Muscular Dystrophy UK
Fighting muscle-wasting conditions

MDUK Oxford Neuromuscular Centre

UNIVERSITY OF OXFORD

MDUK Oxford Neuromuscular Centre Networking Event

17TH SEPTEMBER 2024

ST CATHERINE'S COLLEGE, OXFORD

Networking Event Programme

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Muscular Dystrophy UK
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AGENDA

MDUK Oxford Neuromuscular Centre Networking Event

13:30	-	14:00	***Registration, Tea, Biscuits, & Poster Setup***

Keynote speaker – Chair: Prof Dame Kay Davies

14:00	**-**	**15:00**	**Designing and translating materials for early disease detection and advanced therapeutics** Prof Dame Molly Stevens *John Black Professor of Bionanoscience, Institute for Biomedical Engineering and Department of Physiology, Anatomy & Genetics, University of Oxford |Chair in Emerging Technologies, Royal Academy of Engineering*
15:00	-	17:00	***Posters & Drinks Reception***

VENUE – ST CATHERINE'S COLLEGE

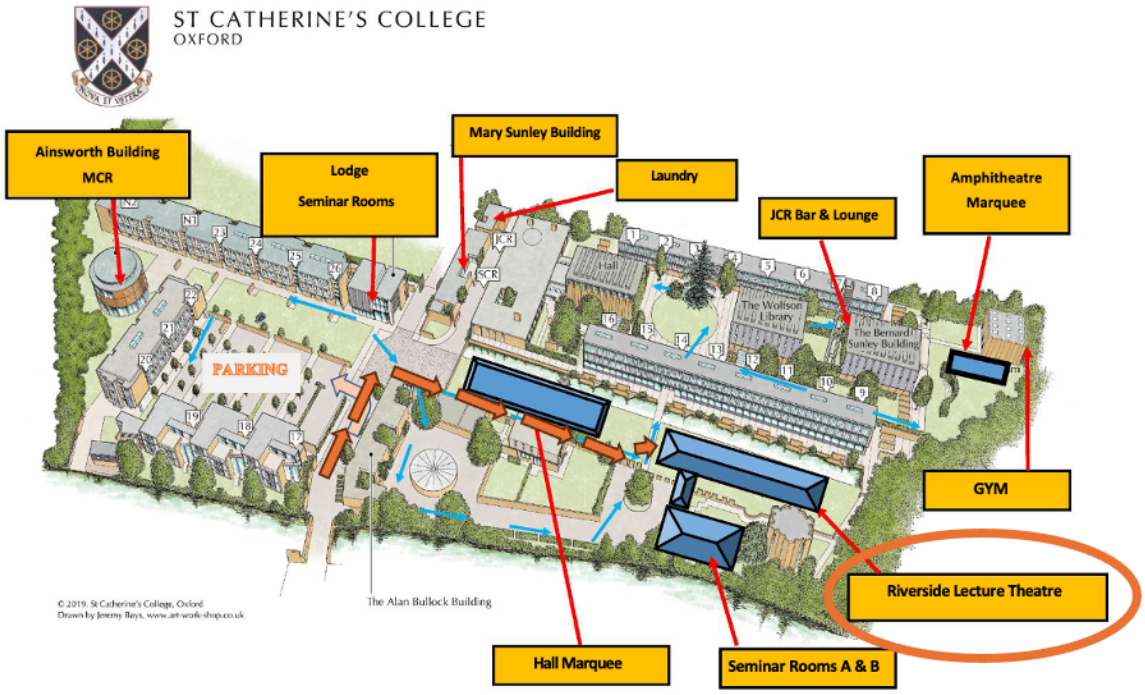
Address

Manor Road, Oxford OX1 3UJ
+44 (0)1865 271700

Venue

St Catherine's College is accessed from St Cross Rd by turning east onto Manor Rd. It is directly accessible via the ST1 and ST2 buses (Manor Rd stop), or it is a 15-min walk from High St or from Keble Rd.

Our event will take place in the **Riverside Lecture Theatre**, which can be accessed by turning right immediately prior to the temporary buildings present on your right side when entering (route shown below in orange). You will pass a temporary lunch hall along your left side, and the Riverside Lecture Theatre is the next building you will come to on the left side. A registration desk will be set-up as you enter the building and meeting volunteers will be present to guide you.



SPEAKER BIOGRAPHIES

Prof Dame Kay Davies

Dr Lee's Professor of Anatomy Emeritus | Co-Director of MDUK Oxford Neuromuscular Centre

Department of Physiology, Anatomy & Genetics, Medical Sciences Division, University of Oxford

Professor Dame Kay Davies, Ph.D., DBE FMedSci FRS is the Dr Lee's Professor of Anatomy Emeritus and co-Director of the MDUK Oxford Neuromuscular Centre in the Department of Physiology, Anatomy and Genetics at the University of Oxford. She received a BA degree in Chemistry and a graduate PhD degree in Biochemistry from the University of Oxford. She was appointed Professor of Genetics in 1996 and then appointed Dr Lee's Professor of Anatomy at the University of Oxford in 1998. She was founding Director of the MRC Functional Genomics Unit 1999-2017 and co-founded the Oxford Centre of Gene Function in 2000 with Professors Ashcroft and Donnelly, a new institute going from genetic association in human disease to function in the whole organism. Kay's research interests lie in the molecular analysis and development of treatments for genetic diseases, particularly Duchenne muscular dystrophy (DMD). She has published more than 400 papers and won numerous awards for her work and co-founded companies to translate her work to the clinic. Kay is a founding Fellow of the Academy of Medical Sciences and a Fellow of the Royal Society. She was appointed Governor of the Wellcome Trust in 2008 and became Deputy Chair 2013-17. She was made Dame Commander of the British Empire for services to science in 2008.

