MABS
Malta Association of Biomedical Scientists

The 2nd national

BIOMEDICAL SCIENCE Conference 2024

Current and future perspectives

5-6 April 2024
Villa Bighi - Kalkara

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WELCOME MESSAGE

Dear esteemed MABS members and guests,

It is my pleasure to invite you to the 2nd National Biomedical Science Conference, which will take place at the majestic Villa Bighi on the 5th and 6th of April, 2024.

This conference aims to showcase the latest advancements in biomedical science, thus promoting our beloved profession while providing a platform for networking and interdisciplinary discussions. This ties in with MABS' mission *to foster and advocate excellence in the practice, education, and management of Medical Laboratory Sciences*. This edition's submitted abstracts for oral and poster presentations represent the overarching theme of *Current and Future Perspectives*, with topics ranging from the comparison of classical and novel mathematical equations for biochemical assays, to the application of new and innovative techniques in diagnostics, surveillance, and translational research. We are also honoured to be hosting local and foreign keynote speakers, joining us in person to share their expertise on the translation of clinical observations into assay design and tumour comprehensive genomic profiling for personalized cancer medicine. The team has put together an exciting programme, even dedicating some time to the mindfulness and wellbeing of all attendees.

In recognition of the ever-present global burden of cancer and in line with Europe's Beating Cancer Plan, a key pillar of the European Health Union, Day 2 of the conference is dedicated to cancer, closing by a multidisciplinary panel discussion on *Comprehensive Cancer Care*, with invitees from the healthcare, research, policy, and patient advocacy spheres. It is also fitting that a proportion of the proceeds from this conference will be donated to Puttinu Cares, a Maltese NGO that helps young cancer patients and their families.

I would like to thank all those involved in the organization of this event: the MABS executive committee, the scientific and organizing sub-committees, our sponsors, and the professional team at Villa Bighi. Last, but certainly not least, I would like to thank you all for your attendance and active participation in what is promising to be another successful conference.

I wish you all a fantastic couple of days enjoying biomedical science with the beautiful views of the Grand Harbour.

Yours sincerely,

Jeanesse Scerri

President
Malta Association of Biomedical Scientists

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ACKNOWLEDGEMENTS

We extend our heartfelt appreciation to the European Association of Biomedical Science (EPBS) for their gracious patronage and support of this event. EPBS's dedication to advancing biomedical science, fostering collaboration, and promoting excellence in research and education is truly commendable, and their support has been instrumental in bringing this event to fruition.

SCIENTIFIC PROGRAM

DAY 1 | Friday 5th April

08:00 – 09:00 **REGISTRATION**

09:00 – 09:30 **OPENING SPEECH**

09:30 – 10:20 **KEYNOTE LECTURE**

Dr. Keith Sacco: Integrating Clinical Insights for Assay Advancement in RUNX1 Haploinsufficiency: A Journey from Bedside to Bench

10:20 – 11:00 **COFFEE BREAK**

11:00 – 12:30 | **ORAL PRESENTATIONS** | **SESSION 1 - CARDIOVASCULAR & GENETICS**

Dr. Jeanesse Scerri: The Maltese Genetic Landscape of Hereditary Cardiovascular Conditions

Mr. Ian Brincat: Optimizing LDL-C Estimation: The Sampson Equation Solution

Ms. Marichela Schembri: Bridging the Gap: The GRITty Quest for Uncovering Osteoporosis Genes

Dr. Graziella Zahra: Whole Genome Sequencing for One Health Surveillance of Antimicrobial Resistance: The Maltese Experience from the FWD-AMR Ref Lab Cap Project

Ms. Nikita Camilleri: Unravelling the Globin Gene Switch Mechanism in Patients with Hereditary Persistence of Foetal Haemoglobin

12:30 – 13:00 | **POSTER SESSION**

Ms. Azra Zejnelagic: Analysis of Structural Variants in Osteoporosis

Ms. Kimberly Fenech: 5-Fluorouracil Resistant Colorectal Cancer Cells Show a Reduced Expression of Key Protein Methyltransferases and No EMT Potential

Ms. Chantelle Sell: The Association of Two Novel Variants in *FBN1* and *SMAD3* with Familial Aortic Diseases

Mr. Mark Briffa: Waste Water Surveillance of SARS-CoV-2 in the Maltese Islands

Mr. Mark Briffa: Innovative Molecular PRofiling of distINct Tumour derived cells in blood (IMPRINT)

13:00 – 14:00 **LUNCH**

14:00 – 15:30	ORAL PRESENTATIONS	SESSION 2 - IMMUNOLOGY & TRANSFUSION MEDICINE

Prof. Byron Baron: Utilisation of Surplus, Expired and Waste Transfusion Products in Proteomics-Based Research

Ms. Gabriella Azzopardi: Prevention of Anti-D Prophylaxis in Pregnant RhD Negative Females Using Non-Invasive Foetal RhD Genotyping in Malta

Ms. Mariana Grima & Ms. Jasmine Spiteri: Phage Display Identifies Single Chain Variable Fragment Antibodies Specific to Immune Checkpoints

Ms. Alessia Sammut Carta: A Case of Disseminated Intravascular Coagulation Complicating Catastrophic Uterine Bleeding Secondary to Adenomyosis

15:30 – 16:00	MINDFULNESS SESSION

Under the guidance of Ms. Charlene Camilleri Duca, we convene for a mindfulness session aimed at finding solace amidst the chaos of the outside world. As we settle into our space, tensions gradually dissolve, and the mind embraces the tranquillity of the present moment. Through this practice, led with care and expertise, we cultivate awareness and resilience, fostering a profound harmony within ourselves.

