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BASIC MEDICAL SCIENCES: INTERNATIONAL SCIENTIFIC CONFERENCE ON MEDICINE AND HEALTH SCIENCES OF THE UNIVERSITY OF LATVIA, 2026

On 26 April 2026, the University of Latvia, in Rīga, hosted the International Scientific Conference on Medicine and Health Sciences.

The section “Basic Medical Sciences” unified several talks on modelling of the diseases, aptamer technology, gene structure and expression in pathology, mitochondrial respiration, some questions of immunology and gasotransmitters.

The topic of disease modelling was represented in the talks, “Development of a calpainopathy *in vitro* model, using patient-derived induced and pluripotent stem cell lines” by J. Stavusis, and “A zebrafish larval xenograft model for pancreatic ductal adenocarcinoma” by K. Kindzule. L. Sproģe and K. Sunteiks. Recent achievements in aptamer technology were represented in the talks entitled “Development and validation of aptamer-based diagnostic tools for malaria detection” by L. Sproģe *et al.* and “Affinity characterisation of the greenb1 aptamer in 2D and organoid models of pancreatic ductal adenocarcinoma under static and microfluidic conditions” by K. Sunteiks *et al*. B. L. Revina reported interesting data on respiration in white blood cells in young men and women, ‘Exploring gender effects on mitochnondrial respiration of PBMCS in healthy young adults”. Questions of the gene structure and expression in relation to diabetes mellitus and multiple sclerosis were summarised in the talks, “FTO gene rs9939609 polymorphism and TCF7L2 gene rs7903146 polymorphism effect on body composition and glucoselevels in young women” (by A. Seržante) and “Proteasome gene expression links to disability and treatment context in a Latvian multiple sclerosis cohort” (by S. Pļaviņa). Our guests from Ukraine presented novel data on immunomodulators (“Size- and dose-dependent immunomodulatory effects of Ti_3C_2 MXenes”, by M. Truhins). Lithuanian colleagues presented very topical data on synergism of two gasotransmitters, nitric oxide and hydrogen sulfide on vasorelaxation (“Involvement of H_2S in nitric oxide relaxations of rat pulmonary arteries”, by A. Tunaitytė).

Nikolajs Sjakste

A ZEBRAFISH LARVAL XENOGRAFT MODEL FOR PANCREATIC DUCTAL ADENOCARCINOMA

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Background. Pancreatic ductal adenocarcinoma (PDAC) remains a clinical challenge due to its dense stroma and late diagnosis. Zebrafish (*Danio rerio*) larvae xenografts, or "zebrafish avatars", have emerged as a promising tool for precision medicine. They allow cell culture within a complex living organism, providing tissue context and allowing high-power drug sensitivity assays within a short timeframe.

Aim. The study aims to establish and characterise a zebrafish xenograft model for PDAC using cell lines and patient-derived organoids to evaluate metastatic potential, tumour-stroma interactions, and drug responses.

Methods. Xenografts were established by injecting MiaPaCa-2 cells or PDAC organoid culture cells into the perivitelline space of AB strain and *MNX:RFP* zebrafish larvae. To study the tumour microenvironment, cells were transplanted alone or in combination with fibroblasts. Cells were pre-labelled with CytoPainter Red for tracking. Whole-mount immunofluorescence (IF) staining was performed for cleaved Caspase-3 (apoptosis) and acetylated tubulin (neuronal tracks). Imaging was conducted via confocal and widefield epifluorescence microscopy. Prior to treatment, a maximum tolerated dose (MTD) was determined for common PDAC chemotherapeutic agents to establish a safe toxicity range in larvae.

Results. We established the non-toxic exposure range for several frontline PDAC chemotherapeutic agents. Notably, for gemcitabine, we identified a tolerated concentration range below 250 μM, which contrasts with previously published observations. We observed high engraftment efficiency (80%) for one patient-derived organoid line, comparable to that of MIA PaCa-2, and detected strong metastatic potential in the primary cells. The presence of fibroblasts altered xenograft morphology, resulting in denser and more rounded tumour structures. Whole-mount xenograft staining enabled efficient axonal labelling and detection by confocal microscopy. In chemotherapy-treated samples, Caspase-3–positive cells were detected and underscoring the need for confocal microscopy and automated analysis for accurate quantification of drug effects in this model Conclusion. Our findings validate the zebrafish xenograft as a robust platform for modelling PDAC. The inclusion of fibroblasts highlights the importance of the stroma in engraftment dynamics. This high-throughput model bridges the gap between in vitro cultures and mammalian studies, offering a scalable system for personalised drug sensitivity testing and the study of tumour stroma interactions in PDAC.

