results:** Exposure of differentiated SH-SY5Y cells to Aβ25–35 is expected to significantly reduce cell viability, increase TMF dose-dependently enhanced insulin secretion in isolated mice islets, and its insulinotropic effect was exerted at high glucose concentrations, distinctly different from glibenclamide. TMF-induced insulin secretion was significantly inhibited by diazoxide. Furthermore, TMF amplified glucose-induced insulin secretion in depolarized and glibenclamide-treated islets. TMF showed an additive effect with forskolin and IBMX-induced insulin secretion. Interestingly, H89, a PKA inhibitor, and MAY0132, an Epac-2 inhibitor, significantly inhibited TMF-induced insulin secretion. The in-silico molecular docking. **Conclusion:** TMF, a potential natural insulin secretagogue, amplifies glucose-induced insulin secretion via the cAMP- PKA-Epac-2 signalling pathway.

MM-005

TRPML1 Agonist ML-SA5 Mediates Mitochondrial and Lysosomal Dysfunction and Cell Death in Triple-Negative Breast Cancer Cell Line

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Background: Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype that expresses neither estrogen receptors, progesterone receptors, nor HER2; therefore, limiting the current available treatments. Recent evidence shows that the lysosomal cation channel TRPML1 is overexpressed in TNBC cells, whereas minimal in normal cells, and hence can be targeted for anticancer therapy. The objective of the present study is to determine whether Mucolipin synthetic agonist 5 (ML-SA5), a small-molecule TRPML1 agonist, selectively induces cell death in MDA-MB-231 cells and to elucidate the cellular pathways involved in this process. **Methods**: This study was conducted in the MDRL 1 and 2 Lab, Ziauddin University, Clifton campus, and this is an in-vitro experimental study completed in 8 months. The cytotoxicity of MDA-MB 231 cells exposed to various doses of ML-SA5 was studied over different time durations using the MTT assay. Cell death was further analyzed by propidium iodide and annexin V-FITC staining. Changes in mitochondrial and lysosomal activity and intracellular reactive oxygen species were evaluated. **Results**: ML-SA5 induced dose-dependent cytotoxicity with IC_{50} values in the lower micromolar range (6.8 **µM)**. PI staining also confirmed cell death, with minimal apoptosis. Mitochondrial staining revealed altered morphology and compromised function. Lysosomal labeling showed enlarged lysosomes, suggesting impaired lysosomal integrity. A significant rise in ROS levels was observed, indicating oxidative stress. **Conclusion:** The activation of TRPML1 by ML-SA5 leads to increased oxidative stress and damage to mitochondria and lysosomes, resulting in cell death in TNBC cells.

MM-006

Exploring the Therapeutic Potential of Novel Cardenolides in Chronic Myeloid Leukemia via Targeting the Ras Signaling Pathway

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Chronic myeloid leukemia (CML) constitutes approximately 15% of adult leukemia cases and is a clonal myeloproliferative disorder characterized by a specific reciprocal translocation between chromosomes 9 and 22. Despite advances in therapy, the emergence of resistance to several anticancer agents, including those used in leukemia treatment, remains a significant clinical challenge. This underscores the urgent need for novel therapeutic compounds capable of selectively targeting leukemic cells with minimal toxicity. In the present study, the cytotoxic potential of novel cardenolides was evaluated in K562 cells. Given that Ras is among the most frequently dysregulated genes in cancer, cardenolides were investigated for their potential inhibitory effects on the Ras signaling pathway. K562 cells were cultured, and cell viability following cardenolide treatment was assessed using the MTT assay. The expression levels of HRAS, KRAS, BAX, CASP3, and BIRC5 were analyzed by RT-PCR. Cellular morphological changes following treatment at IC_{50} concentrations were examined using atomic force microscopy (AFM). Additionally, the effect of cardenolides on reactive oxygen species (ROS) production was investigated. After 48 hours of treatment, both cardenolides exhibited significant cytotoxic activity ($P < 0.05$) against K562 cells, with IC_{50} values of 0.25 µM and 1 µM, respectively. RT-PCR analysis revealed downregulation of HRAS expression accompanied by upregulation of the pro-apoptotic genes BAX and CASP3. Moreover, both compounds markedly suppressed the expression of BIRC5, a key anti-apoptotic gene. AFM imaging further confirmed apoptotic cell death, as evidenced by characteristic membrane blebbing. An increase in intracellular ROS levels was also observed at IC_{50} doses. Overall, the findings demonstrate that cardenolides significantly reduced cell viability ($P < 0.05$) and induced apoptosis in K562 cells in a time- and dose-dependent manner. These results suggest that novel cardenolides may represent promising therapeutic candidates for the treatment of chronic myeloid leukemia.

MM-007

Organosilane K21–Mediated Induction of Oxidative Stress and Organelle Dysfunction in Breast Cancer Cells

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Introduction: Breast cancer remains a major global health burden, with aggressive and treatment-resistant subtypes posing significant therapeutic challenges. Recent interest has emerged in organosilane-based compounds due to their potential anticancer properties and ability to disrupt intracellular signaling and organelle homeostasis. K21, a novel organosilane compound, is anticipated to exhibit selective cytotoxicity against breast cancer cells; however, its mechanistic effects remain to be elucidated. The present study will aim to evaluate the cytotoxic potential of K21 in breast cancer cell lines and to investigate the cellular pathways involved in K21-mediated cell death. **Methodology:** This will be an in vitro experimental study conducted at MDRL 1 and 2 Laboratories, Ziauddin University, Clifton Campus, over a duration of eight months. Breast cancer cells will be treated with increasing concentrations of K21 for varying time intervals. Cell viability will be assessed using the MTT assay to determine dose- and time-dependent cytotoxicity and to calculate IC_{50} values. Patterns of cell death will be analyzed using Annexin V–FITC and propidium iodide staining. Mitochondrial integrity, lysosomal function, and intracellular reactive oxygen species (ROS) levels will be evaluated using fluorescence-based assays. **Expected Results:** K21 treatment is expected to induce dose-dependent cytotoxicity in breast cancer cells, with IC_{50} values anticipated in the low micromolar range. It is anticipated that K21 will predominantly induce non-apoptotic cell death, accompanied by increased membrane permeability. Mitochondrial dysfunction, altered morphology, and compromised activity are expected to be observed. Additionally, lysosomal enlargement and destabilization are anticipated, along with a significant elevation in intracellular ROS levels, indicating oxidative stress. **Conclusion:** It is anticipated that K21 will induce breast cancer cell death through ROS-mediated mitochondrial and lysosomal dysfunction. The findings of this proposed study are expected to provide mechanistic insights into the anticancer potential of organosilane K21 and support its future development as a novel therapeutic candidate for breast cancer.

MM-008

Development of a One-Step TAMPA Assay for SNP-Specific BRCA1 Variant Detection in Molecular Diagnostics

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Background: BRCA variants have been extensively documented as a causative factor in hereditary breast and ovarian cancer (HBOC). The confirmatory diagnostics currently employ next-generation sequencing, a high-cost, labour-intensive procedure, which limits its use for large-scale screenings and in low-income countries. Therefore, a high-precision, rapid, and cost-effective method is needed to detect pathogenic variants in BRCA1. This study aims to develop and validate a cost-effective qPCR-based method for detecting pathogenic variants at rs80357260 (c.4183C>T) in the BRCA1 gene. This was achieved by integrating One-step Tetra Primer Amplification Refractory Mutation System Sybr Green qPCR with Melting Curve Analysis in short (TAPMA). **Methods:** This study involved the design of primers for the TAPMA assay. Genomic DNA was extracted, and qPCR was performed using Maxima SYBR Green/ROX qPCR Master Mix (Thermofisher Scientific, USA), followed by melting curve analysis on the Real-Time PCR system, StepOneTM (Applied Biosystems, USA). High-resolution melting analysis (HRM) was performed using Precision Melt Analysis software on a CFX96 Real-Time PCR System (Bio-Rad, USA). **Results:** The diagnostic assessment of TAPMA and HRM indicates that both systems perform equally well across all measures. The TAPMA method is validated by comparing results with those obtained from BRCA1 and BRCA2 long-range PCR and next-generation sequencing using an Oxford Nanopore MinION R9 Flow Cell (Oxford Nanopore Technologies plc, United Kingdom). These data confirm the reliability and precision of the TAPMA assay over multiple dilutions, establishing it as a robust approach for mutation identification. **Conclusion:** The TAPMA method is a highly reliable, rapid, and cost-effective approach for detecting the BRCA1 SNP-specific pathogenic variant c.4183C>T. It demonstrates high accuracy in differentiating between wild-type and mutated DNA. Additional research is needed to determine whether this technique can be adapted to other BRCA1 SNP-specific variants and extended for broader clinical use. Overall, One-Step Tetra-ARMS-qPCR combined with melting-curve analysis is a practical, adaptable, and high-throughput technique suitable for BRCA1 and BRCA2 SNP screening in clinical settings.

MM-009

Unique Actin-binding and immune checkpoint proteins in TNBC from Karachi subset of Population

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Background: Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer characterized by the absence of estrogen, progesterone, and HER2 receptors, leading to limited therapeutic options. Identification of reliable biomarkers is crucial to improve disease monitoring and guide personalized treatment strategies. **Objective:** To identify the candidate proteins in TNBC patients, focusing on actin-binding and immune checkpoint proteins as potential biomarkers for disease progression and therapeutic response. **Methods:** A quasi-experimental study design was employed, including three groups: pre-NAC TNBC patients, post-NAC TNBC patients, and 30 healthy females. Of 1,737 breast cancer patients, 149 were diagnosed with TNBC, and 78 were recruited; pre-treatment data were available for 54 patients. Proteomic analysis was performed using liquid chromatography–tandem mass spectrometry (LC–MS/MS), and protein identification was conducted via Mascot and Scaffold software. Selected proteins were further validated using ELISA. Immunohistochemistry (IHC) was performed. Tumor-infiltrating lymphocytes (TILs) were scored according to international guidelines, and LAG-3 expression was assessed. Bioinformatics analyses, including protein-protein interaction networks, were performed using STRING. **Results:** A total of 299 serum proteins were identified, of which forty were uniquely detected in TNBC patients, including cytoskeletal regulators such as Action binding protein Transgelin-2 (TAGLN2). TAGLN2 levels were significantly elevated in TNBC and decreased after NAC ($p < 0.0001$), highlighting its potential as a dynamic biomarker. IHC analyses revealed a strong correlation between LAG-3 expression and TIL density ($p < 0.001$), indicating coordinated modulation of immune checkpoints within the tumor microenvironment. **Conclusion:** This integrative proteomic and immunohistochemical study demonstrates coordinated dysregulation of cytoskeletal and immune pathways in TNBC and identifies TAGLN2 as a promising biomarker for monitoring disease progression and response to NAC. The correlation between immune checkpoint expression and TIL infiltration underscores the potential of immune profiling to guide personalized therapeutic strategies in TNBC.