ABSTRACTS

Dr. Keith Sacco

Keith Sacco[1]

[1] Mayo Clinic College of Medicine and Science (Volunteer Faculty), USA

Integrating Clinical Insights for Assay Advancement in RUNX1 Haploinsufficiency: A Journey from Bedside to Bench

This keynote delves into the strategic fusion of clinical observations with assay development, elucidating the specific case of RUNX1 haploinsufficiency. Drawing from comprehensive analyses presented in recent scholarly works we dissect the clinical trajectory and phenotypic spectrum characteristic of this genetic anomaly. Through meticulous examination of patient cohorts, we delineate crucial disease markers and prognostic indicators essential for assay refinement. By harmonizing clinical nuances with laboratory methodologies, we unveil the intricate pathophysiological mechanisms underlying RUNX1 haploinsufficiency. This narrative underscores the imperative of a translational approach, whereby clinical insights inform the design and validation of innovative assays, thereby amplifying diagnostic accuracy and therapeutic efficacy in the realm of rare genetic disorders.

Dr. Jeanesse Scerri

The Maltese Genetic Landscape of Hereditary Cardiovascular Conditions

Jeanesse Scerri[1], Jessica Debattista[1], Mark Abela[2], Jake Schembri[2], Tiziana Felice[2], Maryanne Caruana[2], Mark Adrian Sammut[2], Edith Said[1], Christian Scerri[1].

[1] Department of Pathology, Mater Dei Hospital, Msida, Malta
[2] Cardiology Department, Mater Dei Hospital, Msida, Malta

Introduction: Genetic testing for inherited cardiovascular conditions (ICC) is an important pillar in the diagnosis and management of patients suffering from cardiomyopathies, channelopathies and aortic and other vascular disorders. The Molecular Pathology & Genetics laboratory has been offering cardiogenetic testing since 2018. Existing population databases may not cater for the local gene pool, making novel findings a common occurrence.

Objective: To assess the genetic diagnostic yield and findings in the ICC population referred for genetic testing, according to the referring phenotype.

Methodology: Probands referred for genetic testing between 2018-2023 were included. NGS data were retrospectively reviewed. Patients harbouring (likely) pathogenic variants according to ACMG criteria were labelled as 'genotype positive' for the purpose of this study.

Results: 556 patients were referred for genetic testing (66.1% Male, median age 59), the majority for a cardiomyopathy phenotype (64.2%), followed by arrhythmic disorders (13.1%), aortic/vascular disorders (8.6%), and familial hyperlipidaemia (1.4%). The overall genetic yield was 21.6%; 7 (1.3%) probands harboured two variants; 13 (22.8%) of the various reported variants were novel. A further 24 individuals (4.3%) had an inconclusive result, i.e. variants of uncertain significance warranting further investigation. The highest genetic yield was observed in cardiomyopathies (25.5 %), the most commonly implicated genes being *MYH7* and *MYBPC3* in hypertrophic cardiomyopathy and *TTN, FLNC* and *DES* in dilated cardiomyopathy. Arrhythmias showed an 11% genetic yield; out of 16 long QT syndrome (LQTS) probands, only two (12.5%) were genotype positive, harbouring the same variant in *KCNH2.* Aortic/vascular disorders also showed an 11% genetic yield, with variants in *FBN1* and *SMAD3*.

Conclusion: The genetic diagnostic yields in Malta are mostly comparable to published data, except for LQTS, a phenotype with an established high genetic yield (up to 80%), for which a very low yield was observed locally. Novel variants are regularly observed in Maltese ICC patients.

Mr. Ian Brincat

Optimizing LDL-C Estimation: The Sampson Equation Solution

Ian Brincat[1]

[1] Clinical Chemistry, Department of Pathology, Mater Dei Hospital, Msida, Malta

Cardiovascular disease (CVD) is the primary cause of global mortality, accounting for 44% of noncommunicable deaths in 2019 and 30% of fatalities in Malta in 2020. Elevated low-density-lipoprotein cholesterol (LDL-C) is a pivotal risk factor for CVD. LDL-C levels inform CVD risk stratification, aid in diagnosing genetic dyslipidaemia like familial hypercholesterolemia, and serve as the primary target for lipid-lowering therapy. The gold-standard Beta-quantification method for LDL-C estimation, while accurate, is impractical due to its labour-intensive and costly nature for routine diagnostic use. Alternatively, LDL-C can be indirectly estimated via formulas or directly assayed, a newer approach. Mater Dei Hospital's clinical chemistry laboratory offers both calculated and assayed LDL-C concentrations. Presently, the Friedewald formula is used for LDL-C calculation but is limited when triglyceride (TG) levels exceed 4.5mmol/L. In such cases, direct LDL (DLDL) assays are employed, albeit at increased costs, given the high frequency of lipid profile tests performed annually. Several alternative formulas, like the Sampson and Martin-Hopkins equations, aim to address the Friedewald equation's limitations, especially at TG concentrations surpassing 4.5mmol/L. A study using six months' local data (n = 131,551) compared LDL-C derived from the Sampson formula against those from the Friedewald equation at TG concentrations below 4.5mmol/L and to direct LDL-C assays at concentrations exceeding 4.5mmol/L. Results showed acceptable correlation between the Friedewald and Sampson equations at TG concentrations <4.5mmol/L ($y = 1.0031x + 0.0458$ $R2=0.996$). There is a degree of positive bias between DLDL and Sampson-derived LDL-C at TG concentrations between 4.5 and 9 mmol/L, with DLDL results measuring 0.27 mmol/L higher on average. The study indicates that the Sampson (NIH) equation is the most appropriate formula for LDL-C estimation in hospital laboratories, offering ease of simplicity.