Prof Dame Molly Stevens

John Black Professor of Bionanoscience | Anatomy & Genetics Deputy Director, Kavli Institute for Nanoscience Discovery | Chair in Emerging Technologies, Royal Academy of Engineering

Institute for Biomedical Engineering and Department of Physiology, Anatomy & Genetics, University of Oxford

Professor Dame Molly Stevens DBE FRS FREng is the John Black Professor of Bionanoscience at the University of Oxford and a part-time professor at Imperial College London and the Karolinska Institute. Molly's multidisciplinary research balances the investigation of fundamental science with the development of technology to address some of the major healthcare challenges. She is a serial entrepreneur and the founder of several companies in the diagnostics, advanced therapeutics, and regenerative medicine fields. Her work has been instrumental in elucidating bio-material interfaces. She has created a broad portfolio of designer biomaterials for applications in disease diagnostics and regenerative medicine. Her substantial body of work influences research groups around the world and she has been multiple times listed as Clarivate Analytics Highly Cited Researcher in Cross-Field research.

Molly holds numerous leadership positions currently including Deputy Director of the Kavli Institute for Nanoscience Discovery, Deputy Director of the UK Quantum Biomedical Sensing Research Hub, and Scientist Trustee of the National Gallery. She is a Fellow of the Royal Society and the Royal Academy of Engineering (UK), a Foreign Member of the National Academy of Engineering (USA), an International Honorary Member of the American Academy of Arts and Sciences, and she was recognised with the 2023 Novo Nordisk Prize and the 2024 Royal Society Armourers and Brasiers Company Prize, amongst many other accolades.

KEYNOTE LECTURE ABSTRACT

Designing and translating materials for early disease detection and advanced therapeutics

Professor Dame Molly Stevens DBE FRS FREng
Kavli Institute of Nanoscience Discovery, Department of Physiology, Anatomy and Genetics, Department of Engineering Science, University of Oxford, United Kingdom

This talk will provide an overview of our recent developments in bioinspired materials for applications in advanced therapeutics and biosensing with focus on establishing translational pipelines to bring our innovations to the clinic [1]. Our group has developed fabrication methods to engineer complex 3D architectures that mimic anisotropic and multiscale tissue structures and generate spatially arranged bioinstructive biochemical cues [2]. I will discuss recent advances in our tunable nanoneedle arrays for multiplexed intracellular biosensing at sub-cellular resolution and modulation of biological processes [3]. We are developing creative solutions for targeted and controlled delivery using microrobots with unique bioinspired characteristics that respond to external stimuli to release a payload [4]. Our therapeutic delivery portfolio includes high molecular weight polymer carriers for enhanced delivery of saRNA therapeutics and photo-responsive nanoreactors inspired in the circadian rhythms [5]. We are exploiting the sensing capabilities of functionalised nanoparticles to engineer nanoprobes for in vivo disease diagnostics that produce a colorimetric response ideal for naked eye read-out and for CRISPR-based preamplification free detection of ncRNAs (CrisprZyme) which we have validated with cardiovascular disease patient samples [6]. I will present advances in Raman spectroscopy for high-throughput label-free characterisation of single nanoparticles (SPARTA™) that allow us to integrally analyse a broad range of bio-nanomaterials without any modification enabling exciting biosensing applications using extracellular vesicles as disease biomarkers, a growing area of interest in cardiovascular medicine [7]. Finally, I will explore how these versatile technologies can be applied to transformative biomedical innovations and will discuss our efforts in establishing effective translational pipelines to drive our innovations to clinical application while actively engaging in efforts towards the democratisation of healthcare [8].

References

[1] J. P. K. Armstrong… M. M. Stevens. “A blueprint for translational regenerative medicine.” Science Translational Medicine. 2020. 12(572): eaaz2253.
[2] T. von Erlach, … M. M. Stevens. Nature Materials. 2018. 17: 237-242.
[3] C. Chiappini… M. M. Stevens, E. Tasciotti. Nature Materials. 2015. 14: 532.
[4] X. Song… M. M. Stevens. Advanced Materials. 2022. 34(43): 2204791.; R. Sun… M. M. Stevens. Advanced Materials. 2022. 35(13):2207791.
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[6] C. N. Loynachan, … M. M. Stevens. Nature Nanotechnology. 2019. 14: 883-890.; M. Broto, … M. M. Stevens. Nature Nanotechnology. 2022. 10: 1038.
[7] J. Penders, … M. M. Stevens. Nature Communications. 2018, 9: 4256.; J. Penders, … M. M. Stevens. ACS Nano. 2021, 15, 11, 18192–18205; H. Barriga, … M. M. Stevens. Advanced Materials. 2021, 34(26):2200839.
[8] A. T. Speidel, … M. M. Stevens. Nature Materials. 2022. DOI: 10.1038/s41563-022-01348-5.