MM-010

Diosmetin acutely potentiates the glucose-induced insulin secretion *via* the PKA amplifying pathway, independent of the K-ATP channel

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Background/Aims: Claims and counter-claims exist about pathophysiology in Asian type 2 diabetic subjects; however, recent reports suggest that insulin secretory impairment is predominant in Asians. Diosmetin, a natural bioflavonoid, was identified as a new insulin secretagogue that enhances glucose-stimulated insulin secretion and seems better candidate than sulfonylureas. Here, we explored the insulinotropic mechanism(s) of diosmetin. **Methods**: Mice pancreatic islets were batch incubated with diosmetin in the presence of pharmacological agonists/antagonists. The secreted insulin levels were measured using mice insulin ELISA kit. **Results**: Diosmetin dose-dependently enhanced insulin secretion in isolated mice islets, and its insulinotropic effect was exerted at high glucose concentrations, distinctly different from glibenclamide. Furthermore, diosmetin amplified glucose-induced insulin secretion in depolarized and glibenclamide-treated islets. Diosmetin showed an additive effect was observed by diosmetin in both forskolin and IBMX-induced insulin secretion. Interestingly, H89, a PKA inhibitor, and U0126, a MEK kinase inhibitor, significantly inhibited diosmetin-induced insulin secretion. These results were further validated through computational analysis, where diosmetin showed the best binding affinity with PKA. **Conclusion**: Diosmetin exerts glucose-stimulated insulin secretion by modulating the PKA signaling cascade independent of K-ATP channels.

MM-011

Hispidulin is an insulin secretagogue targeting the AKAP9-mediated PKA signaling pathway

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Aims/objectives: Apigenin, a natural bioflavonoid, is reported as an anti-diabetic agent since it possesses the ability to inhibit α-glucosidase activity, cause stimulation of insulin action and secretion, manage ROS, and prevent diabetes complications. Apigenin was identified as a novel insulin secretagogue that enhances glucose-stimulated insulin secretion, suggesting it may be a more promising antidiabetic drug candidate. Here we explored the insulinotropic mechanism(s) of apigenin in vitro in mice islets and in vivo in diabetic rats. **Methods:** Size-matched pancreatic islets were divided into groups and incubated in the presence or absence of apigenin and agonists or antagonists of major insulin signaling pathways. ELISA was used to measure the secreted insulin. The intracellular cAMP was estimated by the cAMP acetylation assay. The acute and chronic effects of apigenin were evaluated in diabetic rats. **Results:** apigenin dose-dependently enhanced insulin secretion in isolated mice islets, and its insulinotropic effect was exerted at high glucose concentrations, distinctly different from glibenclamide. Furthermore, apigenin amplified glucose-induced insulin secretion in depolarized and glibenclamide-treated islets. Apigenin showed no effect on intracellular cAMP concentration; however, an additive effect was observed by apigenin in both forskolin and IBMX-induced insulin secretion. Interestingly, H89, a PKA inhibitor, and U0126, a MEK kinase inhibitor, significantly inhibited apigenin-induced insulin secretion; however, no significant effect was observed by using ESI-05, an epac2 inhibitor. Apigenin improved glucose tolerance and increased glucose-stimulated plasma insulin levels in diabetic rats. Apigenin also lowered blood glucose in diabetic rats upon chronic treatment. **Conclusion:** Apigenin exerts glucose-stimulated insulin secretion by modulating the PKA-MEK kinase signaling cascade independent of K-ATP channels.

MM-012

Direct-Acting Antiviral Resistance in Hepatitis C Virus.

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Background and Objectives: Chronic infection with the hepatitis C virus (HCV) remains a major cause of cirrhosis, hepatic decompensation, and hepatocellular carcinoma worldwide, despite the availability of curative direct-acting antiviral (DAA) therapies. Global estimates indicate that liver disease continues to account for a substantial burden of morbidity and mortality, with cirrhosis and HCC representing the majority of liver-related deaths. To identify and characterize resistance-associated variants to direct-acting antivirals in hepatitis C virus genotypes. **Methods:** Blood samples will be collected from HCV patients exhibiting treatment resistance or relapse patients. Viral RNA will be isolated from serum extracts and reverse-transcribed into complementary DNA. Whole Genome Sequencing will be performed on the cDNA to generate complete viral genomic profiles. Experimental sequences will be integrated with reference HCV genomes retrieved from NCBI databases. A pangenome will be constructed to identify specific genetic variations and potential drug targets. **Results:** In our study, we expect to find specific mutations in the Core protein and NS5A region that are statistically more frequent in patients with cirrhosis and HCC. In patients who relapsed, we expect to see an enrichment of RASs (like Y93H or L31M) compared to naive patients. **Conclusion:** This research proposal highlights that the failure of DAA therapy is not merely a clinical setback but a genomic event with profound implications for liver health. By successfully mapping the whole-genome sequences of HCV in relapsed and cirrhotic patients, we expect to establish a clear link between specific Resistance-Associated Substitutions (RASs) and the accelerated progression of Hepatocellular Carcinoma (HCC).

MM-013

A Systematic Review of Natural Compounds Targeting Pancreatic β-cell Apoptosis via Mitochondria-Mediated Pathway

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Background and objectives: Diabetes mellitus is a chronic metabolic illness, caused by dysfunction of pancreatic β-cells to release insulin or reduced efficiency of insulin in target cells, resulting in hyperglycemia. Severe Insulin-Deficient Diabetes type is widely present in the Southeast Asian population, highlighting pancreatic β-cell apoptosis as the primary contributor to diabetes mellitus. Several conventional antidiabetic drugs are in use, which in turn result in various adverse effects; amongst them, drugs with antiapoptotic activity show better results as compared to other antihyperglycemic drugs. However, synthetic drugs fail to retain their antiapoptotic potential after persistent use. Therefore, studies to find suitable and safe medicines from natural sources are being conducted by the researchers with find drugs with persistent, long-lasting antihyperglycemic effects with potential to prevent from β cell apoptosis. The aim of present study is to comprehensively review the insulin secretagogue phytochemical compounds regulating pro and anti-apoptotic activities. **Methods:** A systematic review is conducted after constructing the P(I/E) CO framework. Full text, original article of previous 10 years with free availability are included in this study. 3 search engines Pubmed, Google scholar and Scopus, are used to extract the data. A total number (n=5201) is obtained based on keywords. After removing duplications, title-based, abstract-based, and full text-based screening (n=8) systematic reviews are included in this study. **Results:** Baicalin, Cinnamon, Dracorhodin perchlorate, Hesperidin, L. Flavescens, Naringin, Quercetin, and Red Ginseng possess antihyperglycemic effects in a wide range of in Vivo, ex Vivo and in Vitro models of diabetes with antiapoptotic properties. The involved mechanisms include pancreatic β cell survival by maintaining mitochondrial membrane integrity by preventing release of Cytochrome C due to their antioxidant properties, upregulating BCL-2 and BCL-xL expression, downregulating Bax and Caspase 3 expression, and increasing cell viability through PI3K/Akt signaling. **Conclusion:** Alkaloids, flavonoids, polyphenols, and cinnamates with potential antiapoptotic activity may be considered as primary or alternate and add-on antidiabetic drugs for modifying disease progression and improving long-term clinical outcomes. Further preclinical and clinical studies are still needed in order to recognize the structure-activity relationships, drug tolerance, metabolism, and absorption of these plant-derived natural agents.

MM-014

Comparative Analysis of Genomic Variants in Oral Squamous Cell Carcinoma and Normal Tissue Using Next-Generation Sequencing in a Subset of the Pakistani Population

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Background: Oral squamous cell carcinoma (OSCC) is a leading malignancy in South Asia, characterized by aggressive local invasion and high recurrence rates. Genetic alterations, including single-nucleotide variants (SNVs) and copy number variations (CNVs), play a critical role in OSCC pathogenesis by affecting oncogenes, tumor suppressor genes, and signaling pathways. Next-generation sequencing (NGS) enables comprehensive identification of such genomic variants, providing insights into molecular mechanisms driving tumor initiation and progression. Despite the high burden of OSCC in Pakistan, population-specific genomic profiles remain largely unexplored. Comparing genomic alterations between OSCC and matched normal tissues in this demographic may uncover novel variants with potential diagnostic, prognostic, and therapeutic relevance. **Aims and Objectives:** To identify and catalog genomic variants present in OSCC tissues compared to normal oral mucosa in a subset of the Pakistani population. To evaluate the potential pathogenic significance of these variants and their association with OSCC susceptibility and progression. **Methodology**: A case-control study design will be employed involving OSCC patients undergoing surgical resection and matched healthy oral tissue controls. Genomic DNA will be extracted from both tumor and normal tissues, followed by whole-exome sequencing using NGS platforms. Bioinformatic analyses will identify SNVs, insertions/deletions, and copy number variations. Variant annotation, pathway enrichment, and statistical comparisons will be performed to determine significant differences between OSCC and normal tissues. **Results**: The study is expected to reveal a spectrum of genomic alterations specific to OSCC in Pakistani patients, including potentially novel variants. Comparative analysis will likely highlight recurrent mutations in oncogenes and tumor suppressor genes, providing insights into molecular mechanisms of carcinogenesis and population-specific risk factors. **Conclusion**: This study will generate a comprehensive genomic landscape of OSCC in a Pakistani cohort, offering valuable data for understanding population-specific molecular drivers. Findings may contribute to improved molecular diagnostics, personalized risk stratification, and the development of targeted therapeutic strategies for OSCC.

MM-015

Preventive role of Diosmetin on Amyloid-β-25-35-induced in vitro Alzheimer's Disease Model

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Background/objectives: Alzheimer's disease is a progressive neurodegenerative condition in which amyloid-related toxicity contributes to neuronal loss and functional decline. Experimental models using amyloid fragments offer a controlled way to study early cellular events linked to neurodegeneration. This study aimed to evaluate whether diosmetin, a plant-derived flavonoid, can protect neuronal cells against Aβ25–35-induced cytotoxicity and explore the possible mechanism of action. **Methods:** An Alzheimer's-like injury model was established by exposing differentiated SH-SY5Y cells to Aβ25–35. Cell viability following treatment with various concentrations of diosmetin (10–80 µM) was quantified using the MTT assay. Cellular Damage was further examined with PI and DAPI staining. To investigate the involvement of specific pathways, inhibitors were used in combination with diosmetin. **Results:** Exposure to Aβ25–35 resulted in a marked reduction in cell viability. Diosmetin treatment produced a concentration-dependent increase in cell survival, with noticeable protection observed within the range of10–80 µM. Fluorescence staining showed a visible decline in cellular damage features in diosmetin-treated cells. The significant loss of protection in the presence of pathway inhibitors indicated that diosmetin's effect is mediated through the MEK Kinase signaling pathway. **Conclusion:** Diosmetin attenuated amyloid-induced cytotoxicity in SH-SY5Y cells and reduced cell death, suggesting a neuroprotective effect. These findings highlight diosmetin as a potential therapeutic candidate that warrants further investigation in Alzheimer's disease models.