Ms. Marichela Schembri

Bridging the Gap: The GRITty Quest for Uncovering Osteoporosis Genes

Marichela Schembri[1], Donald Friggieri[2], Josanne Vassallo[2,3,4], Melissa Marie Formosa[1,2]

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[3] Department of Medicine, University of Malta, Msida, Malta,
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Osteoporosis is a complex hereditary skeletal disease characterised by low bone mass and microarchitectural deterioration, leading to an increased fracture risk. To date, around 20% of the underlying genetic factors are known, emphasising the need for further research efforts in the field of osteoporosis. From the research and clinical perspective, advances in knowledge about the genetic basis of osteoporosis is beneficial since knowing the casual gene/s underlying the disease will allow healthcare professionals to identify osteoporosis prediction biomarkers and drug targets based on a person's genetic make-up. The GRIT (Genetics of osteoporosis In Malta) project will be using innovative omics techniques with the aim of identifying potentially causal genes and gene variants contributing to low and high bone mass based on bone mineral density results generated from DXA. DNA samples from the Malta Osteoporotic Fracture Study (MOFS) will be utilised in this project. A bioinformatics pipeline has been developed to check the quality, trim, align, call and annotate variants. The resulting genetic findings will be tested in the entire case-control collection of more than 1000 Maltese individuals using real-time PCR to replicate the findings. Replication of top hits will also be sought in international consortia that form part of the GENOMOS and GEFOS consortia. This project has received financial support through the FUSION R&I Technology Development Programme LITE 2022 (R&I-2022-007L) awarded by the Malta Council for Science and Technology (MCST). The wealth of data generated from the GRIT study will widen the knowledge on the genetics of bone pathophysiological mechanisms and pave the way for future follow-up studies, including translational models.

Dr. Graziella Zahra

Whole Genome Sequencing for One Health Surveillance of Antimicrobial Resistance: The Maltese Experience from the FWD-AMR Ref Lab Cap Project

Graziella Zahra[1], Rodianne Abela[2], Christopher Barbara[1], Rebecca Borg[1], Mark Briffa[1], Robert Cassar[2], Chanelle Cilia[1], Christina Gatt[2], Kenneth Mallia[2], Claire Marantidis Cordina[2], Cynthia Schembri[2], Mark Sultana[2]

[1] Molecular Diagnostics-Infectious Diseases, Department of Pathology, Mater Dei Hospital , Msida, Malta
[2] Bacteriology Laboratory, Department of Pathology, Mater Dei Hospital, Msida, Malta

Antimicrobial resistance (AMR) is a critical global concern driven by the overuse, misuse, and/or usage of inadequate antibiotics on humans, animals' agriculture, and as a result of contaminated environments. Through the FWD AMR Ref Lab Cap project and a One Health approach whole-genome sequencing (WGS) was implemented to examine the spread of AMR in Campylobacter and Salmonella. The FWD-AMR-RefLabCap project is a collaborative effort among European national public health reference laboratories specializing in Antimicrobial Resistance (AMR) detection in Salmonella and Campylobacter. The main objectives are to enhance the capabilities of reference laboratories to detect and characterize AMR., to foster collaboration and knowledge exchange among participating laboratories and to improve surveillance and response to AMR threats. Pathogen genomics has the potential to transform public health surveillance by improving outbreak detection and investigation. Increasing the laboratory capacity will help support activities that will enable large scale surveillance of infectious diseases, it will also have a long-term impact on keeping highly skilled Human resources within the country. In addition, all the information gathered from the etiological and epidemiological studies supported by the project will offer critical data as baseline information needed for the health reform policies in Malta. Improvements in service provision since the initiation of the project: As of 1st October 2022, all Salmonella and Campylobacter isolates (clinical, veterinary and from the public health laboratory), as well as human faeces samples that are PCR positive but fail to grow the pathogen, are being analysed further by WGS. WGS results are shared with the Infectious Disease prevention and Control Unit (IDCU), who in turn are looking at the joint data to identify potential clusters.

Ms. Nikita Camilleri

Unravelling the Globin Gene Switch Mechanism in Patients with Hereditary Persistence of Foetal Haemoglobin

Nikita Camilleri[1], Josef Borg[1], Carmen van der Zwaan[2], Kerly Fu[2], Marieke von Lindern[2], Emile van den Akker,[2] Alex E. Felice[3]

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Hereditary persistence of foetal haemoglobin (HPFH) is a benign genetic condition characterised by elevated foetal haemoglobin (HbF) levels that persist throughout adulthood, due to disrupted globin gene switching. Studies have demonstrated that the coexistence of HPFH with other haemoglobinopathies reduces the severity of associated symptoms, attributed to elevated HbF levels. This study focused on 3 Maltese families, encompassing 11 HPFH-affected individuals due to a truncation mutation (p.K288X) in the *KLF1* gene and 11 healthy relatives serving as controls. The primary objective was to gain insights into the underlying mechanisms involved in the regulation of globin gene switching. Whole genome sequencing identified a total of 205 unique variants following a dominant inheritance pattern present in all HPFH-affected individuals. These variants were located on chromosome 19 in close proximity to the *KLF1* gene. Novel variants were discovered in the *LMO2* and *KLF1* genes, which potentially contribute to the onset of HPFH. A subset analysis focusing on four subjects from Fam F1, exhibiting the highest HbF levels (>3%), revealed variants in the *NLRP3* and *RPS9* genes which likely account for higher HbF levels in Fam F1, despite carrying the same *KLF1* mutation as other families. Proteomic analysis using mass spectrometry identified 53 proteins significantly correlated with HbF levels in HPFH-affected subjects, suggesting their involvement in globin gene regulation. In conclusion, this study highlights the importance of adapting an integrative approach to understand the molecular mechanisms underlying *KLF1* deficiency. The identification of potentially causal variants associated with HPFH, provides valuable insights into the onset of this condition. Further investigations involving functional work to confirm the precise impact of the variants and proteins in the upregulation of HbF levels, can provide an extensive understanding of the underlying genetic architecture of HPFH.

Ms. Azra Zejnelagic

Analysis of Structural Variants in Osteoporosis

Azra Zejnelagic[1], Chanelle Cilia[2], Jean-Paul Ebejer[1], Melissa M. Formosa[1,2]

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Introduction: Osteoporosis is a metabolic bone disorder with a strong genetic influence which has been a subject of study in research aiming to pinpoint crucial genes associated with bone health. It has been linked to structural variants (SVs), defined as alterations in chromosome structure exceeding 50 bp in size. This study aimed to comprehensively analyze SV detection tools using whole genome sequencing data (WGS) from a 2-generation Maltese family having multiple relatives affected with osteoporosis and low bone mineral density (BMD).