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PROTEASOME GENE EXPRESSION LINKS TO DISABILITY AND TREATMENT CONTEXT IN A LATVIAN MULTIPLE SCLEROSIS COHORT

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Background. The ubiquitin–proteasome system (UPS) regulates antigen processing and inflammatory signalling, both central to multiple sclerosis (MS). However, how proteasome gene expression relates to clinical severity and treatment context in real-world MS cohorts remains insufficiently characterised.

Aim. To assess associations between expression of selected proteasome genes and clinical parameters in Latvian MS patients, and to explore whether expression profiles differ across disease- and therapy-related strata.

Methods. Gene expression levels of key proteasome/UPS components (including PSMA3, PSMA6, PSMA7, and PSMC6) were quantified in MS patients from a Latvian PSMC6) were quantified in MS patients from a Latvian cohort using PCR-based assays. Clinical variables included disability (EDSS), disease duration, age, and inflamma-

tory/immunological markers (e.g., IgG). Group differences were tested using non-parametric methods (Mann–Whitney/Kruskal–Wallis or ANOVA where applicable), and associations with continuous clinical variables were evaluated by Spearman correlation. Therapy-related analyses compared patients on treatment versus not treated and/or across therapy types (e.g., IFN-β, GA, MX).

Results. Across the MS group, expression medians did not show consistent differences by sex or by broad clinical subtype in the tested comparisons. In contrast, therapy context was associated with expression variability, with significant between-therapy differences detected for multiple genes, including PSMA3 (Kruskal–Wallis $P = 4.95\times10^{-2}$), PSMA7 ($P = 1.43\times10^{-2}$), and a stronger effect for PSMA6 ($P = 1.47\times10^{-3}$). Clinically, higher disability was linked to lower expression: EDSS showed negative correlations with PSMA3 ($\rho = -0.21$; $P = 2.04\times10^{-2}$), PSMA7 ($\rho = -0.20$; $P = 2.18\times10^{-2}$), and PSMA6 ($\rho = -0.20$; $P = 2.22\times10^{-2}$). Disease duration correlated with PSMA3 ($\rho = 0.18$; $P = 3.71\times10^{-2}$). Immunological status also related to UPS expression: IgG correlated negatively with PSMA3 ($\rho = -0.21$; $P = 2.06\times10^{-2}$) and PSMC6 ($\rho = -0.22$; $P = 1.65\times10^{-2}$). Age showed modest inverse associations with PSMA7 ($\rho = -0.20$; $P = 2.15\times10^{-2}$) and PSMA6 ($\rho = -0.15$; $P = 4.7\times10^{-2}$).

Conclusions. Proteasome gene expression was linked to disability and immune markers and differed by therapy, indicating a clinically relevant UPS signature in Latvian MS patients.

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EXPLORING GENDER EFFECTS ON MITOCHONDRIAL RESPIRATION OF PBMCs IN HEALTHY YOUNG ADULTS

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Background. Mitochondria contribute to essential cellular processes [1]. Peripheral blood mononuclear cells (PBMCs) offer a minimally invasive source for monitoring systemic changes and biomarkers, including immune cell mitochondrial respiration for diagnostics and health monitoring [2]. Data on gender differences in PBMC mitochondrial respiratory parameters across OXPHOS pathways, including the fatty acid oxidation (FAO) pathway, remain limited [3].

Aim. To characterise PBMC mitochondrial respiration in healthy 21–29 year-old donors, assess sex differences, and expand limited bioenergetics data for this cell type.

Methods. Twelve healthy participants (aged 21–29 years, no acute/chronic illnesses) were divided into two groups (males (M): n = 4; females (F): n = 8). Procedures followed University of Latvia Local Ethics Committee approval (No. 13-22/179). Participants avoided food supplements, caffeinated drinks, or substances affecting mitochondrial bioenergetics for ≥ 48 h prior. Venous fasting bloeod was collected between 8:4–9:10 A.M. PBMC respiration was assessed via high-resolution respirometry (Oroboros Instruments, Austria) using SUIT-002 protocol [4], measuring oxygen consumption in ROUTINE, OXPHOS, FN-OXPHOS, FNS-OXPHOS, F-OXPHOS, Max-ET, ET-CII and ET-CIV states (≥ 2 replicates/sample). Statistics included multiple t-tests with Holm-Sidak correction, using GraphPad Prism 8.0.2. Results described as mean ± SEM.