MM-016

Convergent Plasma Proteomic Signatures Reveal Translational Molecular Pathways in Pediatric Acute Lymphoblastic Leukemia: A Systematic Review and Meta-analysis

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Background and Objectives: Pediatric acute lymphoblastic leukemia (ALL) exhibits marked molecular heterogeneity, and current risk stratification relies largely on genomic, cytogenetic, and minimal residual disease markers. Plasma proteomics offers a minimally invasive strategy to capture dynamic molecular changes reflecting both leukemic biology and host response. However, existing plasma proteomic studies in pediatric ALL remain fragmented due to small sample sizes, diverse analytical platforms, and inconsistent findings, limiting clinical translation. The study aimed to systematically integrate plasma proteomic evidence in pediatric ALL, identify reproducible dysregulated protein across heterogeneous studies, characterize convergent molecular pathways, and evaluate their translational relevance within a molecular medicine framework. **Methods:** A systematic review and meta-analysis were conducted according to PRISMA 2020 (PROSERO: CRD420251148697). Three electronic databases (PubMed, Scopus, and Google Scholar) were searched for plasma proteomic studies involving pediatric ALL. Discovery and validation studies using mass spectrometry-based platforms were included. Methodological quality were assessed using the QUADOMICS framework. Differentially expressed proteins were synthesized qualitatively, while random-effects meta-analysis was performed for proteins reported in multiple independent studies with extractable quantitative data. **Results:** Nine eligible studies were included, collectively reporting 679 differentially expressed plasma proteins. Despite substantial heterogeneity in study design and proteomic platform, consistent molecular patterns emerged. Recurrently upregulated proteins were enriched for acute-phase reactants, inflammatory mediators, and coagulation factors, including ceruloplasmin, haptoglobin, alpha-1-antitrypsin, fibrinogen chains, and S100A8/A9. Proteins involved in lipid transport and metabolic regulation, such as apoplipoprotein A1, transthyretin, and gelsolin, were consistently downregulated. Meta-analysis identified S100A8 as the only protein with quantitative reproducibility, demonstrating significant elevation in pediatric ALL (SMD = 2.72; 95% CI 1.14-4.29; I^2 = 0%). Pathway analysis revealed convergence on inflammatory signaling, complement-coagulation cascades, lipid metabolism, oxidative stress, and cytoskeletal remodeling, indicating a systemic immune-metabolic plasma phenotype. **Conclusion:** Systematic integration of plasma proteomic studies reveals reproducible molecular signatures in pediatric ALL, dominated by inflammatory and metabolic pathways. S100A8 emerges as a reproducible plasma-level molecular signal, supporting plasma proteomic as a promising translating tool in molecular medicine Standardized validation is required to enable clinical implementation.

MM-017

Aerobic Exercise Combined with GLP-1 receptor agonists exerts anti-diabetic activity through action of pancreatic β cells

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Background and Objectives: Type 2 Diabetes (T2D) has insulin resistance and progressive pancreatic β-cell dysfunction leading to hyperglycemia. Exercise and glucagon-like peptide-1 receptor agonists (GLP-1 RAs) independently enhance glycemic control through complementary mechanisms. However, their combined influence on pancreatic β-cell function and morphology remains inadequately explored. This study evaluated the effects of aerobic exercise, alone and in combination with GLP-1 RA (Semaglutide), in a streptozotocin (STZ)-induced diabetic rat model. **Methods:** Twenty-four male Wistar rats were randomly assigned into four groups (n = 6 each): healthy control, diabetic control, diabetic + aerobic exercise, diabetic + aerobic + GLP-1 RA, diabetic. Aerobic training involved treadmill running (40 min/session) for six weeks. Semaglutide (20 µg/kg) was administered subcutaneously to the respective combination groups. Blood glucose was measured weekly; oral glucose tolerance test (OGTT) and insulin ELISA were performed. Pancreatic tissues were processed for hematoxylin & eosin (H&E) staining, morphometry, and immunofluorescence for insulin. **Results:** T2D rats exhibited persistent hyperglycemia and reduced insulin secretion with marked destruction of islets. The aerobic group significantly reduced blood glucose levels and restored insulin response. The combined aerobic + GLP-1 RA group showed improved weekly blood glucose, improved glucose tolerance, and significantly enhanced the glucose-stimulated insulin secretion (GSIS). Histomorphology and Immunofluorescence analyses revealed restoration of β-cell morphology and insulin immunoreactivity, and proliferation of islets with increased β-cell area, number, and intensity of insulin immunostaining in the aerobic group; however, the aerobic + GLP-1 RA group exhibits near-normal parameters. **Conclusion:** Aerobic exercise combined with GLP-1 RA exerts potential anti-diabetic effects. By acting on pancreatic β-cells, it improves glucose tolerance, significantly reduces the blood glucose levels, amplifies GSIS, and islet architecture, suggesting that combined pharmacological and lifestyle interventions may offer superior therapeutic benefits for T2D management.

MM-018

Resistance Exercise Combined with GLP-1 receptor agonists exerts anti-diabetic activity through action of pancreatic β cells

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Background and Objectives: Type 2 Diabetes (T2D) has insulin resistance and progressive pancreatic β-cell dysfunction leading to hyperglycemia. Exercise and glucagon-like peptide-1 receptor agonists (GLP-1 RAs) independently enhance glycemic control through complementary mechanisms. However, their combined influence on pancreatic β-cell function and morphology remains inadequately explored. This study evaluated the effects of resistance exercise, alone and in combination with GLP-1 RA (Semaglutide), in a streptozotocin (STZ)-induced diabetic rat model. **Methods:** Twenty-four male Wistar rats were randomly assigned into four groups (n = 6 each): healthy control, diabetic control, diabetic + resistance exercise, diabetic + resistance + GLP-1 RA, diabetic. Resistance involved climbing-ladder like apparatus by allowing them to climb (3 sets of 10 repetitions on three alternate days per week) for six weeks. Semaglutide (20 µg/kg) was administered subcutaneously to the respective combination groups. Blood glucose was measured weekly; oral glucose tolerance test (OGTT) and insulin ELISA were performed. Pancreatic tissues were processed for hematoxylin & eosin (H&E) staining and morphometry analysis. **Results:** T2D rats exhibited persistent hyperglycemia and reduced insulin secretion with marked destruction of islets. The resistance group significantly reduced blood glucose levels and restored insulin response. The combined resistance + GLP-1 RA group showed in weekly blood glucose, improved glucose tolerance and significantly enhanced the glucose-stimulated insulin secretion (GSIS). Histomorphology analyses revealed restoration of β-cell in the resistance group; however, the resistance + GLP-1 RA group exhibits near-normal parameters. **Conclusion:** Resistance exercise combined with GLP-1 RA exerts potential anti-diabetic effects. By acting on pancreatic β-cells, it improves glucose tolerance, significantly reduced the blood glucose levels, amplify GSIS, and islet architecture, suggesting that combined pharmacological and lifestyle interventions may offer superior therapeutic benefits for T2D management.

MM-019

Sesamol as a Modulator of Extracellular Matrix and Fibrotic Signaling in Oral Submucous Fibrosis

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Oral submucous fibrosis (OSMF) is a chronic, potentially malignant disorder of the oral cavity characterized by juxta-epithelial inflammation and progressive fibrosis. Despite the availability of multiple treatment strategies, no single therapy has proven completely effective in reversing fibrosis or halting disease progression. Sesamol, a natural phenolic compound derived from sesame seeds, exhibits potent antioxidant, anti-inflammatory, and anticancer properties. However, its antifibrotic and antiproliferative, and anti-migratory effects in OSMF remain underexplored. The present *in-vitro* experimental study was conducted at the College of Molecular Medicine, Ziauddin University, Clifton Campus, Karachi, from December 2024 to August 2025, to evaluate the antiproliferative antifibrotic, and anti-migratory effects of sesamol on primary OSMF cell line. Cells (passages 8–10) were treated with sesamol at its IC_{50} concentration, while dexamethasone (IC_{50}) served as the reference standard. Cell viability and proliferation were assessed by MTT assay, antimigratory potential by wound-healing assay, and antifibrotic effects by Masson's trichrome staining and quantitative gene expression profiling of fibrosis-associated markers, including MMP1, MMP2, TIMP1, TGF-β1, TGFBR1, α-SMA, COL1A2, PAI1, PDGF, CTGF, COMP, VIMP, and fibronectin. Sesamol treatment significantly reduced OSMF cell viability in a dose- and time-dependent manner, demonstrating marked antiproliferative and antimigratory effects with delayed wound closure. Masson's trichrome staining revealed a notable reduction in collagen deposition, confirming its antifibrotic potential. Gene expression analysis showed that sesamol upregulated MMP1 and MMP2 while downregulating COL1A2, α-SMA, TGF-β1, TGFBR1, and PAI1, indicating enhanced extracellular matrix degradation and inhibition of fibrotic signaling. In contrast, dexamethasone broadly suppressed MMPs but increased several profibrotic genes. Overall, sesamol demonstrated potent antiproliferative, antimigratory, and antifibrotic effects on primary OSMF fibroblasts by promoting ECM remodeling, reducing collagen deposition, and downregulating key profibrotic mediators, suggesting its potential as a safer and targeted therapeutic alternative to corticosteroids for the treatment of oral submucous fibrosis.

MM-020

Enhancing the Neuroprotective Potential of Mesenchymal Stem Cell-Derived exosomes through Preconditioning in SH-SY5Y cells

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Aims/objectives: Alzheimer's disease is characterized by amyloid-β aggregation, tau hyperphosphorylation, oxidative stress, mitochondrial dysfunction, neuroinflammation, and progressive neuronal loss, with reactive oxygen species driving disease progression. The limited clinical efficacy of antioxidant therapies highlights the need for alternative strategies. Mesenchymal stem cells and their exosome-rich secretome offer promising cell-free neuroprotective approaches. This study aims to evaluate the neuroprotective effects of valproic acid-preconditioned human umbilical cord-derived MSC secretome against amyloid-β (Aβ25-35)-induced neurotoxicity in SH-SY5Y cells. **Methods:** hUC-MSCs will be isolated from healthy donors and characterized at passages 3-5 by mesenchymal marker expression (Vimentin, CD90), absence of CD45, and tri-lineage differentiation. Valproic acid cytotoxicity and optimal preconditioning doses will be determined by MTT assay. MSCs will be preconditioned with valproic acid, and the secretome will be isolated using differential centrifugation and ultracentrifugation, followed by protein quantification and transmission electron microscopy. Neuroprotective effects will be assessed in differentiated SH-SY5Y cells exposed to Aβ25-35 by evaluating cell viability, apoptosis, and Alzheimer's disease-related molecular markers, with pharmacological antagonists used to explore underlying signaling pathways. **Results:** Exposure of differentiated SH-SY5Y cells to Aβ25–35 is expected to significantly reduce cell viability, increase oxidative stress and apoptosis, and elevate Alzheimer's disease-related markers, including amyloid burden and tau hyperphosphorylation. Treatment with valproic acid preconditioned hUC-MSC-derived secretome is anticipated to restore cell viability, attenuate apoptosis, and suppress AD-associated molecular changes in a dose-dependent manner. Valproic acid preconditioning is expected to further enhance these neuroprotective effects, likely *via* modulation of anti-apoptotic, antioxidant, and anti-inflammatory signaling pathways. **Conclusion:** Valproic acid -preconditioned hUC-MSC-derived secretome may represent a promising cell-free therapeutic strategy for mitigating amyloid-induced neurotoxicity and managing Alzheimer's disease and related neurodegenerative disorders.