Materials and Methods: BreakDancer, Pindel, and Lumpy were selected and computationally assessed on six BAM files generated from short-read WGS. Genotype calling was required for BreakDancer and Lumpy, and additional tools BreakDown and SVtyper were employed. Variants identified by these tools underwent annotation and prioritization based on their relevance to bone physiology. Experimental validation of selected SVs was performed through PCR sizing and Sanger sequencing. Results: Experimental validation confirmed three SVs detected by Lumpy within the *ARHGEF3*, *TBX15*, and *ADAM9* genes, while four SVs identified by Pindel were confirmed within the *SOD2*, *KLF12*, *MCTP1*, and *PTPRM* genes. Lumpy exhibited superiority over Pindel and BreakDancer, showcasing faster runtime, smaller memory footprint for output files, and minimal system requirements.

Conclusion: The findings suggest that the SVs detected by Lumpy and Pindel could potentially be contributing to the genetic architecture of osteoporosis and BMD.

Ms. Kimberly Fenech

5-Fluorouracil Resistant Colorectal Cancer Cells Show a Reduced Expression of Key Protein Methyltransferases and No EMT Potential

Kimberly Fenech[1], Byron Baron[1]

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Colorectal cancer (CRC) is one of the most frequently diagnosed cancers. The poor prognosis of advanced stage CRC is attributed to chemoresistance. There are several mechanisms associated with chemoresistance, including the epithelial-to-mesenchymal (EMT) transition and dysregulation in protein methylation. Dysregulation in protein methylation or in the expression of protein methyltransferases (PMTs) have been associated with the induction of EMT in several cancers. In this study, the impact of 5-FU resistance was investigated on EMT and protein methylation in HCT-116, Caco-2 and DLD-1 CRC cell lines. Cells were exposed to long-term treatment of 5-FU to generate 5-FU resistant cells. Cell viability, wound healing and invasion assays were carried out on parental (untreated) and resistant CRC cells to compare the difference in viability, migration and invasion in the two cell types. Western blotting was carried out to investigate the difference in the protein expression of the EMT markers, E-cadherin, vimentin and Snail in both parental and resistant cells. Additionally, the protein expression of the PMTs Euchromatic histone lysine methyltransferase 2 (EHMT2/G9a), protein arginine methyltransferase 5 (PRMT5) and SET domain containing 7/9 (SETD7/9) together with the lysine and arginine methylation profiles were investigated in the CRC cells before and after treatment. 5-FU resistant cells showed several morphological changes during treatment and tolerated higher doses of 5-FU when compared to parental cells. However, they demonstrated slower migration and invasion rates with lower expression of EMT markers, PMTs and dysregulated global protein methylation profiles. Taken together, although EMT was not one of the mechanisms associated with 5-FU resistance in CRC, dysregulation in protein methylation and in the expression of protein methyltransferases may in part be responsible in the generation of resistant cells.

Ms. Chantelle Sell

The Association of Two Novel Variants in *FBN1* and *SMAD3* with Familial Aortic Diseases

Chantelle Sell[1], Jessica Debattista[1], Christian Scerri[1], Maryanne Caruana[2], Jeanesse Scerri [1]

[1] Molecular Pathology and Genetics Laboratory, Department of Pathology, Mater Dei Hospital, Msida, Malta
[2] Cardiology Department, Mater Dei Hospital, Msida, Malta

Background: Thoracic aortic aneurysm and dissection (TAAD) is the leading cause of sudden cardiac death. Pathogenic variants in *FBN1* and *SMAD3* genes are associated with non-syndromic hereditary TAAD cases. A Maltese family with a history of aortic root dilation and sudden cardiac death was found to harbour two novel variants in the *FBN1* and *SMAD3* genes, whose role in the manifestation of the phenotype has not yet been determined. Aim: To observe the segregation of the novel *FBN1* c.3956G>T and *SMAD3* c.184A>G variants with aortic dilation in the above-mentioned family, and to determine their prevalence in the Maltese population.

Method: The family pedigree was compiled using anonymized genotypic and phenotypic data. 360 randomly selected neonate blood samples were analysed for the presence of both variants by isolating deoxyribonucleic acid (DNA) from blood, genotyping DNA using tetra-primer amplification-refractory mutation system (ARMS) PCR and analysing results using agarose gel electrophoresis. The prevalence of each variant in the general population was assessed and the allele frequencies were used to determine whether the population was in Hardy-Weinberg Equilibrium.

Results: Data obtained from the family pedigree identifies aortic dilation in members presenting with both variants, and no effect on phenotype in members presenting with one variant. The prevalence of each variant in the population was 0%.

Conclusion: Penetrance variability in family members shows that the presence of both variants together might be the causal effect of the aortic root dilation, whilst the statistical data indicates that both variants are rare or absent in the Maltese population.

Mr. Mark Briffa

Waste Water Surveillance of SARS-CoV-2 in the Maltese Islands

Mark Briffa[1] , Christopher Barbara[1], Mattia Caurana[1], Kurt Galea[1], Christopher Grech[1], Elaine Lautier [2], Tanya Melillo[2], Stephanie Portelli[1], Graziella Zahra[1]

[1] Molecular Diagnostics - Infectious Diseases, Department of Pathology, Mater Dei Hospital, Msida,Malta
[2] Infectious Disease Prevention and Control Unit, Department of Health Regulation, Gwardamanga, Malta

In recent years, widespread advances have been made in the field of metagenomics for disease surveillance, particularly through the analysis of wastewater. Theoretically, wastewater surveillance allows public health departments to pre-empt potential infectious disease outbreaks in the general population and monitor vaccine-preventable diseases (Daughton, 2020). This empowers public health departments to implement any necessary interventions as early as possible. Unfortunately, the analysis of metagenomics data obtained from wastewater is complex and prone to inaccuracies due to the extent of contaminating material present in the raw samples. Hence, wastewater surveillance is currently most effective when preceded by capture methods or targeted to specific pathogens of interest.