Results. No statistical differences between males (n = 4) and females (n = 8) in parameters: ROUTINE F (11.12 ± 0.53); OXPHOS F (4.05 ± 0.29); F-OXPHOS F (8.74 ± 0.29); FN-OXPHOS F (8.74 ± 0.29); FNS-OXPHOS F (18.17 ± 0.96); Max-ET F (16.57 ± 0.78); ET-CII (9.63 ± 0.62); ET-CIV F (21.90 ± 1.71); ROUTINE M (9.16 ± 0.71); OXPHOS M (2.59 ± 0.27); F-OXPHOS M (9.37 ± 0.65); FN-OXPHOS M (5.94 ± 0.38); FNS-OXPHOS M (17.74 ± 1.45); Max-ET M (16.48 ± 1.97); ET-CII M (9.88 ± 0.83); ET-CIV M (18.13 ± 0.18). The number of donors (n = 4 (M) and n = 8 (F)) limits generalisability but provides initial reference data for mitochondrial respiration in young healthy human PBMCs.

Conclusion. This pilot study shows no gender differences in PBMC mitochondrial respiration in young adults using a sensitive method, providing novel insights into respiratory parameters, including gender-specific FAO, that address prior inconsistencies. Despite the small sample limiting generalisability, data supports future translational and clinical research.

Acknowledgments. Authors declare no conflict of interest.

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MITOCHONDRIAL ROS PRODUCTION IN PBMCs OF HEALTHY YOUNG ADULTS

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Background. Mitochondria are essential for cellular energy, and many pathologies involve altered bioenergetics and reactive oxygen species (ROS) production [1]. Monitoring alterations and biomarkers, like mitochondrial ROS generation, can be achieved in a minimally invasive manner using peripheral blood mononuclear cells (PBMCs) [2]. However, ROS measurements in PBMCs are challenging due to low flux and high variability [3]. This results in scarce reference data on ROS and respirometry parameters, including fatty acid oxidation (FAO).

Aim. To assess mitochondrial ROS production in PBMCs from healthy adults aged 21–29, examine potential sex-related differences, and expand existing reference data.

Methods. Twelve healthy participants (21–29 years, no acute or chronic illnesses) were divided into two groups (male (M): n = 4; female (F): n = 4). The study was approved by the Ethics Committee of the University of Latvia (No. 13-22/179). No substances that might affect mitochondrial bioenergetics were consumed by participants for at least 48 hours. Fasting venous blood was collected from 8:40 and 9:10 A.M. H_2O_2 (ROS) flux was measured in fresh PBMCs using high-resolution respirometry (Oroboros Instruments, Austria) with modified SUIT-002 for ROS detection [4]. H_2O_2 flux was assessed across key respiratory states: ROUTINE, OXPHOS, FN-OXPHOS, FNS-OXPHOS, F-OXPHOS, Max-ET, and ET-CII states with ≥2 replicates per sample. Statistical analysis included multiple comparison t-tests followed by Holm-Sidak test (GraphPad Prism 8.0.2.). Results defined as mean ± SEM.

Results. PBMCs demonstrated no statistical differences between groups in measured ROS parameters: (F) ROUTINE (0.5038 ± 0.1098); OXPHOS (0.9158 ± 0.1921); F-OXPHOS (0.8740 ± 0.1869); FN-OXPHOS (1.0720 ± 0.2112); FNS-OXPHOS (1.2640 ± 0.2266); Max-ET (1.1230 ± 0.2298); ET-CII (1.2520 ± 0.2549); (M) ROUTINE (0.6848 ± 0.2416); OXPHOS (1.6010 ± 0.5305); F-OXPHOS (1.5360 ± 0.4503); FN-OXPHOS (1.6260 ± 0.5138); FNS-OXPHOS (1.7000 ± 0.5706); Max-ET (1.6500 ± 0.5493); ET-CII (1.6430 ± 0.5479). While the number of donors (n= 4 (M); n= 4 (F)) limits the generalisability of these findings, they provide addition of reference data on mitochondrial ROS production in healthy human PBMCs.