POSTER ABSTRACTS

Table 1: MDUK ONMC Networking Event on 17th Sep 2024 Poster Abstracts List

Optimising immunocapture of extracellular vesicles from cerebrospinal fluid for biomarker discovery in neurodegenerative diseases

Elizabeth R Dellar [1], Iolanda Vendrell [2,3], Roman Fischer [2,3], Alexander G Thompson [#3].

[1] *Nuffield Department of Clinical Neurosciences, University of Oxford.*
[2] *Target Discovery Institute, Centre for Medicines Discovery, Nuffield Department of Medicine, University of Oxford.*
[3] *Chinese Academy of Medical Sciences Oxford Institute, Nuffield Department of Medicine, University of Oxford.*

Introduction

Extracellular vesicles (EVs) are membrane-bound nanoparticles that are released by cells and are secreted from cells in the central nervous system (CNS) into cerebrospinal fluid (CSF), so hold potential as a source of new biomarkers. Current methods for purification such as ultracentrifugation and size exclusion chromatography require large starting volumes of CSF, but immunoaffinity capture methods shows promise to reduce this volume and to separate EVs by cellular origin. Our aim is to optimise methods for capture and proteomic profiling of CNS-EVs from CSF for biomarker discovery in neurodegenerative diseases.

Methods

EVs were purified from 200-1000 µl CSF by immunocapture with M-270 Epoxy Dynabeads conjugated to tetraspanin antibodies (CD81, CD63, CD9), or by size exclusion chromatography (SEC). Bound vesicles were lysed with 5% SDS, for subsequent Lys-C/trypsin digestion with suspension trapping method (Protifi). Liquid chromatography tandem mass spectrometry with library-free data-independent acquisition was used to compare the proteome (1 hour gradient on Orbitrap Ascend).

Results

Here we demonstrate that the immunocapture methodology is robust at CSF volumes down to 200 µl, with detection of core EV marker proteins CD9, CD81, TSG101, Syntenin-1, ALIX, FLOT1 in all samples, and depletion of Apolipoproteins B and A1 relative to SEC. We show high proteomic depth with 811±14 proteins using immunocapture from 200 µl CSF, increasing to 1285±224 or 1266 ±18 in 500 µl or 1000 µl respectively, compared to 812±66 in SEC - isolated samples from 500 µl. In the total dataset, detected proteins were significantly enriched for choroid plexus and astrocytic markers, but with no significant enrichment for neuron markers. We identified proposed neuronal-specific EV capture targets L1CAM, NCAM1 and GAP-43 in the data, but with no peptides mapping to cytoplasmic regions which would enable distinction between membrane-bound and soluble forms of these proteins. We detected cytoplasmic peptides for several neuronal enriched transmembrane proteins in the data, including recently proposed marker ATP1A3.

Conclusions

This work demonstrates that the immunocapture methodology allows for robust EV purification from small volumes of CSF using generic EV markers. This approach can be used to test alternative neuronal, microglial and astroglial-EV capture targets, which we have identified in the dataset.

Unravelling the mechanisms of transcriptional dysregulation in spinal and bulbar muscular atrophy

Francis Grafton[1], Wooi Fang Lim[1], Jonny Gonžić[1], Alfina Ambra Speciale[1], Laura Zanetti-Domingues[2], Christopher J Tynan[2], Matthew J A Wood[1], Carlo Rinaldi[1]

[1] *Institute of Developmental and Regenerative Medicine, University of Oxford, Oxford, UK.*
[2] *Central Laser Facility, Science and Technology Facilities Council, Rutherford Appleton Laboratory, Didcot, Oxfordshire, UK*

BACKGROUND: Spinal and bulbar muscular atrophy (SBMA) is an X-linked neuromuscular disorder caused by a polyglutamine expansion in the Androgen Receptor (AR) protein, a ubiquitously-expressed transcription factor, which critically mediates the response in human tissues to circulating male hormones, such as dihydrotestosterone (DHT). Upon ligand-induced activation, AR undergoes conformational changes, interacts with co-regulators and secondary transcription factors via its intrinsically disordered N-terminal domain (NTD), and binds to cognate DNA motifs to control gene expression. In SBMA cell dysfunction and death results from a combination of toxic gain and loss of function mechanisms which depend on AR binding to its ligand and transcriptional activation. ***What are the mechanisms of transcriptional dysregulation in SBMA and how those contribute to the disease is unknown.***

AIMS: The overarching aim of our research programme is to identify the molecular underpinnings of AR transcriptional activity in skeletal muscle and understand how dysregulation of such functions leads to muscle atrophy and weakness in SBMA.