Locally, surveillance of SARS-CoV-2 from wastewater has been performed on a weekly basis over the past 18 months as part of a collaborative effort between the Molecular Diagnostics – Infectious Diseases laboratory at Mater Dei Hospital, the Water Services Corporation, and the Public Health Department. Weekly deliveries of wastewater from the North, South and Gozo wastewater plants are made to the laboratory. Once delivered, a magnetic bead-based extraction technique is used to extract RNA from the wastewater samples. Amplification and detection of SARS-CoV-2 is then done by real-time quantitative PCR.

This poster will present the methodology used to establish a wastewater surveillance infrastructure in the Maltese Islands. Furthermore, quantitative results obtained during this period of surveillance will be shown.

Ultimately, conclusions will be made on what is required to make meaningful comparisons between SARS-CoV-2 viral loads in wastewater and the presence of SARS-CoV-2 cases in the wider public and reveal whether the current infrastructure allows for the monitoring of other pathogens in wastewater.

This work is funded by the EU as part of Grant Agreement No 060701/2021/864485/SUB/ENV.C2: Support to the Member states to establish national systems, local collection points and digital infrastructure for monitoring COVID-19 and its variants in wastewater.

Mr. Mark Briffa

Innovative Molecular PRofiling of distINct Tumour derived cells in blood (IMPRINT)

Mark Briffa[1], Martina Busuttil[2], Laura Grech[2], Marita Vella[1], Therese Hunter[1], Malcolm Buhagiar[3] Christian Scerri[1], Godfrey Grech[2]

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[2] Department of Pathology, University of Malta, Msida, Malta
[3] Sir Anthony Mamo Oncology Centre, Mater Dei Hospital, Msida, Malta

The detection and identification of cell surface markers on circulating tumour cells (CTCs) is well-documented as a means of early detection of metastasis in cancer-affected patients. The IMPRINT study aims to develop and optimise a method to capture CTCs from peripheral blood samples of cancer-affected patients and profile the cell surface markers present on the CTCs by means of Digital Western Technology (DigiWest®).

This project utilises various methods of identifying and detecting cell surface markers on cancer cells. These include: the QuantiGene Plex assay, a modified version of DigiWest technology (as mentioned above), and in-gel trypsin digest followed by mass spectrometry.

Initial efforts focused on the use of the QuantiGene Plex assay to detect epithelial and mesenchymal markers on different cells.

Subsequently a modified DigiWest methodology was optimised and implemented. The modified DigiWest method begins with the biotinylation of cell surface markers of intact cells and the subsequent lysis of these cells. Early work on this method focused on the development of a technique to successfully biotinylate intact cells. This was followed by an optimisation of lysis methods to ensure that the lysis process did not affect the protein-biotin bond, hence maintaining the integrity of the downstream analysis. Successful biotinylation and lysis of cell surface markers was confirmed by means of western blot analysis using lysates of both unbiotinylated and biotinylated cells, stained with an anti-biotin antibody. The DigiWest process is now being used to analyse the biotinylated lysates.

Incidentally, a unique and interesting band with a molecular weight of approximately 55– 60kDa was observed in the blot for biotinylated MDA-MB-231, a breast cancer cell line. To attempt to identify the protein present, in-gel trypsin digest followed by mass spectrometry was carried out. Results showed that vimentin was identified as the protein with the highest coverage (90%), supporting the findings observed in earlier analysis done using the QuantiGene Plex Assay.

This study entitled Innovative Molecular PRofiling of distINct Tumour derived cells in blood (IMPRINT) is funded by the Malta Council for Science and Technology (MCST) (Contract Number: R&I-2018-037T) as part of the MCST FUSION TDP.

Prof. Byron Baron

Utilisation of Surplus, Expired and Waste Transfusion Products in Proteomics-Based Research

Byron Baron[1], Vanessa Zammit[1,2], Mark Farrugia[1]

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[2] National Blood Transfusion Services, Gwardamanga,Pieta', Malta

The process of preparing blood transfusion products is laborious and expensive but the shelf-life for human use is very short. Nevertheless, expired transfusion products together with the waste components isolated during processing are still viable for research applications. The white blood cells isolated as a waste fraction have been used as an infection model. The surplus plasma has been used to replace the foetal bovine serum in cell culture. The expired platelet concentrates have been converted into a lysate and used as a growth factor supplement for certain cell types. Following the characterisation of these blood components, various standard cell culture practices were optimised in order to set up an effective xeno-free culturing system. The implementation of such a xeno-free culturing system in combination with small molecule cocktails on umbilical cord stem cells provided a setting for differentiation into a neuronal or an insulin producing cell lineage. This allowed for better comprehensive protein characterisation using mass spectrometry-based proteomic techniques, having investigated both the cells at different stages and the exosomes for unique protein signatures and post-translational modifications. This topic is of interest because it is known that xeno-free culturing systems better mimic in-vivo conditions but sourcing all the components might not always be easy or possible. Moreover, this allows for the development of novel techniques for cutting-edge proteomics research, offering new insight into cell biology questions.

