8th International Wolfram Syndrome Symposium
Surrey, England 16th-18th April 2023

Meeting Notes

We express our deepest gratitude towards the ***Be A Tiger Foundation*** for its support and generosity in this year's symposium.

Summary

These meeting notes have been developed to provide a helpful summary of the International Symposium for attendees, other professionals working in Wolfram Syndrome research and for the WS community of affected people and their families.

These notes include the following:

- Meeting agenda
- Abstracts provided by each researcher (where they can be shared)
- Points noted during the presentation (that does not represent a summary of the work – which has / will be published in peer reviewed journals).
- Action steps – developed by Dr Urano which were also circulated to attendees by email 20th April 2023.
- Meeting attendee list.

Agenda: Day 1 (17th April 2023)

Presentations

(08:45-9:00) Welcome Stephanie Gebel – Snow Foundation & Tracy Lynch – Wolfram Syndrome UK

(9:00-9:45) New function of Wolframin in regulating the monocarboxylate transporter 1 (MCT1) in glial cells in brain and retina. Dr Vania Broccoli - CNR - National Research Council Institute of Neuroscience, Milan. Italy

(9:45-10:30) Depletion of WFS1 compromises mitochondrial function in hiPSC-derived neuronal models of Wolfram syndrome. Dr Malgorzata Zatyka – Birmingham University, UK.

(11:00-11:45) Descriptive analysis of 68 patients with Wolfram syndrome with emphasis on sensorineural involvement and possible phenotype-genotype correlation. Dra. Gema Esteban- Bueno, Dr. Juan R. Coca, Dr. Nicolas Fernández-Fernández, Leticia Fernandez Amores, Miguel Navarro Cabrero, Dra. Aida Berenguel-Hernández and Spanish Multidisciplinary Wolfram Syndrome Group. Spain.

(11:45-12:30) Pathophysiology of type 2 Wolfram syndrome: implications for therapy. Prof Gil Leibowitz – President of the Israel Endocrine Society (IES), Hadassah Medical center, the Hebrew University, Jerusalem, Israel

(12:30- 1.30) Lunch

(1:30- 2:00) Exploring novel ocular biomarkers of disease in Wolfram syndrome. Dr Xuehao Cui - PhD student and research fellow University of Cambridge

(2:00 – 2:15) Pridopidine: A selective and potent S1R agonist as a potential treatment for Wolfram Syndrome. Randal Hand PhD - Director of Neuroscience Research, Prilenia Therapeutics

(2:15- 3:00) Role of oligodendrocytes and cell metabolism in Wolfram syndrome; retinal phenotype of Wolfram syndrome mouse models. Prof Lies de Groef - KU Leuven Stem Cell Institute, Leuven, Belgium

(3:30 – 4:15) Advances on the pharmacological and gene therapies approaches. Dr Benjamin Delprat - Inserm Research Director, Montpelier, France

(4:15 – 5:00) Helios – A Phase II Study of Safety and Efficacy of AMX0035 in Wolfram Syndrome. Dr. Leinders – Amylyx Pharmaceuticals Inc. Cambridge, MA, USA **.**

(5:00-5:45) iPSC-based modeling of Wolfram Syndrome and therapeutic genome editing approaches. Prof Catherine Verfaillie - Stem Cell Institute, KU Leuven, Belgium

(5:45-6:00) Rounding up of the day's sessions to create action points. Fumihiko Urano, M.D.

(7:00-7:30) Reception Drinks

(7:30) Dinner

Agenda: Day 2 (18th April 2023)

Presentations

(9:00-9:30) Audiowolf : Description, contribution and interest of this protocol. Dr Christophe Orssaud - Functional Unit of Ophthalmology, Paris, France

(9:30-9:45) Introduction from Camelot BioCapital. Josh Sharan - D.O (Cand.) Vice President of Camelot BioCapital

(9:45-10:30) Update on TREATWOLFRAM clinical trial. Prof Timothy Barrett – University Hospital Birmingham, UK

(11:00-11:45) Exploring dual-incretin agonists as a novel therapeutic modality for Wolfram Syndrome. Dr Mario Plaas – University of Tartu, Estonia

(11:45-12:30) Novel Therapies for Wolfram Syndrome. Fumihiko Urano, MD, PhD - Samuel E. Schechter Professor of Medicine, Washington University School of Medicine, St. Louis, USA

(12:30- 1.30) Lunch

(1:30- 2:15) Pharmacological targets to correct deficient ER-mitochondrial Ca2+ homeostasis in the neuronal models of Wolfram Syndrome. Dr Allen Kaasik - University of Tartu, Estonia

(2:15- 3:00) Rounding up of the day's sessions to create action points & closing comments. Fumihiko Urano, M.D.