METHODS AND RESULTS: We have mapped the AR transcriptional landscape by performing chromatin immunoprecipitation followed by deep sequencing (ChIP seq) in healthy and SBMA skeletal muscle, testing the working hypothesis that polyQ AR alters chromatin regions of AR occupancy in SBMA. By integrating these results with the transcriptomic profiles of muscle biopsies from SBMA individuals and age- and sex-matched controls, we have identified target genes directly under the control of AR. In order to investigate mobility and patterns of chromatin binding of AR molecules in real-time and at single molecule resolution, we engineered human myoblast lines to express wild type, polyQ or NTD-lacking (NTL) AR tagged with Halo-Tag and subjected these cells to single-molecule tracking analysis.

CONCLUSIONS: Our results support a mechanism of polyQ AR-driven transcriptional hijacking and represent an advancement in the molecular understanding of a driving mechanism of pathogenesis in SBMA.

Effects of S-nitrosylation on Androgen Receptor transcriptional activity and SBMA pathogenesis

Yasumasa Hashimoto [1, 2, 3], Divya Seth [4, 5], Antonio Garcia-Guerra [2, 3], Kseniya Korobchevskaya [6, 7], Taichi Fukunaga [2, 3], Thomas Goedert [2, 3], Ka Long Ko [6, 7], Helena Coker [6, 7], Marco Fritzsche [6, 7], Richard Premont [4, 5], Jonathan S. Stamler [4, 5], Matthew J A Wood [1, 2, 3], Carlo Rinaldi [1, 2, 3]

[1] *MDUK Oxford Neuromuscular Centre, University of Oxford, UK*
[2] *Institute of Developmental & Regenerative Medicine (IDRM), University of Oxford, UK*
[3] *Department of Paediatrics, University of Oxford, UK*
[4] *Department of Medicine, Institute for Transformative Molecular Medicine, Case Western Reserve University School of Medicine, Cleveland, Ohio, USA*
[5] *Harrington Discovery Institute, University Hospitals Cleveland Medical Center, Cleveland, Ohio, USA*
[6] *Kennedy Institute for Rheumatology, University of Oxford, UK*
[7] *Oxford-Zeiss Centre of Excellence, University of Oxford, UK*

[Background] Spinal and bulbar muscular atrophy (SBMA) is an X-linked and late-onset progressive neuromuscular disease caused by a CAG repeat encoding a poly-glutamine (polyQ) expansion in the Androgen Receptor (*AR*) gene. S-nitrosylation (SNO) is a redox-triggered post-translational modification that governs protein functionality through the covalent reaction of nitric oxide (NO)-related compounds with a cysteine thiol group found on the target protein. In physiological conditions, SNO can be a significant regulator of signal transduction pathways, much like phosphorylation. However, ageing or exposure to environmental toxins can result in anomalous SNO reactions that impact several functions, including protein misfolding, mitochondrial fragmentation, synaptic function, apoptosis, and autophagy. These alterations are significantly known to contribute to the mechanism of several cancers and neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases. Importantly, SNO is therapeutically targetable. How SNO contributes to SBMA pathogenesis is unknown. Here, we hypothesise that SNO regulates AR genomic activity by affecting the ability of AR protein to form biomolecular condensates and that SNO is an attractive therapeutic target for SBMA.

[Methods/Materials] We have employed the resin-assisted capture (SNO-RAC) assay for protein S-nitrosothiols, coupled with mass spectrometry in human-derived myoblasts from unaffected and affected individuals to identify the sites of S-nitrosylation within AR protein sequence. We generated cell lines expressing AR proteins with relevant Cysteine-to-Serine (Cys-to-Ser) mutations. The effect of S-nitrosylated Cys-to-Ser mutants on wild-type and mutant polyQ-AR biology has been tested in these mutant cells using several molecular biology techniques, from transactivation assays to confocal microscopy using fluorescence recovery after photobleaching (FRAP).

[Results] S-nitrosylation of three specific cysteines within AR critically controls the ability of AR to promote the assembly and functionality of the transcriptional machinery recruited onto the chromatin, regulating the ability of AR to transactivate its target genes.

[Conclusion] Our findings suggest that the specific S-nitrosylated Cys-to-Ser play a pivotal role in AR transcriptional activities, affecting the assembly of the transcriptional machinery onto chromatin, which is a key mechanism of SBMA pathogenesis. This discovery opens exciting avenues for therapeutic intervention in SBMA and underscores the importance of further research in unravelling the intricate connections between SNO and neuromuscular disorders.

Revising a clinical pathway for patients diagnosed with Spinal Muscular Atrophy via Newborn Screening in the UK: A case study on the first patient identified through the Thames Valley and Wessex Pilot (NBS SMA).

Francesca Henderson (Nee Taylor), BSc[1], Charlotte Lilien, MSc[2], Alison Skippen, BM, BSc, PhD[3], Isabel Hatami, MChem, MSc[3], Laurent Servais, MD, PhD[2,4], Hayley Ramjattan MSc[1], Sithara Ramdas MBBS, FRCPCH[3,5].