Ms. Gabriella Azzopardi

Prevention of Anti-D Prophylaxis in Pregnant RhD Negative Females Using Non-Invasive Foetal RhD Genotyping in Malta

Gabriella Azzopardi[1], Stefan Laspina[1,2], Jesmond Debono[1], Charmaine Lia[4], Jessica Debattista[3], Laura Grech[5], Godfrey Grech[2]

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[4] Obstetrics and Gynaecology Department, Mater Dei Hospital, Msida, Malta
[5] Department of Applied Biomedical Sciences, Faculty of Health Sciences, University of Malta, Msida, Malta

The discovery of cell-free foetal DNA (cffDNA) in maternal peripheral blood has revolutionised the field of non-invasive prenatal testing (NIPT), eliminating the need for invasive procedures to obtain a foetal sample. In recent years, routine antenatal anti-D prophylaxis (RAADP) for all RhD negative women has been subject to ethical considerations. This led to the concept of targeted RAADP, where women are treated only if the foetus is determined to be RhD positive through foetal RHD genotyping. The aim of this study is to introduce NIPT foetal RHD genotyping in Malta with the goal of reducing prophylactic anti-D usage by 42%. To achieve this, the study set out three objectives: Design and validate a highly sensitive and specific RHD genotyping test suitable for the Maltese population; compare the effectiveness of two PCR platforms - real-time PCR (qPCR) and droplet digital PCR (ddPCR); and confirm the PCR results by comparing them with the current postnatal serological gold standard test - the Cord Blood test. A 9mL blood sample was collected from 31 RhD negative women at around 28 weeks of gestation. Cell-free DNA was extracted using a semi-automated method, quantified using a low-throughput automated microfluidic capillary electrophoresis platform, and analysed for RhD (RHD5 and RHD7) and gender (SRY) genotyping using qPCR and ddPCR. The results from both PCRs were confirmed with and compared statistically to the serological cord blood test. qPCR achieved a remarkable 98.39% accuracy (97.22% specificity; 100% sensitivity) while ddPCR achieved a 96.77% accuracy (100% specificity; 92.31% sensitivity). These results align with findings from studies conducted in other countries, thereby supporting the use of both PCR platforms and the introduction of NIPT in Malta. In conclusion, the implementation of NIPT foetal RHD genotyping in Malta holds promising potential for reducing prophylactic anti-D treatment by 42%.

Ms. Mariana Grima

Phage Display Identifies Single Chain Variable Fragment Antibodies Specific to Immune Checkpoints

Małgorzata Lisowska[1], Giuseppina Monda[2], David Saliba[2], Jasmine Spiteri[2], *Mariana Grima*[2]

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Tumour cells use immune checkpoints to evade the immune system, which act as molecular brakes that prevent attacks on healthy cells. Immune checkpoint inhibitors (ICIs) are a type of immunotherapy that blocks these checkpoints, allowing the immune system to attack cancer cells. FDA-approved examples include antibody-based inhibitors of Programmed Death-Ligand 1 (PD-L1) and Cytotoxic T-Lymphocyte Associated Protein 4 (CTLA-4) for melanoma and lung cancer. We are investigating additional co-inhibitory molecules to improve efficacy and reduce resistance. To identify new monoclonal antibodies for cancer immunotherapy, we used phage display biopanning to target immune checkpoints. We targeted V-Domain Ig Suppressor of T Cell Activation (VISTA) and PD-L1, expressed on myeloid cells and certain cancer types, as well as T cell Immunoglobulin and Mucin domain 3 (TIM-3) and TIM-4, expressed on T cells and antigen-presenting cells, respectively. Phage display produces monoclonal antibodies more quickly than traditional methods. The process involves immobilising the target protein on a surface, expressing scFv antibodies on M13 phages, and competitively binding them to the immobilised target. Unbound phages are removed, and target-specific phages are eluted and subjected to four rounds of selection for high target affinity. We validated 30 monoclonal antibodies for specificity using ELISA and determined their amino acid sequences through Sanger sequencing. Different amino acid sequences were identified for the anti-TIM-3 and anti-TIM-4 antibodies. We are currently evaluating the specificity of anti-VISTA and anti-PD-L1 antibodies and assessing the amino acid sequence of each clone. This research demonstrates the potential of phage display technology in identifying monoclonal antibodies targeting novel immune checkpoint targets for cancer immunotherapy.

Ms. Alessia Sammut Carta

A Case of Disseminated Intravascular Coagulation Complicating Catastrophic Uterine Bleeding Secondary to Adenomyosis

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A 41-year-old female presented to hospital with severe vaginal bleeding, with this having started a few days prior but which had become torrential in nature. En route to hospital, she suffered a tonic-clonic seizure, control of which was achieved with benzodiazepines. On clinical examination, she was noted to be hypotensive, pale and tachycardic, in view of which she was transfused several units of blood. Magnetic resonance imaging studies showed the presence of an acute intraparenchymal haemorrhage in the region of the right basal ganglia. Computed tomography studies showed a bulky uterine mass with extensive endoluminal haemorrhage. Blood investigations showed severe anaemia and thrombocytopenia, with an acute associated coagulopathy compatible with disseminated intravascular coagulation (DIC). Despite intensive multiorgan support, further intervention was deemed futile and life support was withdrawn after twenty-four hours. A post-mortem examination was carried out. The uterus was enlarged and bulky, extending beyond the pelvic cavity to the supraumbilical region, comparable to a 28-week gestation. Both adnexae were grossly unremarkable. On sectioning, the endometrium contained copious blood clots. The uterine wall had a diffusely trabeculated appearance, with microcysts containing inspissated blood. Histological analysis confirmed the macroscopic impression of diffuse uterine adenomyosis. The brain was diffusely oedematous and, on sectioning, a focus of intraparenchymal haemorrhage was observed, centred on the right caudate nucleus. This bleed extended into the ventricular system, with associated diffuse bilateral subarachnoid haemorrhage. The cause of death in this case was deemed due to intraparenchymal cerebral haemorrhage secondary to DIC complicating catastrophic uterine bleeding caused by adenomyosis. We herein describe a vanishingly rare case of DIC arising in the unusual setting of adenomyosis, in the absence of conventional associated factors.