Day 1 (17th April 2023)

1. Depletion of WFS1 compromises mitochondrial function in hiPSC-derived neuronal models of Wolfram syndrome. Dr Malgorzata Zatyka - Institute of Cancer and Genomic Sciences, University of Birmingham, Edgbaston, Birmingham. UK

Abstract: Mitochondrial dysfunction involving mitochondria-associated ER membrane (MAM) dysregulation is implicated in the pathogenesis of late-onset neurodegenerative diseases, but understanding is limited for rare early-onset conditions. Loss of the MAM resident protein WFS1 causes Wolfram syndrome (WS), a rare early-onset neurodegenerative disease that has been linked to mitochondrial abnormalities. However, contradictory reports on mitochondrial functionality in non-human or nonclinical cell models and lack of data in disease-affected settings have precluded biomedical exploitation.

Here we demonstrated mitochondrial dysfunction in human induced pluripotent stem cell-derived neuronal cells of WS patients. VDAC1 was identified to interact with WFS1, whereas loss of this interaction in WS cells could compromise mitochondrial function. Genetic rescue by WFS1 restoration or pharmacological agents modulating mitochondrial function improved the viability and bioenergetics of WS neurons. Our data implicate a role of WFS1 in regulating mitochondrial functionality and highlight a therapeutic target for WS and related rare diseases with mitochondrial defects.

Points noted:

- Mitochondrial dysfunction and increased oxidative stress are seen in human induced PCS derived neuronal cells from WS patients.
- Not all typical biochemical mitochondrial markers were seen in WS patient derived cells.
- Treatment to rescue WSF1, rescued mitochondrial function and reduced cell death.
- WSF1 interacts with VDAC1 and may regulate VDAC1 function formation.
- Not yet assessed investigational compounds in this model to evaluate potential benefits.

2. New function of Wolframin in regulating the monocarboxylate transporter 1 (MCT1) in glial cells in brain and retina. Dr. Vania Broccoli - CNR - National Research Council Institute of Neuroscience, Milan. Italy

Abstract: A key pathological manifestation in Wolfram syndrome is the progressive optic atrophy which leads to relentless visual loss. Although some of the pathological mechanisms caused by wolframin mutations have been unravelled in the recent years, how they impinge on visual deficits it remains unclear. Through genomics and proteomics analyses on retinal tissues isolated from wolframin mutant mice, we identified a significant reduction of the monocarboxylate transport isoform 1 (MCT1) and its partner basigin that are highly enriched on retinal glia and myelin-forming oligodendrocytes in optic nerve together with wolframin. Loss of MCT1

causes a failure in lactate transfer from glial to neuronal cell bodies and axons leading to a chronic hypometabolic state that can cause retinal ganglion cell (RGC) degeneration.

This metabolic dysfunction occurs months before the frank RGC degeneration suggesting an extended time-window for intervening with new therapeutic strategies focused on boosting retinal and optic nerve bioenergetics in WS1.

Points noted:

- Retinal ganglion cells (RGCs) are vulnerable in WS, the genetic link to symptoms still needs to be understood.
- Mice WS KO model(s) were shared from Estonia (Dr Mario Plaas) – nerve damage and visual acuity loss are detected early (6-8 months) before very significant RGC loss (12 months).
- Developed working hypothesis of biochemical pathway alterations that lead to lack of energy metabolites and neuron loss.
- One future goal is to understand the structure of wolframin protein, (which is not easy!). Therefore, intend to collaborate with expert group based in New York.

3. Descriptive analysis of 68 patients with Wolfram syndrome with emphasis on sensorineural involvement and possible phenotype-genotype correlation.

Dra. Gema Esteban- Bueno, Dr. Juan R. Coca, Dr. Nicolas Fernández-Fernández, Leticia Fernandez Amores, Miguel Navarro Cabrero, Dra. Aida Berenguel-Hernández and Spanish Multidisciplinary Wolfram Syndrome Group. Spain.