[1]Oxford University Hospitals NHS Foundation Trust, Oxford, UK; [2]STRONG, MDUK Oxford Neuromuscular Centre, Department of Paediatrics, University of Oxford and NIHR, Oxford, United Kingdom; [3]Department of Paediatric Neurology, John Radcliffe Hospital, Oxford ,UK; [4]Centre Hospitalier Régional de Références des Maladies Neuromusculaires, Hospital of la Citadelle and University Hospital Liège, Liège, Belgium; [5]MDUK Oxford Neuromuscular Centre, Department of Paediatrics, University of Oxford, Oxford, United Kingdom

Spinal Muscular Atrophy (SMA) is an autosomal recessive condition characterised by progressive muscle weakness. Patients are deficient in the *SMN1* gene which codes for the survival motor neuron protein and is critical to motor neuron health. They are therefore reliant on the *SMN2* gene however, approximately 90% of the protein produced by *SMN2* is non-functional.

Nusinersen, onasemnogene abeparvovec and risdiplam are disease modifying therapies available for SMA treatment under the UK Managed Access Agreement. Current literature evidences improved motor and respiratory outcomes with the greatest gains seen the earlier a patient is treated.

Newborn screening (NBS) is already established in many countries to facilitate early treatment but is yet to be implemented in the UK. To provide real world evidence to the UK NBS committee, a feasibility study is currently underway in the Thames Valley and Wessex regions (NBS SMA). Following successful screening of 15,166 newborns the first confirmed SMA case has been identified.

This has prompted revision of the current clinical pathway for children identified through NBS in the UK, including tertiary centre assessments and community management. The aim of this project is to formulate a suggested pathway that is feasible within the NHS model for this emerging cohort.

When formulating this pathway it will be crucial to learn lessons from countries with established NBS and hear the experience of the first family diagnosed through NBS SMA and the professionals involved in their journey.

Learning points already identified:

- The importance of understanding the disease-trajectory and specific needs of these patients.
- Outcome measures previously effective at assessing older symptomatic children may not be sensitive enough in mildly impacted or clinically silent newborns.
- Symptoms of cognitive involvement have been noted in those treated after symptom presentation. Effective outcome measures will therefore be vital in monitoring cognitive involvement in NBS infants.

Identifying, Characterising And Evaluating Antibodies In Guillain-Barré Syndrome (GBS)

Mariya Misheva[1], Alexander J. Davies[1], IGOS consortium, Simon Rinaldi[1]

[1] *Nuffield Department of Clinical Neurosciences, University of Oxford, UK*

Guillain-Barré syndrome (GBS) is a disabling immune-mediated neuropathy which affects ~100,000 people every year. In most cases, the precise underlying immunological mechanism is unknown, and therapeutic approaches have not advanced in over 20 years. In a minority of patients, pathogenic ganglioside antibodies (targeting neuronal surface sialylated glycolipids) arise following infection through a process of molecular mimicry. Although other antibodies undoubtedly exist, their antigenic targets and pathogenic potential are currently undefined. Our project aims to detect and characterise novel GBS-associated antibodies.

Using myelinating co-cultures of human induced-pluripotent stem cell (hiPSC)-derived sensory neurons and rat Schwann cells, we have screened over 300 GBS sera from 5 different cohorts, including over 200 samples from the International GBS Outcome Study (IGOS), for IgG peripheral nerve reactivity. Using this platform, between 10-20% (including 34/217 from IGOS) are positive. Reactivity can be seen directed against axons, myelin, non-myelinating Schwann cells and nodal/paranodal structures. In live cultures, myelin reactive IgG can be shown to induce demyelination in the presence of a source of complement.

In an initial round of immunoprecipitation/mass spectrometry-based proteomics, we have identified a glial membrane protein as a leading candidate for the target of Schwann-cell reactive IgG in one patient. Validation of the mass spectrometry results through Western blot and cell-based assays is ongoing.

In future work, we will expand our screening platform to include hiPSC-derived motor neurons and Schwann cells. Further rounds of IP/MS will be performed using sera with the strongest and most specific binding patterns. The frequency of any identified reactivities will be tested in wider GBS cohorts and correlated with clinical features.

Thus, results to date indicate the presence of peripheral nerve reactive antibodies against novel targets in a substantial proportion of GBS cases. Their antigenic targets and pathogenic relevance remain to be determined.

New generation of aryl hydrocarbon receptor (AhR) antagonists: utrophin modulation for the treatment of Duchenne muscular dystrophy.