SCIENTIFIC PROGRAM

DAY 2 | Saturday 6th April

08:00 – 08:30 REGISTRATION

08:30 – 08:45 OPENING OF DAY 2

08:45 – 09:30 KEYNOTE LECTURE

Dr. Brigitte Maes: The Belgian Approach for Local Laboratory Extensive Tumor Testing (BALLETT)

09:30 – 10:00 COFFEE BREAK & POSTER SESSION

10:00 – 11:30 | ORAL PRESENTATIONS | SESSION 3 – CANCER SPECIAL

Dr. Matthew Farrugia: TFE-3 Rearranged Renal Cell Carcinoma, a Case Report

Dr. Ian Said Huntingford: A Retrospective Analysis of Lung Cancer Diagnoses Made by Endoscopic Bronchial Ultrasound, a Histological and Molecular Analysis

Dr. David Pisani: A Case of Primary Testicular Follicular Lymphoma

Ms. Jessica Debattista: Tumour Budding-Specific Signatures in Colorectal Carcinoma

Ms. Patricia Brincat: Serendipity in Science

11:30 – 13:00 | PANEL DISCUSSION

Comprehensive Cancer Care Panellists:

- Dr. Miriam Dalmas
- Dr. James DeGaetano
- Ms. Martina Fenech
- Dr. Nick Refalo
- Prof. Christian Scerri

12:30 – 13:45 CLOSING & LUNCH

ABSTRACTS

Dr. Bridgette Maes

The Belgian Approach for Local Laboratory Extensive Tumor Testing (BALLETT)

Bridgette Maes[1]

[1] Laboratory of Molecular Diagnostics, Jessa Hospital, Hasselt, Belgium

Background & objectives: Comprehensive Genomic Profiling (CGP) in cancer diagnostics is gaining interest but broad access is still limited. The nationwide BALLETT study aimed to have a fully standardized CGP analysis available, potentially increasing access to innovative drugs for Belgian cancer patients.

Methods: BALLETT has recruited about 872 patients with a metastatic solid tumor in 12 Belgian hospitals. CGP was weekly performed on pooled samples using the TSO500® kit (Illumina) by one of the 9 hospital labs collaborating in the BALLETT lab consortium. Results were discussed weekly in a virtual, national Molecular Tumor Board (nMTB) resulting in CGP-based treatment recommendations.

Results: From June 2021 to October 2023 tumor samples of 756 patients with 32 different tumor types were successfully tested by CGP, with a final test success rate of 93 %. Median turnaround time from informed consent to nMTB discussion was 26 days. Actionable genomic alterations were recorded in 83 % of cases. The nMTB discussions resulted in a CGP-guided treatment recommendation in 531 patients (70 %): reimbursed treatments (n=58), participation to a clinical trial (n=425) or a medical need program (n=10) or off-label drug use (n=38). In 21 % of patients, the treatment recommendation was followed by the treating physicians. Follow-up on potential hereditary cancer predisposition was recommended for 74 patients (12 %).

Conclusion: BALLETT has resulted in a broad access to CGP for patients with metastatic cancer in Belgium. Clinically relevant biomarkers were identified and CGP-based treatment recommendations were made for the large majority of patients. The outcome of the patients will be followed up within the project. Furthermore, the BALLETT laboratory consortium combined with the nMTB is a valuable platform for reducing turnaround time, exchanging expertise and standardization of CGP methodology and treatment recommendation. ClinicalTrials.gov:NCT0505893.

Dr. Matthew Farrugia

TFE-3 Rearranged Renal Cell Carcinoma, a Case Report

Matthew Farrugia [1], David Pisani [1] , Charlene Busuttil [1]

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TFE-3 (transcription factor E3) rearranged renal cell carcinoma (RCC) represents a rare but well recognised histological type of renal cell carcinoma.

We present a case report of 36-year-old female who presented to the emergency department with a six-day history of visible painless haematuria associated with urgency. No dysuria or episodes or retention were documented. She claimed that she had had two episodes of a urinary tract infection (UTI) in the previous month. Ultrasound studies of the urological tract showed a tumour occupying the lower half of the right kidney. This was followed up with computed tomographic imaging, showing an enhancing tumour in the interpolar region of the right kidney measuring 10cm in maximum dimension. A right radical nephrectomy was subsequently carried out. On macroscopy a well-circumscribed 95mm friable tumour was identified, having a tan, focally cystic cut surface. On histological examination, the tumour was comprised of papillary fronds and glands lined by large neoplastic cells with distinct cell membranes, variably eosinophilic-to-clear cytoplasm and vesicular nuclei with readily discernible nucleoli. No calcifications were seen. The tumour was noted to infiltrate the pelvicalyceal system of the kidney. Strong nuclear expression of PAX8, apical CD10 expression and diffuse AMACR expression was noted. No expression of CK7, CD117 or melanocytic markers was noted. An ALK1 stain was also negative. Strong and diffuse nuclear TFE3 immunohistochemistry was confirmed**.**

The morphological and immunohistochemical findings, together with the patient's demographic were suspicious for a translocation-associated renal neoplasm. Molecular studies performed in the United Kingdom established a TFE-3 translocation (locus at Xp11.23) confirming a TFE-3 rearranged renal cell carcinoma.

These tumours are very rare molecularly-defined neoplasms which account for around 2% of all adult renal tumours, with a higher frequency in the paediatric population. These tumours typically follow and aggressive course and present at an advanced stage, as in the case outlined above. Adjuvant therapy is typically indicated in these patients.

Dr. Ian Said Huntingford

A Retrospective Analysis of Lung Cancer Diagnoses Made by Endoscopic Bronchial Ultrasound, a Histological and Molecular Analysis

Ian Said Huntingford[1], Michael Pace Bardon[2], Jessica Saliba[2], Justine Borg[3], Jade Camilleri[3], Dr. Joanne D'Amato[3]

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Background: Linear convex probe Endoscopic Bronchial Ultrasound (EBUS) is one of the main diagnostic and staging modalities used in lung cancer. This was initially introduced to Mater Dei Hospital in 2016. Besides providing a histological diagnosis, this technique can accurately stage lung cancers and help to determine subsequent management. This analysis serves to assess the adequacy of tissue samples obtained by such a technique. We also provide a local perspective on PD-L1 status, molecular profile and attempt to correlate smoking status with histological subtype, PD-L1 status and molecular profile.