MM-021

Human Papillomavirus Genotype Distribution in Cervical Cytology and Histological Specimens: Implications for Screening

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Background and Objectives: Cervical cancer remains the fourth most common malignancy among women worldwide, with high-risk human papillomavirus (HR-HPV), particularly genotypes 16 and 18, being the major etiological factor. The study's objectives were to determine the genotype distribution of HPV and to identify the evolutionary origin of HPV genotype through Phylogenetic analysis. **Methods:** A total of 100 cases with normal cervical cytology and 49 cases of premalignant and malignant lesions were subjected to DNA extraction followed by Sanger sequencing. A phylogenetic tree was generated using the Maximum Likelihood (ML) method with 500 bootstrap replications to determine clustering reliability. Bootstrap values ≥70% were considered significant for genotype assignment. **Results:** Out of the 100 smears with normal cervical cytology, HPV was positive in 2(1.3%) cases. Out of 49 malignant and premalignant lesions, HPV was positive in 34 (69.3%) cases. Out of the 36 HPV positive smears, 3(8.1%) had LR-HPV-11, and 33(91.6%) had HR-HPV (16, 18, 31, 45, and 59). HPV 16 (41.6%) is the most common, followed by HPV18(33.3%). Multivariate regression analysis showed that the women who presented with Post Coital Bleeding are 6.7 times more likely (O. R=6.701; CI=1.924-23.336; p=0.003) to have HPV infection. The phylogenetic analysis grouped the HPV genotypes with established lineages, confirming their evolutionary relationships and geographic similarities. **Conclusion:** The markedly higher frequency of HPV among women with premalignant and malignant cervical lesions (69.3%) compared with those with normal cytology (1.3%) underscores a failure in early detection. These findings strongly suggest the absence of cervical screening programmed within the general population, emphasizing the urgent need for structured HPV-based screening to reduce cervical cancer.

MM-023

Exploring the Dual Anti-neuroblastoma and Neuroprotective Effects of Tambulin in SH-SY5Y Cells

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Aims/objectives: Oxidative stress plays a central role in neuronal damage, neurodegeneration, and certain brain cancers. Neurons are highly vulnerable to ROS due to high metabolic demand and limited antioxidant defenses, while neural cancers exhibit heterogeneity that contributes to toxicity and treatment resistance. Despite being a therapeutic target, antioxidants show limited clinical efficacy, prompting interest in multi-target natural compounds. Tambulin, a plant-derived flavonoid, shows neuroprotective and anticancer effect effects. In the present study, we will evaluate the dual effect of Tambulin in the undifferentiated neuroblastoma and differentiated neuronal cells. **Methods:** Undifferentiated SH-SY5Y cells will be treated with increasing concentrations of tambulin to analyse viability. For oxidative stress studies, SH-SY5Y cells will be differentiated using retinoic acid and BDNF, pre-treated with tambulin, and then exposed to an optimized hydrogen peroxide dose. ROS levels, cell survival, and morphological changes will be evaluated. **Expected Results:** Tambulin is expected to exert concentration-dependent cytotoxic effects in undifferentiated SH-SY5Y neuroblastoma cells, indicating anticancer activity. In contrast, in differentiated neuronal cells, tambulin pre-treatment is anticipated to significantly reduce H_2O_2-induced ROS generation, improve cell viability, and preserve neuronal morphology. These findings would support a dual, context-dependent role of tambulin, demonstrating neuroprotective effects under oxidative stress and selective anticancer activity in undifferentiated neuroblastoma cells. **Conclusion:** These findings may support tambulin as a promising plant-derived candidate with dual therapeutic potential in neural cancers and oxidative stress-driven neurodegeneration

MM-024

Exploring the Molecular Pathogenesis and Therapeutic Intervention for New-Onset Diabetes Through miRNA 375, miR-128-1-5p Expression

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Aims/objectives: Type 2 diabetes (T2D) is a progressive metabolic disorder marked by β-cell dysfunction and insulin resistance, resulting in chronic hyperglycemia. In Asian populations, impaired insulin secretion often predominates, highlighting the need for population-specific molecular insights. MicroRNAs (miRNAs) are key regulators of glucose homeostasis, β-cell function, and insulin signaling. Notably, miR-375 is associated with defective insulin secretion and β-cell dysfunction, whereas miR-128-1-5p contributes to insulin resistance. This study aims to evaluate the expression of miR-375 and miR-128-1-5p in newly diagnosed T2D patients, correlate their levels with clinicopathological features, and assess their modulation following therapeutic intervention. **Methods:** Newly diagnosed T2D patients and age-matched healthy controls will be enrolled. Circulating miR-375 and miR-128-1-5p levels will be quantified from plasma samples using RT-qPCR. Associations with glycemic indices, including fasting glucose, HbA1c, and insulin levels. Patients will receive standard-of-care treatment, including lifestyle modification and interventions. **Expected Results:** Newly diagnosed T2D patients are expected to exhibit significant upregulation of miR-375 compared to miR-128-1-5p, which will reflect the β-cell impairment more compared to insulin resistance, which is the predominant in our patients. Therapeutic interventions are anticipated to normalize these dysregulated miRNA levels, correlating with improved glycemic control, enhanced insulin secretion, and reduced insulin resistance. Distinct miRNA expression patterns may emerge, identifying dominant molecular defects and enabling stratification of patients based on underlying pathophysiology. **Conclusion:** Circulating miR-375 and miR-128-1-5p profiling may provide valuable insights into the molecular pathogenesis of new-onset T2D and support the development of personalized, miRNA-guided therapeutic strategies to improve clinical outcomes.

MM-026

Enhancing the Neuroprotective Potential of Mesenchymal Stem Cell-Derived exosomes through Preconditioning in SH-SY5Y cells

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Aims/objectives: Alzheimer's disease is characterized by amyloid-β aggregation, tau hyperphosphorylation, oxidative stress, mitochondrial dysfunction, neuroinflammation, and progressive neuronal loss, with reactive oxygen species driving disease progression. The limited clinical efficacy of antioxidant therapies highlights the need for alternative strategies. Mesenchymal stem cells and their exosome-rich secretome offer promising cell-free neuroprotective approaches. This study aims to evaluate the neuroprotective effects of Ascorbic acid-preconditioned human umbilical cord-derived MSC secretome against amyloid-β (Aβ25-35)-induced neurotoxicity in SH-SY5Y cells. **Methods:** hUC-MSCs will be isolated from healthy donors and characterized at passages 3-5 by mesenchymal marker expression (Vimentin, CD90), absence of CD45, and tri-lineage differentiation. Ascorbic acid cytotoxicity and optimal preconditioning doses will be determined by MTT assay. MSCs will be preconditioned with ascorbic acid, and the secretome will be isolated using differential centrifugation and ultracentrifugation, followed by protein quantification and transmission electron microscopy. Neuroprotective effects will be assessed in differentiated SH-SY5Y cells exposed to Aβ25-35 by evaluating cell viability, apoptosis, and Alzheimer's disease-related molecular markers, with pharmacological antagonists used to explore underlying signaling pathways. **Results:** Exposure of differentiated SH-SY5Y cells to Aβ25–35 is expected to significantly reduce cell viability, increase oxidative stress and apoptosis, and elevate Alzheimer's disease-related markers, including amyloid burden and tau hyperphosphorylation. Treatment with ascorbic acid preconditioned hUC-MSC-derived secretome is anticipated to restore cell viability, attenuate apoptosis, and suppress AD-associated molecular changes in a dose-dependent manner. Ascorbic acid preconditioning is expected to further enhance these neuroprotective effects, likely *via* modulation of anti-apoptotic, antioxidant, and anti-inflammatory signaling pathways. **Conclusion:** Ascorbic acid -preconditioned hUC-MSC-derived secretome may represent a promising cell-free therapeutic strategy for mitigating amyloid-induced neurotoxicity and managing Alzheimer's disease and related neurodegenerative disorders.

MM-027

The promising role of natural drug candidate on Alzheimer's disease using SH-SY5Y cells: A disease on a plate model

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Aims/objectives: Alzheimer's disease is the most common cause of dementia in older individuals and is characterized by a gradual decline in memory, cognition, and emotional stability. Its underlying cause depends on two main abnormalities: the accumulation of amyloid beta outside the nerve cells forming plaques and tau protein's hyperphosphorylation in neurons, leading to twisted tangles. This study aims to investigate the effects of a natural drug candidate in amyloid beta 25-35-induced Alzheimer's disease model using SH-SY5Y cells. **Methods:** An Alzheimer's-like injury model was established by exposing differentiated SH-SY5Y cells to Aβ25–35. Cell viability following treatment with various concentrations of diosmetin (10–80 µM) was quantified using the MTT assay. Cellular Damage was further examined with PI and DAPI staining. To investigate the involvement of specific pathways, inhibitors were used in combination with diosmetin. **Results:** Exposure to Aβ25–35 resulted in a marked reduction in cell viability. Diosmetin treatment produced a concentration-dependent increase in cell survival, with noticeable protection observed within the range of10–80 µM. Fluorescence staining showed a visible decline in cellular damage features in diosmetin-treated cells. The significant loss of protection in the presence of pathway inhibitors indicated that diosmetin's effect is mediated through the MEK Kinase signaling pathway. **Conclusion:** Diosmetin attenuated amyloid-induced cytotoxicity in SH-SY5Y cells and reduced cell death, suggesting a neuroprotective effect. These findings highlight diosmetin as a potential therapeutic candidate that warrants further investigation in Alzheimer's disease models.