Conclusion. This pilot study demonstrated detectable mitochondrial ROS in PBMCs from young healthy adults, showing no sex differences. Despite the limited sample size, the study offers new reference values for FAO-linked ROS and supports future translational research.

Acknowledgments. Authors declare no conflict of interest.

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IN VITRO ASSESSMENT OF SIZE-DEPENDENT BIOEFFECTS OF TITANIUM-BASED MXENES

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Background. MXenes, emerging two-dimensional transition metal carbides, exhibit exceptional properties, including high photothermal conversion efficiency and biocompatibility. Titanium-based MXenes (e.g., $Ti_3C_2T_x$) exhibit relatively good biocompatibility compared to other compositions, positioning them as promising candidates for biomedical applications, including wound healing, drug delivery, and tissue engineering. However, the role of lateral

flake size — a key physicochemical parameter — on cellular responses remains poorly understood and may critically influence safety profiles.

Aim. This study aimed to compare the biocompatibility of titanium-based MXene flakes of different lateral sizes in human immortalised keratinocytes (HaCaT), focusing on viability, DNA damage, and long-term proliferative capacity.

Methods. Two size fractions of delaminated $Ti_3C_2T_x$ MXene were prepared: small flakes (t nm) and large flakes (>1000 nm), verified by dynamic light scattering and electron microscopy. HaCaT cells were exposed to MXene suspensions at concentrations of 6.25, 25, and 100 μg/ml for 4 h, 24 h, and three days. Biocompatibility was evaluated by using: a) Live/Dead fluorescence microscopy (Calcein AM/PI) for membrane integrity and viability; b) Immunofluorescence detection of phosphorylated histone γH2AX as a sensitive marker of DNA double-strand breaks; c) Clonogenic (colony-forming) assay to assess long-term reproductive survival after 7–10 days.

Results. These multi-assay results demonstrate a clear size-dependent toxicity profile, with large titanium-based MXene flakes exerting substantially higher cytotoxic and genotoxic effects on the HaCaT cell line than after three or more days of coincubation. Both MXene sizes demonstrated dose-dependent effects on cell viability, DNA integrity, and clonogenicity. Short-term co-incubation (< 24 h) did not produce significant changes in cellular responses.

Conclusion. The findings suggest that flake size is an essential factor governing MXene–cell interactions, along with the dose, and it is crucial for developing future biomedical applications.

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FTO GENE RS9939609 POLYMORPHISM AND CF7L2 GENE RS7903146 POLYMORPHISM EFFECT ON BODY COMPOSITION AND GLUCOSE LEVELS IN YOUNG WOMENE

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Background. Obesity constitutes a major public health concern, affecting a substantial proportion of the population. It is a multifactorial condition arising from complex interactions between genetic susceptibility and individual lifestyle factors. Genetic characteristics, such as gene polymorphisms, contribute to an individual's predisposition to obesity. In addition, body composition and postprandial glucose levels provide insight into the body's ability to regulate blood glucose and can help identify individuals at an early stage who are at risk of developing metabolic disorders, including type 2 diabetes.

Aim. The aim of the study was to evaluate the potential effects of the Fat mass and obesity-associated (FTO) gene polymorphism rs9939609 on body composition, as well as the effects of the Transcription factor 7–like 2 (TCF7L2) gene polymorphism rs7903146 on body composition and postprandial glucose levels. An additional objective was to develop a methodology for detecting these polymorphisms using PCR primers.

Methods. Twenty-one women for rs9939609 and twenty-eight women for rs7903146 aged 20 to 40 years were selected to participate in the study. Body mass index was calculated. Ultrasound was used to determine fat mass. After a carbohydrate-rich meal glucose levels were measured. A questionnaire was administered to collect lifestyle-related data. DNA was extracted from blood samples, and participant genotypes were determined using PCR and agarose gel electrophoresis.

Results. Data dispersion differences in body mass index and total fat mass were observed between rs9939609 genotype groups ($p < 0.05$), with alleles AA showing higher adiposity indicators. PCR optimisation for rs9939609 was achieved at an annealing temperature of 61 ^{o}C with 0.625 μl of each primer. For the rs7903146 polymorphism, primers flanking the polymorphic site were designed, and DNA amplification for sequencing was successful, providing a foundation for further genomic-metabolic correlation analysis. Preliminary observations suggest a potential correlation between body composition and postprandial glucose levels.