Methods: 321 lung cancer patients were retrospectively analysed over the period of 2018-2022. Of these, 42 lung cancer patients had their diagnosis established by EBUS. The histological subtype, adequacy of the sample, PD-L1 status, EGFR and ALK-1 profile were assessed. In addition, the smoker status of the patients was correlated with the histological subtype, PDL-1 and EGFR status. Results: 26 cases were diagnosed as adenocarcinoma, 7 cases as squamous cell carcinoma, with the remainder of the cases divided into various other histological subtypes. All cases tested for PD-L1 status (39) were adequate, and all cases submitted for EGFR, ALK1 testing were sufficient. 9.4% of cases demonstrated an EGFR mutation. 96.4% of all squamous cell carcinoma cases had a smoking history compared to 70% of all adenocarcinoma cases. Statistical significance was not obtained when comparing smoking status with PD-L1 status and molecular profile.

Conclusion: Despite being a relatively recent innovation locally, EBUS is an effective and readily available means of obtaining a histological diagnosis in lung cancer patients. Furthermore, material provided is sufficient for assessment of PD-L1 status and molecular (EGFR, ALK-1) testing.

Dr. David Pisani

David Pisani[1]

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A Case of Primary Testicular Follicular Lymphoma

A 29-year-old male was referred in view of a firm left testicular mass which was serially followed up by ultrasound given positive Chlamydia urinary PCR studies, but which persisted despite antibiotic therapy. A left radical orchidectomy was performed, with the testicle and epididymis involved by a grossly evident tumoural mass. Histology showed a neoplastic lymphoid infiltrate with a follicular morphology, comprised predominantly of centroblasts, which also exhibited foci of diffuse growth. Fluorescence in situ hybridisation was negative for t(14;18) BCL2:IGH translocation. Despite a high grade cytomorphology, disease was confined to the testicle on imaging studies. Bone marrow analysis showed no evidence of disease involvement. The overall disease pattern confirmed with a primary testicular follicular lymphoma (PTFL). The patient was given R-CHOP chemotherapy and remained well, with no evidence of disease recurrence. PTFL represents a lymphoma entity which is not currently classified as a unique entity in the 5th Edition of the WHO Classification of Lymphoid Tumours. These are exceedingly rare tumours which typically arise in the paediatric and adolescent setting, with tumours usually being confined to the testicle or showing epididymal and paratesticular infiltration but no evidence of nodal or marrow involvement. They are characteristically t(14;18) negative tumours and thus cannot be classified as a conventional follicular lymphoma subtype. Despite high grade histological features, these lymphomas are typically indolent and carry an excellent prognosis.

Ms. Jessica Debattista

Tumour Budding-Specific Signatures in Colorectal Carcinoma

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Colorectal malignancy accounts for the second most common cause of cancer-related mortality. The search for prognostic biomarkers subserving to improve tumour classification and to stratify therapeutic patient management groups is actively ongoing. A promising histomorphological biomarker in colorectal cancer (CRC) is tumour budding; characterised by single isolated tumour cells or small clusters of up to five cells (buds) at the invasive tumour front. Budding is associated with both nodal and distant metastasis and is therefore a promising surrogate prognostic marker.

A retrospective analysis was performed on 44 formalin-fixed paraffin-embedded (FFPE) tissue sections with at least stage 3 CRC burden. The three discreet tumoural compartments histologically selected were (a) the central portion of the tumour, (b) an invasive front lacking tumour budding and (c) areas of tumour budding at the invasive front. A 0.5mm core was obtained from each respective compartment from the specimen block. These cores were lysed for 18-22 hours at 58°C. Fifty different genes related to CRC pathology were analysed. Data analysis was carried out by comparing expression of each of these gene against that of house-keeping genes, with mean values subsequently compared by virtue of paired t-test analysis.

The cell motility and cytoskeletal genes *TRIB1* and *TPX2*, the cell cycle promoter *CCND3* and the antiapoptotic factor *MCL1* were increasingly expressed progressing from the central compartment of the tumour to invasive fronts lacking tumour buds to areas rich in tumour buds. Statistical significance was reached when specifically comparing the central tumour with areas rich in tumour buds ($p = 0.009$ [*TRIB1*], 0.04 [*TPX2*], 0.04 [*CCND3*], 0.008 [*MCL1*]).

Identification of such genes may offer an approach to establishing tumour budding-specific signatures that seem to be responsible for local and distant metastases. Furthermore, tumour budding has potential implications in liquid biopsy analysis.

Ms. Patricia Brincat

Serendipity in Science

Patricia Brincat [1], Jacklyn Saliba[1], David James Camilleri[1], Alexander Gatt[1], Mark Grech[1], David Pisani[1]

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Serendipity in science refers to unexpected yet very important discoveries made whilst the actual research is focused on a different line of thought. Throughout history, these discoveries have led to very important breakthroughs, leading to significant unanticipated achievements. Examples of such discoveries include penicillin, X-rays and Microwaves. Unexpected findings are also encountered during routine diagnostic laboratory practice. A routine check-up may trigger specialised investigations, which in turn uncover unexpected diagnostic findings and previously unsuspected conditions. This presentation will look into three case studies which clinical assessment and initial diagnostic workup (Blood Counts and morphology) indicated a specific type of Haematological malignancy. However, upon further immunophenotypic investigations, results revealed either additional concurrent disorders or a diagnosis completely different from what was initially predicted. The three cases described were initially being investigated for A) Acute Myeloid Leukaemia, B) Myelodysplastic Syndrome and C) T-cell Lymphoma, however other Haematological malignancies were found to be present which were totally unanticipated. These unforeseen findings had major impact on patient management, including treatment plans, prognosis, and patient monitoring. These case scenarios are few examples which highlight the importance of adopting an open minded and receptive diagnostic approach in the laboratory, thus capturing unexpected outcomes, both in the diagnostic and in the research field. Serendipity often occurs when scientists are vigilant, curious, observant, and willing to explore the unknown, even when it deviates from the original clinical question.

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