Marc Panosetti[1], Carole JR Bataille[1,3], Sarah E Squire[2], Lee Moir[2], Massimiliano Runfola[3], Kay E Davies[2], Angela J Russell[1,3]

[1]*Chemistry Research Laboratory, University of Oxford, Oxford OX1 3TA, United Kingdom*
[2]*Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford OX1 3PT, United Kingdom*
[3]*Department of Pharmacology, University of Oxford, Oxford OX1 3QT, United Kingdom*

Upregulating utrophin is a promising strategy to compensate for the lack of dystrophin in Duchenne muscular dystrophy (DMD), offering a genotype-independent approach. Ezutromid, a first-in-class utrophin modulator, was developed through phenotypic screening and advanced to a Phase 2 clinical trial. Initial results after 24 weeks showed efficacy and target engagement, but these effects were lost after 48 weeks. Further investigation revealed that ezutromid's sub-optimal solubility, metabolic stability, and CYP1A induction limited its clinical efficacy. It was discovered that ezutromid binds with high affinity to the aryl hydrocarbon receptor (AhR), with AhR antagonism driving utrophin upregulation. This confirmed AhR as a target for DMD therapies[1].

Following ezutromid, second-generation phosphinate derivatives were designed with improved solubility and stability but were halted due to hepatotoxicity[2,3]. A third generation was then created to improve stability while maintaining activity, with promising in vitro results. However, in vivo efficacy did not match. CYP1A1, a key downstream protein in the AhR pathway, was identified as a critical marker, and its inhibition correlated with AhR antagonism[4].

To refine the assay, CYP1A1 expression in HepG2 cells became the primary screening tool, supplemented by testing in mouse hepatocytes and dystrophin-deficient murine cell lines. This workflow led to the identification of a fourth generation of novel compounds with high potential as in vivo candidates. The project aims to advance these compounds toward an IND-ready candidate for treating all DMD patients, regardless of mutation.

1. I. V. L. Wilkinson, K. J. Perkins, H. Dugdale, L. Moir, A. Vuorinen, M. Chatzopoulou, S. E. Squire, S. Monecke, A. Lomow, M. Geese, P. D. Charles, P. Burch, J. M. Tinsley, G. M. Wynne, S. G. Davies, F. X. Wilson, F. Rastinejad, S. Mohammed, K. E. Davies and A. J. Russell, *Angewandte Chemie International Edition*, 2020, **59**, 2420-2428.

2. A. Babbs, A. Berg, M. Chatzopoulou, K. E. Davies, S. G. Davies, B. Edwards, D. J. Elsey, E. Emer, S. Guiraud, S. Harriman, C. Lecci, L. Moir, D. Peters, N. Robinson, J. A. Rowley, A. J. Russell, S. E. Squire, J. M. Tinsley, F. X. Wilson and G. M. Wynne, *J Med Chem*, 2020, **63**, 7880-7891.

3. M. Chatzopoulou, E. Emer, C. Lecci, J. A. Rowley, A. S. Casagrande, L. Moir, S. E. Squire, S. G. Davies, S. Harriman, G. M. Wynne, F. X. Wilson, K. E. Davies and A. J. Russell, *ACS Med Chem Lett*, 2020, **11**, 2421-2427.

4. E. Wincent, J. Bengtsson, A. Mohammadi Bardbori, T. Alsberg, S. Luecke, U. Rannug and A. Rannug, *PNAS*, 2012, **109**, 4479-4484.

Mitophagy in Modulating mtDNA Heteroplasmy: Therapeutic Implications

Danyang Li[1], Fitz Silao[1], Nancy Liang[1], Sara Maxwell[1], **Joanna Poulton[1]**

[1] *Department of women's and Reproductive Health, Oxford University*

Background: Mitochondrial DNA (mtDNA) mutations cause a variety of debilitating disorders with limited treatment options. Understanding the intricate interplay between mitophagy activation and physiological conditions in modulating mtDNA heteroplasmy holds promise for therapeutic interventions. By recycling damaged mitochondria, mitophagy contributes to mitochondrial quality control. However, its importance in modulating mtDNA heteroplasmy, particularly under physiological conditions, remains poorly understood.

Objective: Our objective was to elucidate how alterations in oxygen, glucose, and spermidine levels influence mtDNA heteroplasmy and mitophagy, providing insights into potential therapeutic strategies for mitochondrial disorders.

Methods: We employed a combination of cell culture techniques, molecular assays, and high-content imaging to investigate the effects of varying oxygen concentrations (1-8%), glucose levels (3-25mM), and spermidine concentrations (0.1-10µM) on mtDNA heteroplasmy and mitophagy dynamics. Quantitative PCR confirmed was used to assess heteroplasmy levels. Mitophagy was assessed through colocalization studies using high-content imaging, complemented by Bromodeoxyuridine (BrdU) labeling to differentiate between mitophagy-mediated and replication-driven heteroplasmy shifts.

Results: Preliminary findings demonstrated that exposure to 5% oxygen reduced the mutation load from 75% to 70%, while spermidine treatment (5µM for 24 hours) decreased it from 78.4% to 69.8%. Activation of mitophagy via altering glucose concentrations, oxygen levels, and spermidine treatment led to dynamic shifts in mtDNA heteroplasmy linked to altered mitophagy dynamics on imaging. under different conditions, suggesting a potential mechanism for heteroplasmy modulation.

Conclusion: Our study suggests that physiological factors are able to modulate mtDNA heteroplasmy by activating mitophagy. Understanding these mechanisms may pave the way for targeted therapeutic interventions in mitochondrial disorders, highlighting the importance of considering mitophagy modulation as a potential strategy for disease management.