Conclusion. Differences in body composition associated with FTO rs9939609 alleles suggest a potential genetic influence on adiposity-related parameters; however, further investigation in larger cohorts is required to confirm these findings. Methodological improvements to the PCR protocol enhanced detection of the rs9939609 polymorphism, and primers suitable for flanking the rs7903146 polymorphism were successfully designed.

Acknowledgements. The authors declare no conflicts of interest. The authors would like to thank Dalius Butkauskas for his involvement in the design of DNA primers and sequencing of samples. The authors thank all participants for their voluntary participation.

DEVELOPMENT AND VALIDATION OF APTAMER-BASED DIAGNOSTIC TOOLS FOR MALARIA DETECTION

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Background. Despite long-standing efforts by the World Health Organization to control and eliminate malaria, the disease remains a major cause of mortality, particularly among children under five and pregnant women. Caused by *Plasmodium* parasites, malaria spreads quickly through the bloodstream, resulting in serious complications. In the UL Department of Pharmaceutical Sciences, we identified a promising panel of aptamers specific to *Plasmodium* spp. lactate dehydrogenase (LDH) and their potential application in diagnostic devices warrants further investigation.

Aim. The aim of this study is to characterise the potential application of *Plasmodium falciparum* and *Plasmodium vivax* specific aptamers Pf-LDH3t and Pv-LDH2, respectively, in an aptamer-based biosensor.

Methods. To characterise how protein source affects aptamer affinity towards the target, aptamers Pf-LDH3t and Pv-LDH2 were tested towards in-house *Plasmodium* spp. LDH, SPAN Diagnostics and Creative Diagnostics proteins Pf-LDH and Pv-LDH using Enzyme-Linked Oligonucleotide Assay (ELONA). Next, sandwich ELONA method was employed to validate aptamers as capture and detection tools, simulating diagnostic device conditions also comparing how different pLDH sources affect the results.

Results. Aptamer Pf-LDH3t exhibited Kd values of 0.21 ± 0.06 nM, 130.80 nM and 0.29 nM towards SPAN Diagnostics, Creative Diagnostics and in-house Pf-LDH, respectively. Aptamer Pv-LDH2 demonstrated Kd values of 1.75 ± 0.25 nM, 12.58 nM against SPAN Diagnostics and in-house Pf-LDH while insufficient saturation was reached towards Creative Diagnostics Pf-LDH. Since aptamer Pf-LDH3t does not recognise Pv-LDH, only Pv- LDH2 was tested for Pv-LDH binding. Pv-LDH2 exhibited Kd values of 0.63 ± 0.14 nM, 6.68 nM and 1.21 nM towards SPAN Diagnostics, Creative Diagnostics and in-house Pv-LDH proteins, respectively. In Sandwich ELONA using amino plates, only aptamer pair Pf-LDH3t-NH2 and Pf-LDH3t-Biotin (capture and detection aptamers, respectively) yielded a significant signal over the background signal. However, when tested with the in-house protein the result showed a low signal-to-noise ratio. Use of strepatavidin plates in sandwich ELONA did not improve binding efficiency.

Conclusion. The data reveals significant variability in Plasmodium spp. LDH specific aptamer affinity depending on the protein source. The aptamer pair Pf-LDH3t-NH2 and Pf-LDH3t-Biotin demonstrated the best performance in sandwich ELONA using amino plates, however did not show signal when streptavidin plates were used. Optimisation of the aptamer-plate binding strategy should be considered, or alternatively, the capture aptamer should be replaced with a more different capture molecule.

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DEVELOPMENT OF A CALPAINOPATHY *IN VITRO* MODEL, USING PATIENT-DERIVED INDUCED PLURIPOTENT STEM CELL LINES

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Background. Calpainopathy is a progressive autosomal recessive limb-girdle muscular dystrophy (LGMDR1) caused by calpain 3 (*CAPN3*) gene variants. We previously identified a hypomorphic intronic variant, c.1746- 20C>G, common in Latvia (MAF 0.24) and other Eastern European countries, classified as having conflicting interpretations of pathogenicity. We showed it causes aberrant splicing and contributes to a disease phenotype when in a compound heterozygous state with another pathogenic variant in the gene[1]. We have established and validated three patient-specific iPSC lines (LUi001-A, LUi002-A, and LUi003-A), which are now registered in the hPSCreg registry[2].