Abstract: Our work consists in providing greater knowledge of what already exists in the Wolfram Syndrome phenotype with special attention to hearing loss, through a descriptive and longitudinal study of two sets of patients affected from Spain and Portugal. The first set (descriptive study) contain a registry with patients that have been appearing since 1999, and the second set collects data from multidisciplinary assessments that have been carried out in Spain since 2011 year after year (longitudinal study).

In turn, a brief study of the genotype-phenotype relationship of the hearing loss that exists in patients of Spain and Portugal with Wolfram Syndrome has been carried out, based on the genetic data collected by our team.

Point noted:

- Spanish and Portuguese families are evaluated through a multi-disciplinary team, who are in regular contact – to listen to families; accumulate experience and provide biopsychosocial support.
- Currently assessing 2 patient populations (second set is a sub-set of the first).
- No definitive pattern can be established for hearing loss or progression. Homozygous genetic changes are more severe.
- The team actively participates in the WS Global Awareness Day and plan more activities for 2023.

- The team are keen to collaborate with other groups / researchers to help drive change for families – valuable dataset.
- University of Birmingham group (through Dr Renuka Dias) has been assessing gonadal function – connecting with the team in Spain may be helpful.

4. Pathophysiology and treatment of type 2 Wolfram syndrome. Prof Gil Leibowitz – President of the Israel Endocrine Society (IES), Hadassah Medical center, the Hebrew University, Jerusalem, Israel.

Abstract: Type 2 Wolfram syndrome results from a missense mutation in the CISD2 gene, encoding NAF-1, which transfers Fe-S clusters from the mitochondria to cytosolic acceptor proteins. The carrier rate of CISD2 missense mutation among the Palestinian population in the Middle East is 1:40, suggesting a founder effect. Type1 and type 2 Wolfram syndrome have common and distinct clinical features, suggesting heterogeneity in disease phenotype and pathophysiology. NAF-1 deficiency leads to increased labile iron accumulation in the mitochondria with subsequent development of mitochondrial dysfunction and oxidative stress, resulting in neurodegeneration and diabetes. Treatment of NAF-1 deficient cells by iron chelation, N-acetylcysteine and GLP-1-RA reduced mitochondrial iron overload and alleviated oxidative stress and mitochondrial dysfunction. I will discuss the therapeutic implications of these findings.

Points noted:

- T2WS is not extremely rare in the Middle east region (compared to WFS1). This is very different from the situation reported elsewhere (e.g. in US – almost all patients have WFS1 mutations rather than CISD2 mutations).
- CISD2 gene mutation (Glutamate – Glutamine, 8 amino acid frameshift with abnormal splicing and stop sequence) generates a protein which is 25% of the size of the native protein that is rapidly degraded. Unrelated families can carry the same mutation.
- Combined GLP-1-RA (e.g. exenatide) and N-acetylcysteine generate increased effects, compared with single treatment (e.g. beta cell protection).
- Better understanding of T2WS has implications for more common forms of Diabetes Mellitus.
- Early intervention is likely to be needed.
- RCTs need broad international collaboration.
- Heterozygous mutations were not studied to date.

5. Exploring novel ocular biomarkers of disease in Wolfram syndrome

Dr Xuehao Cui - PhD student and research fellow University of Cambridge and Moorfields Eye Hospital

Abstract: Visual loss in Wolfram syndrome arises primarily due to the selective loss of retinal ganglion cells resulting in optic nerve degeneration. We are exploring various ocular imaging modalities that would provide us with additional non-invasive tools to monitor this process more closely in affected individuals. Progressive brainstem and cerebellar atrophy are also well-recognised neuroradiological features of Wolfram syndrome and these could potentially be assessed by more detailed eye movement recordings. Our objective is to validate ocular biomarkers of disease in Wolfram syndrome that are both applicable to clinical practice and as outcome measures for treatment trials.

Points noted:

- Aim to protect RGCs to stabilise vision / slow down vision loss.
- Currently exploring a number of approaches to assess vision [e.g. OcuMet (ocular mitochondrial health); Flavoprotein fluorescence (early signs of mitochondrial dysfunction) Optical Coherence Tomography (OCT; different layers of the retina); eye movement recording (level of OA)].
- Recruiting patients (n=30) to a new study during May and June 2023; BWCH will refer patients to help boost numbers for the study.