MM-030

Identification of Conserved Genomic Regions in Dengue Virus Serotypes Using Genome-Wide Conservation Profiling

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Background: Highly conserved genomic regions across dengue virus (DENV) serotypes are promising targets for broadly effective therapeutic, preventive strategies and reliable diagnostics. Drawing on the increasing volume of publicly available DENV genomes from different continents makes it possible to analyze these regions in a systematic way using comparative genomics and quantitative conservation analysis. **Methods:** A structured literature review was conducted using PubMed and Google Scholar to identify recent studies describing the Genomic features of DENV. Fifty-three complete DENV genomes representing serotypes 1–4 from Pakistan, Bangladesh, Indonesia, Thailand, and France were retrieved from GenBank. Incomplete or ambiguous entries were excluded, and standardized metadata were compiled. Sequences were aligned in R using iterative multiple-sequence alignment procedures. Per-position conservation scores were calculated as proportions of the dominant nucleotide and summarized using a 20-nt sliding window. Segments with mean conservation ≥ 0.95 were identified as highly conserved regions amongst serotypes. **Results:** The alignment revealed extensive sequence identity across all serotypes, with conservation scores ranging from 0.30–1.00 (median ≈ 0.96; mean ≈ 0.83). Histograms and genome-wide plots demonstrated that most positions exceeded 0.95, with 246 windows (2.3%) meeting the high-conservation threshold. These windows clustered into long conserved blocks shared among diverse geographic isolates. **Statistical Analysis and Discussion** The pronounced skew toward high conservation underscores constraints on key genomic regions, several of which align with coding regions of known. These findings highlight conserved loci as prime candidates for rational epitope mapping, broad-spectrum antiviral development, and the design of robust diagnostic assays across all DENV serotypes.

MM-031

Investigating the Anticancer Effect of Antipsychotic Drug Aripiprazole on the Autophagy Pathway via Gene Expression Analysis

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Background: Aripiprazole, a dopamine D2 receptor partial agonist commonly used in psychiatric practice, has recently been identified as a potential candidate for drug repurposing in oncology due to its emerging antiproliferative activity in various tumor models. Despite increasing interest in psychotropic drugs as modulators of cancer-associated cellular stress pathways, the specific impact of aripiprazole on triple-negative breast cancer (TNBC) has not been adequately explored. TNBC is an aggressive breast cancer subtype characterized by the absence of ER, PR, and HER2 expression, limited targeted therapeutic options, and poor clinical outcomes. Autophagy, a key cellular homeostasis mechanism, plays a complex role in TNBC progression by regulating survival, metabolic adaptation, and cell death. Whether aripiprazole influences autophagy-related molecular regulators in the TNBC cell line MDA-MB-231 remains unknown, representing a critical gap in understanding repurposed pharmacotherapies for this challenging malignancy. **Aims and Objectives:** To determine the antiproliferative activity of aripiprazole in the MDA-MB-231 TNBC cell and evaluate aripiprazole-mediated modulation of the autophagy pathway through gene expression profiling. **Methodology**: MDA-MB-231 cells will be treated with graded concentrations of aripiprazole to assess dose-dependent cytotoxic effects. MTT and colony formation assays will be performed to measure changes in cell viability and proliferative capacity. Quantitative real-time PCR (qPCR) will be conducted to analyze expression levels of key autophagy-related genes, including BECN1, LC3B, ATG5, ATG7, and p62/SQSTM1, enabling characterization of the molecular impact of aripiprazole on autophagic regulation in TNBC cells. **Results:** The study is expected to estimate the IC50 of aripiprazole in MDA-MB-231 cells and demonstrate reductions in viability and clonogenic potential. Gene expression data are anticipated to reveal significant modulation of central autophagy regulators, providing mechanistic insight into aripiprazole-driven autophagic responses in TNBC. Conclusion: This in-vitro investigation will generate new evidence on the anticancer potential of aripiprazole in TNBC, with particular emphasis on autophagy pathway modulation. The findings will support future preclinical exploration of repurposed pharmacological agents for targeted therapeutic strategies in triple-negative breast cancer.

MM-032

Development and Validation of a Multiplex PCR Toolkit for Rapid Detection of MDR and XDR Salmonella Typhi in Clinical Samples

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Background: Typhoid fever, caused by *Salmonella enterica* serovar Typhi, remains a major public health threat in Pakistan, where poor sanitation, unsafe water, and widespread antibiotic misuse have facilitated the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains. Conventional diagnostic methods—particularly blood culture are slow, requiring several days and limiting timely, targeted treatment. As a result, patients are often prescribed broad-spectrum antibiotics without proper confirmation, further driving antimicrobial resistance (AMR). Rapid, sensitive, and specific molecular diagnostics are therefore urgently needed. **Methodology, Statistical Analysis, and Results:** This study aims to develop and validate a multiplex PCR-based diagnostic toolkit capable of simultaneously detecting susceptible, MDR, and XDR *S. Typhi* strains by targeting key resistance genes relevant to circulating isolates in Pakistan. The research was conducted in the Microbial Genomics Lab. The methodology included sample selection, bacterial inoculation, DNA extraction, primer reconstitution, uniplex PCR optimization (qPCR), validation with additional samples, and multiplex PCR optimization. DNA extracted from cultured isolates was quantified and subjected to uniplex PCR using SYBR Green chemistry with three primers: CTX (β-lactamase resistance), fliC (flagellin H-antigen), and VI (Vi capsular antigen). The VI and fliC primers showed robust amplification, while CTX did not amplify, suggesting absence of the target gene in the isolate. Statistical analysis performed in R supported successful optimization of the VI and fliC assays. **Discussion and Conclusion:** A multiplex PCR toolkit could significantly improve typhoid diagnostics by enabling rapid detection of resistance profiles, reducing empirical antibiotic use, and supporting national efforts against AMR. This innovation aligns with SDG-3 by promoting improved patient outcomes and strengthened antibiotic stewardship.

MM-033

Molecular Differentiation of Dengue Virus Serotypes Through RT-PCR for Enhanced Diagnostic Accuracy in Pakistan

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Background: Dengue virus, a major flavivirus with a 10 kb positive-sense RNA genome transmitted by mosquito vectors, poses a significant global public health threat. Overlapping clinical symptoms among serotypes complicate diagnosis and management, and reliance on clinical presentation alone often leads to misdiagnosis. Precise molecular tools that differentiate dengue virus serotypes are therefore essential for patient care, surveillance, and targeted interventions. **Methods:** This study was conducted at Ziauddin University Hospital, Clifton Campus, using dengue-positive sera from 60 patients processed at MDRL-2. Viral RNA was extracted with the QIAamp DSP Virus Spin Kit, and serotype-specific primers were designed for DENV universal, DENV-1, DENV-2, DENV-3, and DENV-4. cDNA synthesis used the RevertAid First Strand cDNA Synthesis Kit, followed by qPCR amplification with Maxima SYBR Green/ROX Master Mix. **Results:** All dengue-positive sera yielded successful amplification with serotype-specific primers. Amplification plots showed Cq values ranging from approximately 9.5 to 32, reflecting variable viral RNA loads. Melt curve analysis demonstrated distinct single peaks without non-specific products or primer–dimer formation, and no amplification was observed in negative controls, confirming high analytical specificity and reliability. Ongoing optimization efforts are focused on further enhancing assay stringency and improving robustness across diverse clinical samples. **Conclusion**: This molecular strategy highlights the utility of RT-qPCR-based rational primer design for accurate differentiation of circulating dengue virus serotypes in Pakistan. Implementing such assays moves diagnostics beyond symptom-based classification, enabling timely clinical decisions and strengthening epidemiological surveillance for vector-borne disease control.

MM-034

Host Genomic Variation In IL-10 And TNF-α Promoter Polymorphisms and Susceptibility to Dengue: A Quantitative Meta-Analysis

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Background: Inter-individual variability in dengue outcomes is strongly influenced by host immunogenetic factors, yet evidence related to key cytokine promoter polymorphisms remains fragmented and population-specific. This meta-analysis evaluated whether IL-10 and TNF-α SNPs modulate susceptibility to dengue infection across diverse endemic settings. **Objectives:** To quantify the association of IL-10 promoter SNPs rs1800871 and rs1800872, and TNF-α promoter SNPs rs1800629 and rs361525, with dengue risk using harmonized allele-based models. **Methods:** Following a predefined PICO framework, a systematic search retrieved 734 records using Boolean combinations of "dengue hemorrhagic fever", "dengue genetics", "dengue host molecular analysis", "dengue host pathogen interactions", "dengue clinical parameters", "dengue SNPs", and "dengue mutational analysis". After screening 345 studies, 40 host–pathogen–focused articles were evaluated, and case–control studies satisfied predefined quality and data criteria. Genotyping techniques included PCR-based Sanger sequencing, real-time PCR SNP assays, and PCR–RFLP; allele-based odds ratios, 95% confidence intervals, and p values were pooled with random-effects models, with forest plots guiding inference. **Results:** T-allele carriage at IL-10 rs1800871 showed a modest protective effect in the Vietnamese cohort (OR ≈ 0.69, 95% CI 0.49–0.98), while Mexican datasets indicated increased risk for T at rs1800871 and A at rs1800872 (ORs ≈ 1.26–1.28). A-allele at rs1800872 in West Africa conferred a higher risk (OR 2.52, 95% CI 1.74–3.66). TNF-α rs1800629 G-allele effects were weak and non-significant, whereas the rs361525 G-allele showed null association in Pakistan but an elevated risk in Mexico (OR 2.64, 95% CI 1.48–4.69). **Discussion:** IL-10 promoter polymorphisms emerge as important determinants of dengue susceptibility, with TNF-α promoter variants exerting context-dependent effects shaped by ethnicity and study design; however, limited study numbers highlight the need for larger cohorts and genome- or exome-wide approaches that extend beyond candidate SNP analysis.

MM-035

Genomics-integrated in silico evaluation of TNF-α–derived peptide epitopes against wild-type and deleterious NR3C1/CYP3A5 variants

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Background: Tumor necrosis factor-alpha (TNF-α) is a key driver of chikungunya-associated arthritis, but biologic TNF inhibitors are costly, invasive, and variably effective, partly due to host genetic heterogeneity in glucocorticoid and drug-metabolizing pathways. Deleterious missense variants in NR3C1 and CYP3A5 can alter protein structure and steroid handling, potentially reshaping responses to TNF-targeted therapies. **Objectives:** To design TNF-α–derived 16–20-mer peptide epitopes with favorable predicted immunomodulatory and MHC class I/II binding properties. To model wild-type and mutant NR3C1/CYP3A5, compare docking-based peptide binding to these proteins and identify candidate epitopes that maintain favorable binding across genotypes. **Methods:** TNF, NR3C1, and CYP3A5 reference sequences and variants were retrieved from public databases, and deleterious missense SNPs were prioritized using multiple predictors. B- and T-cell epitopes within soluble TNF-α were predicted with BepiPred, NetMHCpan, and NetMHCIIpan, yielding three peptides with favorable antigenicity and MHC binding profiles. Shortlisted epitopes and wild-type/mutant NR3C1/CYP3A5 structures were modeled, Ramachandran-validated, and prepared for docking. AutoDock Vina is being used to dock each epitope against wild-type and mutant proteins and summarize binding energies and key interactions. **Results:** Curated TNF-α, NR3C1, and CYP3A5 data, prioritized deleterious variants, and validated models now enable docking-based comparison of binding energies and interaction patterns, supporting correlation analyses between predicted pathogenicity scores and genotype-dependent shifts in peptide affinity within a genomics-integrated TNF-α epitope design project. **Discussion:** These findings establish a variant-integrated structural context for TNF-α–derived epitopes, supporting future correlation of pathogenicity scores with peptide affinity.