Aim. This study aimed to differentiate the calpainopathy patient-derived iPSC lines into myotubes and characterise the *in vitro* disease model.

Methods. All three patient-derived lines, as well as KOLF 2.1J iPSCs, were differentiated into myoblasts and myotubes using the appropriate commercial differentiation media (Amsbio^{TM} Skeletal Muscle Differentiation Kit and Stemcell Technologies^{TM} MyoCult Differentiation Kit). Cell morphology and molecular marker (PAX3, PAX7, MYOD, MYOG, MF20) expression were evaluated at differentiation days 10, 18, 25, 27, and 29 using immunofluorescent staining and microscopy. Expression levels of selected markers (PAX3, PAX7, MYOD, MYOG, CAPN3, DMD, TNNT1, MYH3, MYH7, and MYH8) were assessed by quantitative real-time PCR in all cell lines and all differentiation stages.

Results. The cell morphology — long, fused, and multinucleated fibres — and staining show that the iPSC lines have been successfully differentiated into myotubes. The differentiated cells express the expected molecular markers PAX3 and MYOD for myoblasts, and MYOG, DMD, TNNT1, MYH3, MYH7, and MYH8 for myotubes.

Conclusion. Patient-derived iPSC lines provide a valuable *in vitro* model for studying calpainopathy, offering a platform for future investigations of disease mechanisms and therapeutic strategies, including muscle-on-chip approaches.

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AFFINITY CHARACTERISATION OF THE GREENB1 APTAMER IN 2D AND ORGANOID MODELS OF PANCREATIC DUCTAL ADENOCARCINOMA UNDER STATIC AND MICROFLUIDIC CONDITIONS

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Background. Although in recent years increased attention has been paid to expanding the possibilities for diagnosis and therapy of malignant tumours, pancreatic ductal adenocarcinoma (PDAC), due to the peculiarities of the pancreatic environment, remains one of the most aggressive types of cancer demanding an effective and innovative targeted therapy approach. One of the strategies for targeted therapy is aptamers and their conjugates. Aptamers are single-stranded oligonucleotides that, due to their unique three-dimensional structure, can bind to target molecules with high affinity and selectivity. GreenB1 is an integrin β1 (ITGB1) specific aptamer, and since ITGB1 is often overexpressed in tumour cells, its characterisation in various model systems and conditions would enable understanding of integrin expression and GreenB1's potential application as a vector for targeted therapy. The use of various PDAC cell lines, organoids, and organ-on-chip model systems would provide in-depth information about this aptamer's ability to bind to tumour cells under different environmental conditions.

Aim. The aim of the current study was to characterise the affinity of GreenB1 in PDAC cell lines and organoids obtained from patient biopsies under static and microfluidic conditions, for possible development of a targeted drug delivery approach.

Methods. Experimental condition optimisation and GreenB1 affinity testing for established cancer cell lines (CAPAN, MIA-PaCa), PDAC and normal pancreatic organoid cultures were done using ImagestreamX MarkII (Cytek Biosciences). Organ-on-chip model systems with and without endothelial channels were made using 4-channel CellBox Labs microfluidic chips. GreenB1 binding validation in microfluidic conditions was done using immunofluorescence.

Results. FC data indicate that Capan cells have a superior affinity for GreenB1; thus further optimisation was performed using the Capan cell line, where we found that one hour of 500 nM staining yields the best results. Capan cells were further used in an organ-on-chip system in which the binding of GreenB1-FAM could not be detected by IF due to low signal intensity. Signal amplification improved the detection of GreenB1 binding in static conditions and allowed visualisation in a microfluidics chip.

Conclusion. GreenB1 binding conditions were optimised for the Capan cell line. Preliminary data demonstrate that GreenB1 binding is detectable in an organ-on-chip system. Together, these findings support the feasibility of extending this work toward a proof-of-principle targeted therapy approach, in which GreenB1 could be conjugated to extracellular vesicles to enable targeted drug delivery to PDAC.