Quantifying splice-switching and gapmer antisense oligonucleotides using ligation-based qPCR systems

Abinaya Ramesh[1], Nina Ahlskog[1], Matthew J.A. Wood[1], Alyssa C. Hill[1]

[1] *Institute of Developmental and Regenerative Medicine, Department of Paediatrics, University of Oxford, Oxford, United Kingdom.*

Antisense oligonucleotides (ASOs) are therapeutic agents used to treat a variety of conditions, including neuromuscular diseases such as Duchenne muscular dystrophy (DMD; eteplirsen, golodirsen, viltolarsen, casimersen) and spinal muscular atrophy (SMA; nusinersen). ASOs are typically 15-30 nucleotides long, and they are divided into splice-switchers or gapmers based on their mechanism of action. Because in their natural, unmodified form nucleic acids make poor drugs, various structural modifications have been made to ASOs to improve their pharmacokinetic and pharmacodynamic properties. Common modifications include the phosphorothioate backbone and 2'-*O*-methyl and 2'-*O*-methoxyethyl additions to ribose. Although the mode of gene correction using ASOs is understood, aspects of their delivery into cells, trafficking into subcellular compartments, and potency need further research to maximize their effectiveness. The quantitative detection of ASOs within tissues and cells can be done using qPCR, aided by a chemical or enzyme-assisted ligation step. Such systems use a pair of DNA probes that recognize and bind to the ASO and are ligated to form a single, complementary DNA oligonucleotide. This DNA oligonucleotide is then amplified and detected in a routine qPCR set-up. The decision to use a chemical or enzyme-assisted ligation depends on the chemistry of the ASO being detected. The studies presented here probe the applicability of the chemical and enzyme-assisted ligation techniques to ASOs of different chemistries, attached to different conjugate moieties, and in different matrices, including both in vitro and in vivo samples.

Myonuclear domain-associated and central nucleation-dependent spatial restriction of dystrophin protein expression

Katarzyna Chwalenia[1,2], Vivi-Yun Feng[1,2,3], Nicole Hemmer[1,2,4], Eric T. Wang[5,6], Annemieke Aartsma-Rus[7], Maaike van Putten[7], Matthew J.A. Wood[1,4,8], **Thomas C. Roberts[1,2,8]**

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[7] Department of Human Genetics, Leiden University Medical Center, Leiden, the Netherlands
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Here we describe a novel genetic mouse model of DMD, mdx52-XistΔhs, which expresses dystrophin at variable levels from birth as a consequence of skewed X-chromosome inactivation. The mdx52-XistΔhs syncytial myofibers are heterokaryons containing a mixture of myonuclei expressing either wild-type or mutant dystrophin protein. Analysis of isolated single myofiber and sections from 6-week-old mdx52-XistΔhs skeletal muscles showed apparent myonuclear domain-restricted distribution of dystrophin and associated proteins. This pattern of dystrophin expression models the situation observed in female carriers, and DMD muscle in which dystrophin has been incompletely restored by partially-effective experimental therapeutics. Dystrophin patchiness was maintained in aged (60-week-old) mdx52-XistΔhs mice suggesting that accumulation of dystrophin-positive myofibers and myonuclei via positive selection over time is insufficient to resolve this pattern of expression. Non-uniform dystrophin was protective against pathology-related muscle turnover in an expression-level-dependent manner in both adult and aged mdx52-XistΔhs mice. Similarly, extracellular myomiR biomarkers abundance was correlated with patchy dystrophin in adult animals. In contrast, utrophin levels in the mdx52-XistΔhs muscle did not correlate with dystrophin abundance and the protein was found localized predominantly in newly-formed myofibers. Notably, systematic classification of individual mdx52-XistΔhs myofibers showed profound differences associated with central nucleation. These include higher numbers of myonuclei and the more substantial disorganisation of the microtubule network in centrally-nucleated myofibers. Intriguingly, dystrophin was found to be translationally repressed in centrally-nucleated regions of mdx52-XistΔhs myofibers.

Deconvoluting New Druggable Targets for Utrophin Upregulation

Massimiliano Runfola[1,2], Sarah E Squire[3], Marc-Antoine Panosetti[1,2], Carole JR Bataille[1,2], Lee Moir[3], Kay Davies[3], Angela J Russell[1,2]

[1] *Department of Pharmacology, University of Oxford, OX13QT, Oxford, United Kingdom*
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Duchenne Muscular Dystrophy (DMD) is a rare genetic degenerative disease affecting approximately 1 in 3500 male births every year worldwide. It is marked by a progressive loss of muscle integrity due to the lack functional dystrophin, that lead to severe limb disability before adolescence often resulting in cardiac or respiratory arrest in early adulthood. Current treatments are mainly palliative and gene therapy approaches are not suitable for all patients. Small molecule-mediated tissue repair - i.e., inducing tissue-selective regenerative processes to restore cellular integrity - could represent a promising therapeutic option for next-generation DMD therapeutics, regardless of gene mutation.