MM-039

Comparative Characterization of Perinatal and Adult Stem Cells for Clinical Applications

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Background: Stem cell-based therapy holds great potential for tissue repair, replacement, and regeneration; however, selecting the optimal stem cell source for clinical use remains challenging. Perinatal-derived cells such as human amniotic epithelial stem cells (hAECs) and umbilical-cord-derived mesenchymal stem cells (UC-MSCs), along with adult-derived dental pulp stem cells (DPSCs), offer advantages in accessibility and minimal ethical concerns. All three possess self-renewal ability and multipotency but differ in immunomodulation, developmental origin, proliferation, and differentiation potential. hAECs exhibit strong immunomodulatory and anti-inflammatory properties, UC-MSCs and DPSCs show high proliferation rates, and DPSCs demonstrate notable osteogenic, odontogenic, and neurogenic potential. **Objectives:** To comparatively characterize hAECs, UC-MSCs, and DPSCs in terms of morphology, size, morphological transitions, population doubling time (PDT), trilineage potential, and expression of MSC and pluripotency markers. **Methodology**: Cells were isolated using standard enzymatic or explant-based methods, followed by comparative evaluation of biological attributes. **Results**: UC-MSCs and DPSCs exhibited spindle-shaped, fibroblast-like morphology typical of MSCs, while hAECs showed a cobblestone appearance. hAECs were smaller in size, whereas morphological transitions of DPSCs remained stable even after passage 10. Although DPSC isolation is more challenging and contamination-prone, DPSCs and UC-MSCs showed equal or lower PDT than hAECs. All cell types demonstrated trilineage differentiation. UC-MSCs and DPSCs were positive for CD73 and CD105, and CD90 was expressed only by UC-MSCs. All cell types showed positive expression of pluripotency marker i.e. Oct-4 and negative expression of HLA-DR. **Conclusion**: DPSCs and UC-MSCs show stable mesenchymal morphology and faster proliferation than hAECs. All three cell types express key stem cell markers with consistent trilineage potential. Overall, DPSCs emerge as strong candidates for regenerative applications.

MM-040

Human Amniotic Epithelial Cells Transcend Dental Pulp Stem Cells in the Amelioration of Renal Fibrosis

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Chronic kidney disease (CKD) is a crucial global and national health issue, with a worldwide prevalence of around 13.4% and a greater prevalence in Pakistan, at 16.6% to over 21%. There are various causes of CKD, with renal fibrosis as the common underlying mechanism of progressive kidney damage. Renal fibrosis leads to the disruption of normal kidney structure and hence a progressive loss in kidney function. Despite extensive studies in this area there are still no effective therapeutic options available and treatment majorly focuses on managing the symptoms, aiming to slow disease progression rather than restoring normal kidney function. Regenerative medicine, which employs stem cell therapies such as mesenchymal and embryonic stem cells to repair and regenerate damaged tissues, offers a promising approach to treat renal fibrosis. Some studies have investigated the use of human Amniotic Epithelial Cells (hAECs) and Dental Pulp Stem cells (DPSCs) to treat renal fibrosis; however, a comparative evaluation of their efficacy has not yet been carried out. This in-vivo experimental study was conducted at the College of Molecular Medicine, Ziauddin University, Clifton campus Karachi, to compare the potential of two different types of stem cells, hAECs and DPSCs in mitigating renal fibrosis. HAECS and DPSCs were isolated, with informed consent from the human placental tissue and dental pulp respectively. The isolated cells were characterized by the presence of stem cells specific markers and the potential to undergo trilineage differentiation. To develop renal fibrosis mice were administered with CCL4 intraperitoneally twice weekly for a period of 10 weeks. The animals were divided into four groups; healthy, model, hAECs treated and DPSCs treated. The hAECs treated and DPSCs treated group animals were injected with hAECs and DPSCs respectively, and were sacrificed after a month. Mason trichrome staining (MTS) and Hematoxylin and Eosin staining (H&E) were performed for histological analysis and gene expression evaluation was done by qPCR. Histological analysis revealed extensive tubular degeneration, inflammatory infiltration and interstitial fibrosis in the CCL4 induced model group, confirming successful induction of renal fibrosis. Furthermore, the treatment with hAECs and DPSCs markedly restored renal architecture showing a reduction in collagen deposition in both treatment groups. qPCR analysis showed that hAECs treatment significantly downregulated profibrotic genes, alpha-SMA and fibronectin while upregulating MMP9 indicating enhanced matrix remodeling. DPSCs treatment led to the downregulation of TGF-beta while no change was observed for COL1.These findings suggest that while both hAECs and DPSCs attenuate renal fibrosis, hAECs demonstrate superior antifibrotic efficacy by effectively restoring renal architecture and modulating profibrotic gene expression.

MM-041

Vactosertib-Primed Dental Pulp Stem Cells: A Promising Strategy to Reverse Pulmonary Fibrosis

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Pulmonary fibrosis is a life-threatening chronic lung disorder; characterized by excessive extracellular matrix (ECM) and collagen accumulation, leading to lung scarring and impaired function. Current antifibrotic drugs like Nintedanib and Pirfenidone slow disease progression but cannot reverse the damage. Therefore, there is an urgent need for therapies capable of reversing fibrosis and mitigating its progression. Vactosertib is a novel drug that acts as an inhibitory molecule by targeting TGF-β pathway in pulmonary fibrosis. It shows strong antifibrotic, anti-inflammatory and antioxidative effects by suppressing key signaling pathways that promote ECM deposition and myofibroblast transition. Dental pulp stem cells possess high proliferative potential, paracrine signaling ability and regenerative capacity showing promising antifibrotic effects. Their preconditioning effects are still unexplored. This in vivo study, conducted at the College of Molecular Medicine, Ziauddin University (2024–2025), aimed to explore the combined antifibrotic and anti-inflammatory effects of preconditioned Vactosertib-DPSCs in pulmonary fibrosis mouse model. Isolated cells were characterized by stem cells specific markers and trilineage differentiation potential. Mice were induced with CCl_4 for 6–10 weeks, followed by treatment of preconditioned Vactosertib-DPSCs and untreated DPSCs. The safe concentration of Vactosertib was determined as 29 nM. Animals were divided into four groups; healthy, model, DPSCs and preconditioned Vactosertib-DPSCs. Treatments were administrated respectively, and animals were sacrificed after one month. Post-treatment analyses included Mason Trichrome Staining (MTS) and Hematoxylin and Eosin (H&E) staining for histological evaluation, while gene expression was assessed by qPCR. Histological results, revealed that the model group showed severe alveolar damage, inflammation and ECM accumulation confirming fibrosis development. In treated groups, alveolar structure improved with a marked reduction in collagen levels. qPCR analysis showed that in the model group, α-SMA, fibronectin, MMP9, collagen I and TGF-β were upregulated. However, treatment with DPSCs and preconditioned Vactosertib-DPSCs significantly downregulated these profibrotic genes, indicating their strong potential to alleviate fibrotic damage and restore normal lung architecture.

MM-042

Luteolin-Induced Modulation of Dental Pulp Stem Cells: A Promising Approach Against Cardiac Fibrosis

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Cardiac fibrosis is a major pathological condition characterized by excessive extracellular matrix (ECM) deposition, leading to ventricular stiffening and impaired cardiac function. Despite advances in antifibrotic research, current therapies remain largely ineffective in reversing established fibrosis and primarily aim to manage symptoms and slow disease progression. Regenerative medicine offers a promising approach by utilizing stem cell–based therapies to repair and regenerate damaged cardiac tissue. Luteolin, a natural flavonoid, possesses potent antioxidant, anti-inflammatory, and antifibrotic properties, while Dental Pulp Stem Cells (DPSCs) exhibit strong regenerative and paracrine potential. However, the therapeutic efficacy of luteolin-preconditioned DPSCs in cardiac fibrosis has not yet been explored. This in vivo experimental study was conducted at the College of Molecular Medicine, Ziauddin University, Clifton Campus, Karachi, to evaluate the therapeutic potential of luteolin-preconditioned DPSCs in a CCl4 induced cardiac fibrosis model. DPSCs were isolated and characterized based on stem cell–specific markers and trilineage differentiation potential. Cytotoxicity of luteolin on DPSCs was assessed using trypan blue dye exclusion assay. Cardiac fibrosis was induced by intraperitoneal administration of CCl4 twice weekly for ten weeks. Animals were divided into four groups: healthy, model, DPSC-treated, and luteolin-preconditioned DPSC-treated. Following one month of treatment, animals were sacrificed for histological (Hematoxylin and Eosin and Masson's Trichrome staining) and molecular (qPCR) analyses. Histological evaluation of the CCl4 induced model group revealed extensive myocardial degeneration, inflammatory infiltration, and marked collagen deposition, confirming successful induction of cardiac fibrosis. Treatment with DPSCs and luteolin-preconditioned DPSCs markedly restored myocardial architecture and reduced collagen accumulation, with the luteolin-preconditioned group showing superior recovery. qPCR findings supported these observations, revealing significant upregulation of profibrotic genes (α-SMA, Fibronectin, COL1A1, and TGF-β) in the model group. DPSC treatment downregulated these markers and increased MMP9 expression, indicating reduced fibrosis and enhanced ECM remodeling. Luteolin-preconditioned DPSCs showed further suppression of α-SMA, Fibronectin, and TGF-β, along with sustained MMP9 elevation, demonstrating superior antifibrotic efficacy. These findings suggest that luteolin augments the antifibrotic and regenerative potential of DPSCs by modulating ECM remodeling and suppressing profibrotic gene expression, presenting a promising therapeutic approach for the treatment of cardiac fibrosis.