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SIZE- AND DOSE-DEPENDENT IMMUNOMODULATORY EFFECTS OF Ti_3C_2 MXENES

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Background. MXenes are an emerging class of two-dimensional transition metal carbides and nitrides with strong potential as drug delivery platforms due to their high surface area and readily tunable surface chemistry. However, their interaction with immune cells remains insufficiently characterised, and understanding size- and dose-dependent immune effects is essential to reduce inflammatory risks and guide safe biomedical translation.

Methods. Primary immune cells were isolated from the peripheral blood of a healthy donors. Cells were incubated for 24 hours with Ti_3C_2 MXenes of two different sizes (small flakes 300–500 nm and large 1000–1200 nm) at concentrations of 6.25 and 25.0 μg/ml. After incubation, culture supernatants were collected for enzyme-linked immunosorbent assay (ELISA), while cells were harvested for flow cytometry and RT-PCR analysis. Activation markers for T and B cells, as well as NK cell degranulation markers, were assessed by flow cytometry. Cytokine expression and secretion (IL-6, IL-8, IL-10, TNF-α, and IFN-γ) were quantified using ELISA and RT-PCR.

Results. Ti3C2 MXenes induced a clear size- and dose-dependent immune response. At equivalent concentrations, smaller particles induced higher levels of immune activation markers than larger particles. Increasing MXene concentration resulted in elevated cytokine secretion. No significant activation of T or B cells was detected, however, larger MXenes caused a modest increase in NK cell degranulation.

Conclusion. Ti-based MXenes demonstrated activation of immune cells activation, and controlling particle size may help minimise or avoid unwanted immune activation, thereby reducing the risk of inflammatory side effects during therapeutic use.

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INVOLVEMENT OF H2S IN NITRIC OXIDE RELAXATIONS OF RAT PULMONARY ARTERIES

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Background. Hydrogen sulphide (H_2S) is an endogenous gaseous signalling molecule involved in the regulation of pulmonary vascular tone. Impaired H_2S production and increased oxidative stress contribute to endothelial dysfunction and altered nitric oxide (NO) signalling in pulmonary hypertension. NO-induced vasorelaxation is mediated predominantly via activation of soluble guanylate cyclase (sGC), which is highly sensitive to oxidative modification. The interaction between H_2S, sGC activity, and oxidative stress in pulmonary arteries is not fully understood. Understanding H_2S signalling pathways is essential for identification of novel therapeutic targets.

Aim. The aim of the study was to investigate the role of H_2S in modulating NO-mediated relaxations of rat pulmonary arteries under conditions of sGC inhibition and oxidative stress.

Methods. Small pulmonary arteries isolated from adult male Wistar rats were mounted in wire myographs for isometric tension recordings. Vessels were normalised and precontracted with U46619. The involvement of soluble guanylate cyclase (sGC) in vascular relaxation was examined using the sGC inhibitor ODQ. Oxidative stress was simulated by incubating vessels with the superoxide generator pyrogallol. The ability to restore impaired relaxations was evaluated by supplementation with sodium hydrogen sulfide (NaHS) or the antioxidant tempol during relaxation induced by nitric oxide donors or sGC stimulators.

Results. NaHS induced a typical biphasic response in the pulmonary arteries. Inhibition of sGC with ODQ significantly attenuated the contractile phase of the NaHS response, while the relaxation phase remained similar to the control group. This suggests that NaHS-induced relaxation occurs primarily through cGMP- independent mechanisms. In contrast, ODQ effectively abolished relaxations induced by the nitric oxide donor sodium nitroprusside (SNP). Furthermore, exposure to pyrogallol caused a marked reduction in both endothelium-dependent and sGC-mediated (riociguat-induced) relaxations, suggesting impaired endothelial nitric oxide availability and sGC dysfunction. While incubation with NaHS during pyrogallol exposure significantly restored acetylcholine-induced relaxations, it failed to fully recover riociguat-mediated responses. SNP-induced responses remained largely unchanged after pyrogallol

treatment. Superoxide scavenging with tempol did not significantly improve relaxations, suggesting that scavenging alone is insufficient to reverse the functional deficits induced by pyrogallol.

Conclusions. Hydrogen sulfide mediates relaxation in rat intrapulmonary arteries through mechanisms largely independent of the NO–sGC–cGMP axis. While H_2S can mitigate the loss of endothelial nitric oxide bioavail- ability under oxidative stress, it does not fully restore oxidized sGC function. Targeting both sGC activation and H_2S enhancement is a potential strategy for pulmonary hypertension.

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