Over the past decades, we have focused on inducing tissue regeneration in DMD via upregulation of utrophin. Utrophin is an autosomally-encoded paralogue of dystrophin that can compensate for lack of dystrophin by restoring muscle integrity and improving the dystrophic phenotype. We validated this approach through the clinical development of ezutromid, a first-in-class utrophin modulator. Although a Phase 2 clinical trial (PhaseOut DMD) confirmed its target engagement and demonstrated a degree of efficacy, ezutromid was ultimately discontinued due to lack of sustained efficacy[1,2]. This led us to the development of a new screening assay and the subsequent identification of a new class of highly potent 2-pyrimidinecarbohydrazide utrophin upregulators with a distinct mode of action from ezutromid.[3,4] Unfortunately, the pathway leading to utrophin upregulation remains elusive for this class of compounds. Deciphering this pathway and the main mediators of this process could lead to substantial improvement of drug design for therapeutic repair of muscular dystrophy.

To this aim, we successfully designed and synthesized a new set of photoactivatable chemical probes that preserve potency in promoting utrophin regulation. Using these probes along with state-of-the-art proteomics, RNA-sequencing and a comprehensive biological investigation we are deconvoluting their molecular targets, unravelling the complex pathway of utrophin modulation.

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Investigating RNA editing dysregulation in spinal and bulbar muscular atrophy

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Background: RNA editing by deamination is a widespread and essential post-transcriptional modification, catalysed by the Adenosine Deaminase Acting on RNA (ADAR) enzymes. These enzymes bind to double-stranded RNAs and convert adenosine into inosine (A-to-I editing) which is recognised as guanosine by the translation machinery. A-to-I editing can have significant effects on cellular functions, including altering amino acid sequences (recoding) and modifying pre-mRNA splicing patterns. Recent advancements in high-throughput sequencing techniques have revealed that neurons exhibit some of the highest levels of RNA editing, with millions of A-to-I conversion events across the transcriptome. Dysregulated RNA editing has been identified in various neurological disorders, though the underlying mechanisms and consequences remain largely unknown. Whether RNA editing is compromised in SBMA and other polyglutamine diseases is unknown.

Aims: The overarching aim of my DPhil project is to explore the role of RNA editing in the mechanisms of toxicity in polyglutamine diseases, using spinal and bulbar muscular atrophy (SBMA) as disease model. Specifically, I will test the working hypothesis that the transcriptomic dysregulation in polyglutamine disorders is disrupting the ADAR interactome, preventing efficient canonical editing.

Methods/Materials: Using deep transcriptomic data-sets from SBMA patient-derived iPS-motor neurons and diseased tissues, we profiled the RNA editing events in this disorder. In parallel, using the same data-sets and co-IP techniques, we are now investigating differentially expressed ADAR interactors. Additionally, we employed single-molecule tracking microscopy techniques to characterise the dynamic behaviour of ADAR enzymes within neuronal cells in disease contexts.

Results: Our editome analysis, revealed a decrease in overall editing within the non-coding sequences in SBMA, along with differential editing of targets involved in neuronal survival in SBMA motor neurons. These results are coupled with evidence of altered enzyme dynamics and binding activity in presence of the polyglutamine-expanded Androgen Receptor.

Conclusion: With this work, we aim to unravel a previously unrecognised mechanism of pathogenesis in repeat expansion disorders, focusing on RNA editing impairment. Successful completion of this project may pave the way to identify novel therapeutic approaches, targeting restoration of ADAR editing activity.

Engineered Extracellular Vesicles (EVs) for Efficient Delivery of CRISPR-Cas9 systems

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Clustered regularly interspaced short palindromic repeats-associated protein 9 (CRISPR-Cas9) system is currently the most efficient and promising technique of gene editing, which has great therapeutic potential in treating various genetic diseases. However, the traditional way to deliver CRISPR-Cas9 system as DNA or RNA would result in high off-target effects and insertional mutagenesis. In comparison, delivering them as ribonucleoproteins (RNPs) could help to avoid these risks due to their ability to rapidly target genomic sites and quickly degrade thereafter. In this study, we aim to apply endogenous engineered extracellular vesicles (EVs) to deliver CRISPR-Cas9 RNPs, as the EVs could both accommodate the relatively large payload size and achieve safe and efficient delivery. We so far explored the optimising strategies for generating EVs encapsulating CRISPR-Cas9 systems, including: 1) exploring transmembrane/membrane-interacting EV sorting domains to be fused with Cas9 proteins; 2) designing different gRNA scaffold to increase stability; 3) optimising the dosage ratio of different plasmids during EV production. So far, the CRISPR-Cas9 system encapsulated EVs we produced could achieve an *in vitro* InDel efficiency reaching 90% in the reporter cell line and 30% in primary cardiomyocytes. Further, we engineered EVs to encapsulate base editors and after optimisation, the *in vitro* base editing efficiency could reach 99% with no bystander editing. Overall, the results established engineered EVs as a safe and efficient delivery platform for CRISPR nucleases, which could be further applied to conduct gene therapy for specific diseases.