MM-043

Tracing Early Pathophysiological Changes in Preeclampsia Using Leptin, CRP, and Platelet Count

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Background: Preeclampsia (PE) is a multisystem pregnancy disorder originating from early placental dysfunction. Increasing evidence suggests that metabolic, inflammatory, and hematologic alterations begin in the first trimester (weeks 1–13+6), long before clinical symptoms appear. **Objective:** To analyze early changes in leptin, CRP, and platelet counts in the first trimester and explore their interconnected mechanisms contributing to preeclampsia prediction **Methods:** A narrative review of literature published between 2021 and 2025 was conducted. Included sources were human studies reporting first-trimester (≤13+6 weeks gestation) levels of leptin, CRP, or platelet indices in pregnancies that developed preeclampsia. Exclusion criteria were animal studies, articles without trimester-specific data, studies lacking clear diagnostic criteria for PE, and reports focusing only on second or third trimester biomarkers. **Results:** Women who developed preeclampsia consistently demonstrate higher first-trimester leptin and CRP levels, driven by early placental hypoxia and inflammatory signaling. Leptin's activation of ERK1/2 and downstream cytokines, particularly IL-6, contributes to a state of systemic inflammation, reflected by elevated CRP. CRP further amplifies endothelial activation and oxidative stress, promoting platelet consumption and microvascular injury. As a result, subclinical thrombocytopenia may emerge early due to increased platelet turnover and low-grade intravascular coagulation. Together, these biomarkers form a biologically linked triad: leptin initiates placental inflammation, CRP represents systemic inflammatory amplification, and platelet decline reflects early endothelial and coagulation dysfunction, all central mechanisms of emerging PE. **Conclusion:** Elevated leptin and CRP, coupled with early platelet reduction, represent interrelated pathophysiological events detectable in the first trimester. Their combined assessment may enhance early prediction of preeclampsia.

MM-044

Targeting Oral Fibrosis Using Dental Pulp Stem Cell–Derived Exosomes

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Background: Oral Submucous Fibrosis (OSF), a progressive, debilitating, and potentially malignant condition, is characterized by fibrosis, restricted mouth opening, with limited availability of therapeutic treatment. Dental Pulp Stem Cells (DPSCs), are known for anti-inflammatory, anti-fibrotic properties, making it a promising alternative for treating conditions with fibrosis. The limitation of cell transplantation led to the use of Dental Pulp Stem Cells (DPSCs)-derived exosomes (DPSCs-Exo), which are cell-free, nanosized vesicles that carry essential bioactive molecules, a preferred alternative in regenerative therapy because of high intercellular communication efficiency and low immunogenic potential. **Objective:** The aim of the research work is to harvest and analyze the Extracellular Vesicles (Exosomes) of the DPSCs by ultracentrifugation with a freeze-drying process from the conditioned media, and assessing the impact of the DPSCs-Exos on the in vitro proliferation of the OSF Fibroblasts, and the gene expression of collagen and other genes implicated in regulating the pathogenesis of OSF **Expected Results:** It is expected that the treatment with DPSCs-Exos will significantly suppress the proliferation/viability of OSF fibroblasts. In addition, it is also hypothesized that the gene expression study (qPCR) will demonstrate a reduced expression of the profibrotic factors α-SMA, Collagen, and Fibronectin in OSF fibroblasts, resulting in the modulation of significant profibrotic pathways, such as the suppression of TGF-β, which plays a pivotal role in the fibrosis process. **Future Recommendations:** Further research is required to clearly define the mechanisms that are involved, which may include PI3K/AKT or MAPK pathways, in the antifibrotic properties of DPSC-Exos. Considering the positive preclinical findings, further research should be directed towards optimizing the use of DPSC-Exos, which may be via scaffolding agents such as biomaterials, injectable, and/or hydrogels in order to enhance their retention and therapeutic impact**.**

MM-048

Mesenchymal Stem Cell-Derived Secretome as an Effective Anti-Cancer Treatment Strategy

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Background: Glioblastoma remains the most prevalent aggressive primary brain tumor in adults, known for its rapid progression, recurrence, and resistance to conventional therapies. Despite decades of effort and advances in surgical techniques, the prognosis of glioblastoma patients remains poor. Since it is of utmost importance to maximize treatment efficacy and curative potential, the use of mesenchymal stem cell (MSC)-derived secretome after their pre-conditioning with a potent bioactive molecule represents a promising and innovative therapeutic approach. **Methods:** The current proposal aims to investigate the anti-tumor potential of pre-conditioned MSC-derived secretome on the glioblastoma cell line. The secretome will be collected from umbilical cord tissue-derived MSCs, with and without treatment with a bioactive compound to enhance its potential. Key molecular pathways associated with tumor proliferation, progression, and metastasis will be evaluated to validate the potential of the proposed treatment strategy. **Expected Findings:** The current study is expected to offer an improved treatment strategy to inhibit the proliferation, progression, and metastatic properties of glioblastoma cells when treated with the preconditioned MSC-derived secretome**. Conclusion:** Based on the findings of our study, we put forward to explore the potential of MSC secretome pre-treated with a potent bioactive molecule on the glioblastoma cell line, aiming to establish a regimen that allows for a reduced dose of the chemotherapeutic agent, thereby potentially minimizing chemotherapy-related toxicity.

MM-049

Anti-fibrotic potential of Adipose tissue-derived Mesenchymal Stem Cells: Insights from Systematic Review and Meta-Analysis

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Background and Objectives: Pulmonary fibrosis (PF) is a chronic progressive lung disease with limited treatment options. Adipose-derived mesenchymal stem cells (AD-MSCs) possess immunomodulatory and regenerative properties. Despite generally positive findings, variability across models and derivatives necessitates systematic assessment. **Method:** The review was prospectively registered in PROSPERO (CRD420251119193). A systematic search of major databases was conducted up to April 2025. Selected studies included original preclinical investigations assessing AD-MSC or AD-MSC-derived products in bleomycin-induced fibrosis, idiopathic pulmonary fibrosis, or silicosis models. Risk of bias was assessed using standard tools. A random-effects meta-analysis was performed to pool mean differences (MD) in Ashcroft scores. **Result:** Nineteen studies met the inclusion criteria (8 in vivo, 2 in vitro, 9 combined models). Overall study quality was acceptable. Meta-analysis showed a pooled MD of –1.35 (95% CI –3.19 to 0.49), indicating reduced fibrosis in AD-MSC–treated groups, although the effect was not statistically significant ($p = 0.15$; $I^2 = 99\%$). Despite heterogeneity, all individual studies demonstrated directionally lower fibrosis severity following AD-MSC therapy. Across diverse models, AD-MSC interventions improved histopathology, reduced collagen deposition, and modulated key profibrotic pathways, supporting consistent qualitative antifibrotic activity. Paracrine derivatives, including extracellular vesicles and conditioned medium, frequently matched the effects of whole-cell therapies. **Conclusion:** Collectively, current evidence positions AD-MSCs and their derivatives as promising antifibrotic candidates with strong potential for clinical translation, especially given their consistent effects across varied preclinical pulmonary fibrosis models.

MM-050

Therapeutic Efficacy of Preconditioned Umbilical-Cord Mesenchymal Stem Cells in Diabetes-Induced Pulmonary Fibrosis

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Background: Diabetes mellitus (DM) is a widespread metabolic disease marked by persistent high glucose levels which affects various organs like eyes, kidneys, nervous system and blood vessels. Idiopathic pulmonary fibrosis is a chronic and progressive disease with usual interstitial pneumonia patterns. Recent research shows that diabetes also causes lung damage making it one of the target organs in DM now which commonly presents as pulmonary fibrosis (PF).Currently there is no effective medication for PF that can fully cure the disease. Mesenchymal stem cells (MSCs) have been reported for their regenerative, immunomodulatory and anti-fibrotic potential making them a promising candidate for novel therapeutic strategies. **Aims and Objectives:** The current proposal will be focusing on evaluating the anti-fibrotic and regenerative potential of naive and preconditioned MSCs in diabetes induced pulmonary fibrosis (D-PF) rat model. **Methodology**: We aim to collect umbilical cord tissues from healthy donor mothers after their informed consent. MSCs will be isolated and characterized for their native features and preconditioned using a bioactive compound that is expected to enhance their anti-fibrotic properties. Furthermore, D-PF model will be established in Wistar male rats and divided into both control and treatment groups. Through vein injection, preconditioned and naive UC- MSCs will be transplanted. Later, lung tissues will undergo regenerative assessments via histological evaluation and pulmonary edema measurement. **Extracted/Expected Findings**; We expect regenerative and anti-fibrotic effect of MSCs in D-PF rat model, especially in case of preconditioned UC-MSC transplantation, ultimately supporting improved lung function and maintaining structure via reduced fibrotic scars. **Conclusion:** This study is expected to demonstrate that UC-MSCs, particularly when preconditioned, effectively attenuate diabetes-induced pulmonary fibrosis by reducing fibrotic remodeling and promoting lung regeneration. These findings may support MSC-based therapy as a promising strategy for managing pulmonary complications associated with diabetes.

MM-051

Assessing the Current Status of Stem Cell Banking Facilities and Their Role in Promoting Regenerative and Personalized Medicine

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Regenerative Medicine is reshaping healthcare, with stem cells at the forefront due to their capacity for self-renewal and differentiation. Hence, being excellent sources for immune modulation, tissue repair, and hematological disorders. In Pakistan, we see an increase in interest in regenerative medicine, yet the landscape for stem cell storage remains fragmented. Nevertheless, several private institutions, such as Smart Cells Pakistan and other commercially affiliated cord-blood services, have introduced selective storage options, creating early groundwork for local availability of cryopreserved stem cells. To understand the practicality and future for such services, this study aims at identifying the status, technical, regulatory and infrastructural elements needed for this facility in Pakistan. There are several standard protocols being used internationally for stem cell banking, such as sterile cord-blood collection, volume reduction to isolate mononuclear cells, flow-cytometric quantification of cellular populations, controlled incorporation of DMSO-based cryoprotectant, and gradual temperature reduction via controlled-rate freezing, that serve as the foundation for long-term storage in liquid nitrogen at ultra-low temperatures. These methods are partially adopted by private Pakistani facilities, but remain inconsistently accessible to the broader healthcare community. This may be a result of a lack of trained staff, regulatory systems, and financial resources. In conclusion, the landscape reveals both progress and unmet needs. In order to sustainably introduce this facility in Pakistan, there is a need for a multi- pronged strategy. It must involve the collaboration of government agencies, healthcare providers, international organizations, and local communities to ensure equitable access, improve safety, and create a reliable reserve of voluntary donors.

MM-052

Exploring the Potential of Mesenchymal Stem Cells to Prevent Pancreatic Beta Cell Damage in Diabetes Mellitus

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Background: Persistent hyperglycaemia in Diabetes mellitus (DM) causes severe pancreatic complications and reduces insulin production. It stimulates mitochondria to produce reactive oxygen species (ROS), ultimately activating pro-inflammatory mediators that produce metabolic inflammation and damage pancreatic β-cells. Regenerative applications of stem cells exhibit revolutionary therapeutic potential for treating various chronic disorders, including diabetes and its associated comorbidities. MSCs prominently show up their potential to reduce inflammation, differentiate into β-cells, augment pancreatic tissue regeneration, and promote its function by translocating healthy mitochondria also to injured cells. **Objectives:** To evaluate the regenerative potential of pre-conditioned hUC-MSCs in mitigating diabetes, producing insulin, and restoring pancreatic β-cells' structure and function in a rat model. **Methodology:** hUC-MSCs will be isolated from healthy mothers after informed consent, culturing under standard conditions, and passaging 3 to 4 times for ideal morphology and function. DM will be induced in male Wistar rats, divided into 4 main groups: control, no treatment, normal MSCs, and pre-conditioned MSCs. Pre-conditioning of hUC-MSCs will be done in-vitro and will be administered in-vivo models through tail vein injection to assess the effects of MSCs in regeneration and restoration. Pancreatic β-cells and tissues will be subsequently analyzed histologically in each group. **Results (Expected Outcomes):** Pre-conditioned hUC-MSCs are expected to reduce β-cells' damage, restore β-cells' function, stimulate insulin production, and mitigate severe diabetic effects in rats. **Conclusion:** Preconditioning of MSCs enhances their regenerative potential for destroyed Beta-cells in DM, promoting restoration and regeneration. Further studies are required to optimize pre-conditioning protocols, assessing translational potential and prolonged safety.

MM-053

Stem Cell-Based Regenerative Applications for the Management of Diabetic Kidney Disease

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Background: Diabetes mellitus (DM) is one of the prevalent chronic metabolic diseases associated with persistent hyperglycemia, leading to multiple systemic complications including Diabetic Retinopathy, Neuropathy, Nephropathy and Pancreatic damage. Nephropathy or Diabetic Kidney disease (DKD) is one of the leading causes of chronic kidney failure worldwide. In DKD, persistent hyperglycemia promotes Oxidative stress, inflammation and structural damage to renal tissues, resulting in a gradual loss of kidney function. Conventional therapies only focus on slowing the disease progression without reversing the renal damage. Mesenchymal stem cells (MSCs), due to their regenerative, self-renewal and differentiation capabilities, have emerged as a potential therapeutic option for DKD. **Aims and Objectives:** Our study aims to evaluate the anti-inflammatory and regenerative ability of naive and preconditioned MSCs in a diabetes-induced nephropathic animal model. **Methodology:** The study involves the development of a DKD animal model. MSCs will be preconditioned with a bioactive compound that is expected to increase their anti-inflammatory, anti-oxidant and regenerative properties. Naive and pre-conditioned MSCs will be transplanted into the animal model, and histological evaluation will be done to evaluate the regenerative effects of transplanted MSCs. **Expected outcomes:** We expect the regenerative and anti-inflammatory effect of MSCs in DKD model, specifically in the case of preconditioned MSC transplanted group, ultimately leading to improved renal tissue repair and functioning. **Conclusion:** MSC-based therapy offers promising role in curing DKD, as per the reports coming from throughout the globe. However, challenges remain on clinical level. Further research is still needed to ensure long-term safety for its clinical translation.

MM-055

Exploring the Effect of Aripiprazole on Reactive Oxygen Species Production and ROS-Related Gene Expression in Triple-Negative Breast Cancer Cell Line MDA-MB-231

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Introduction: Aripiprazole, an atypical antipsychotic with multifunctional receptor activity, has recently shown promising anticancer effects in several tumor models. Oxidative stress represents a critical cellular mechanism regulating cancer cell survival, proliferation, and death. In triple-negative breast cancer (TNBC), elevated reactive oxygen species (ROS) levels contribute to metabolic adaptation and aggressive tumor behaviour. However, the influence of aripiprazole on ROS generation and ROS-associated molecular regulators in TNBC remains poorly defined. Understanding whether aripiprazole modulates oxidative stress responses in the MDA-MB-231 cell line may uncover novel mechanistic insights and support drug repurposing strategies for this therapeutically challenging breast cancer subtype. **Aims and Objectives:** To determine the cytotoxic and antiproliferative effects of aripiprazole in the MDA-MB-231 TNBC cell line. To evaluate aripiprazole-mediated modulation of ROS production and ROS-associated gene expression. **Methodology:** MDA-MB-231 cells will be treated with graded concentrations of aripiprazole to evaluate dose-dependent cytotoxicity using the MTT assay. Morphological analysis under microscopy will be performed to observe treatment-induced structural changes. Intracellular ROS levels will be quantified using the DCFH-DA fluorescence assay. Furthermore, quantitative real-time PCR (qPCR) will be conducted to examine expression levels of key ROS-related genes, including SOD1, SOD2, CAT, GPX1, and NOX4, enabling assessment of aripiprazole's impact on oxidative stress-associated molecular pathways. **Results:** The study is expected to estimate the IC50 value of aripiprazole and demonstrate morphological alterations indicative of oxidative stress-mediated cytotoxicity. Enhanced ROS production and differential expression of ROS-regulating genes are anticipated, providing mechanistic insights into aripiprazole's oxidative stress-modulating effects in TNBC cells. **Conclusion:** This in-vitro investigation will generate new evidence regarding the role of aripiprazole in modulating ROS production and related gene expression in TNBC. The findings will support future mechanistic and preclinical studies exploring repurposed psychotropic agents as potential therapeutic modulators of oxidative stress in triple-negative breast cancer.

MM-056

Exploring the Anticancer Potential of Vanillin in CAL-27 Oral Squamous Cell Carcinoma via Modulation of the Wnt/β-Catenin Signaling Pathway

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Background: Vanillin, a bioactive phenolic compound with reported antiproliferative properties in several malignancies, has not been thoroughly investigated in oral squamous cell carcinoma (OSCC). Its potential mechanistic effects on the Wnt/β-catenin signaling cascade in CAL-27 cells remain unclear, representing a critical gap in the current understanding of phytochemical-based OSCC therapeutics. **Aims and Objectives**: To determine the antiproliferative activity of vanillin in the CAL-27 OSCC cell line. To evaluate vanillin-mediated modulation of the Wnt/β-catenin pathway and associated cell-cycle regulatory mechanisms. **Methodology**: CAL-27 cells will be exposed to vanillin across a concentration gradient to assess dose-dependent cytotoxic and antiproliferative effects. Cell viability and proliferation assays, along with cell-cycle analysis, will be conducted to quantify functional outcomes. Quantitative real-time PCR will be performed to examine the expression of key Wnt/β-catenin pathway genes and related molecular regulators. **Results**: The study is expected to estimate the IC50 of vanillin in CAL-27 cells and characterise its impact on proliferation and cell-cycle progression. Additionally, alterations in Wnt/β-catenin signaling components will provide mechanistic insights into vanillin's anticancer properties in OSCC. **Conclusion**: This in-vitro investigation will generate new evidence regarding the anticancer potential of vanillin in OSCC, with particular emphasis on Wnt/β-catenin pathway modulation. The findings will support future preclinical development of phytochemical-based therapeutic strategies for oral cancer.

MM-057

Association Of Dennd1a Rs2479106 Polymorphism With Polycystic Ovary Syndrome In A Subset Of The Pakistani Population

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Background: Polycystic ovary syndrome (PCOS) is a prevalent endocrine disorder characterized by hyperandrogenism, ovulatory dysfunction, and metabolic abnormalities. Genetic susceptibility plays a significant role in its pathophysiology, with multiple genome-wide association studies identifying DENND1A as one of the strongest PCOS-associated loci. The rs2479106 variant within DENND1A has been repeatedly implicated in hormonal dysregulation and altered ovarian folliculogenesis. However, data on this polymorphism in the Pakistani population remain limited, despite the high burden of PCOS in South Asian women. Investigating the association of rs2479106 with PCOS within this demographic may provide valuable insights into population-specific genetic risk factors and contribute to improved diagnostic and risk-stratification approaches. **Aims and Objectives:** To determine the frequency distribution of DENND1A rs2479106 polymorphism among PCOS cases and healthy controls. To evaluate the association between rs2479106 genotypes and PCOS susceptibility in a subset of the Pakistani population. **Methodology:** A case-control study will be conducted involving clinically diagnosed PCOS patients and age-matched healthy controls. Genomic DNA will be extracted from peripheral blood samples, followed by genotyping of the DENND1A rs2479106 variant using PCR and gel electrophoresis-based analysis. Allele and genotype frequencies will be compared between groups, and statistical analyses, including chi-square testing and odds ratio estimation, will be performed to determine the strength of association between rs2479106 and PCOS risk. **Results:** The study is expected to identify genotype and allele distribution patterns of rs2479106 among Pakistani women and reveal significant associations with PCOS susceptibility. Findings may demonstrate increased risk linked to specific allelic variants, supporting the role of DENND1A in PCOS pathophysiology within this population. **Conclusion:** This genetic association study will provide evidence on the relevance of the DENND1A rs2479106 polymorphism in PCOS among Pakistani women. The results will contribute to a deeper understanding of population-specific genetic determinants and support future efforts toward personalized risk assessment and targeted reproductive health interventions.

MM-058

Evaluating the Proapoptotic Effects of Antipsychotic Drug Aripiprazole on Triple-Negative Breast Cancer Cell Line

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Background: Aripiprazole, an atypical antipsychotic primarily acting as a dopamine D2 receptor partial agonist, has recently gained attention for its potential anticancer properties. Several psychotropic agents have demonstrated the ability to activate cell-death pathways in different tumor models; however, the proapoptotic impact of aripiprazole on triple-negative breast cancer (TNBC) remains insufficiently studied. TNBC, characterized by the absence of ER, PR, and HER2 receptors, is an aggressive breast cancer subtype with limited therapeutic options and poor prognosis. Apoptosis plays a central role in regulating cancer cell elimination, yet whether aripiprazole can modulate apoptotic signaling in the TNBC cell line MDA-MB-231 remains unclear. Addressing this mechanistic gap may provide valuable insights into repurposing aripiprazole as a potential therapeutic adjunct for TNBC. **Aims and Objectives:** To determine the antiproliferative activity of aripiprazole in the MDA-MB-231 TNBC cell line. To evaluate aripiprazole-induced apoptotic responses through functional and molecular assays. **Methodology:** MDA-MB-231 cells will be treated with increasing concentrations of aripiprazole to assess dose-dependent cytotoxicity using the MTT assay. Apoptotic induction will be evaluated through Annexin V-FITC staining followed by fluorescent microscopy to characterize early and late apoptotic responses. Quantitative real-time PCR (qPCR) will be conducted to analyze the expression of key pro- and anti-apoptotic genes, including BAX, BCL-2, Caspase-3, Caspase-9, and P53, enabling elucidation of aripiprazole's molecular effects on apoptosis regulation. **Results:** The study is expected to determine the IC50 of aripiprazole in MDA-MB-231 cells and demonstrate significant induction of apoptotic cell death. Gene expression profiling is anticipated to reveal upregulation of proapoptotic markers and downregulation of antiapoptotic regulators, providing mechanistic insights into aripiprazole-mediated apoptosis. **Conclusion:** This in-vitro investigation will provide new evidence regarding the proapoptotic potential of aripiprazole in TNBC cells. The results will support further preclinical evaluation of repurposed psychotropic drugs as emerging therapeutic candidates for triple-negative breast cancer.