6. Pridopidine: A selective and potent S1R agonist as a potential treatment for Wolfram Syndrome

Randal Hand PhD - Director of Neuroscience Research, Prilenia Therapeutics

No abstract provided.

Points noted:

- Pridopidine is currently in clinical trials for neurodegenerative conditions (e.g. ALS (Ph II moving into Ph III); Huntington's (Ph III).
- Currently being explored in WS due to commonalities in cellular pathways.
- Rescue with Pridopidine has been shown to reduce ER stress and improve mitochondrial respiration in WS models.
- Nausea and dizziness have been reported side-effects (fairly typical side-effects).

7. Oligodendrocytes in Wolfram syndrome: bystanders or partners in crime?

Karan Ahuja[1], Marjan Vandenabeele[2,3], Arefe Nami[1], Catherine Verfaillie[1], Lieve Moons[2], Lies De Groef[3]
[1]Development and Regeneration Department, KU Leuven Stem Cell Institute, Leuven, Belgium
[2]Neural Circuit Development and Regeneration Research Group, Biology Department, KU Leuven Brain Institute, Leuven, Belgium
[3]Cellular Communication and Neurodegeneration Research Group, Biology Department, KU Leuven Brain Institute, Leuven, Belgium

Abstract: Up till today, the neurodegenerative pathology associated with Wolfram syndrome (WS) is unstoppable. This treatment gap is at least in part due to the limited

understanding of the underlying cellular mechanisms. In particular, it is becoming increasingly clear that –although neurons eventually die– there is a central role for glial cell types in most neurodegenerative disorders and it is essential to determine which of these cell types is the catalyst of the disease processes leading to WS, so that future therapies can be targeted to this cell type. In this study, based on recent evidence suggesting that the neurodegenerative component of WS could be driven by an oligodendrocyte rather than a neuronal pathology, we aimed to investigate what the effect of these 'diseased' oligodendrocytes is on the function of 'healthy' neurons, focusing on ER stress, mitochondria and cell metabolism as potential underlying mechanisms.

Our studies in iPSC-derived oligodendrocytes from WS patients reveal that these may be more vulnerable to ER stress and display signs of mitochondrial dysfunction. This, together with their seemingly reduced capacity to transfer metabolites and thereby support axons, suggests that oligodendrocyte dysfunction may, at least partially, be underlying the neurodegenerative component of WS. Next, we validated these findings *in vivo*, by investigating the retina and optic nerve of the *Wfs*1 KO mouse. We found that functional and glial cell alterations precede structural neuronal changes, and that these animals have problems with the oligodendrocyte cell lineage, leading to a decreased oligodendrocyte precursor cell number, a thinner myelin sheet and more signs of axonal degeneration in the *Wfs*1 KO animals. Finally, MRI studies of the brain of these *Wfs*1 KO mice showed a reduction in the volume of several brain regions, including the cerebellum, brainstem and corpus callosum –which are also affected in WS patients– as well as changes in the apparent diffusion coefficient, pointing towards neurodegeneration and changes in myelination.

Based on these data, it is tempting to speculate that the white matter changes and neuronal loss observed in WS patients is at least partly caused by problems with the supportive functions of oligodendrocytes: signal transduction via myelination and metabolic support of axons. This suggests that future WS therapies may need to target oligodendrocytes, rather than or in addition to neurons. All in all, our findings indicate that the eye is a window to the brain, with the retina reflecting the pathological processes ongoing in the brain.

Points noted:

- *Wfs*1 KO mice were shared from Estonia (Dr Mario Plaas). Final time point studied 7.5 months. Some changes detected very early (from 3 months).
- Also shared preliminary in vitro data where ER stress, mitochondrial dysfunction and cell death were observed in WS patient derived cell lines.

8. Advances on the pharmacological and gene therapies approaches

Dr Benjamin Delprat - University of Montpellier, France

Abstract: Wolfram syndrome is a rare autosomal recessive disease affecting many organs with life-threatening consequences and currently no treatment is available. Therefore, the need to find a cure is imperative. The pathology is related to the deficient activity of wolframin, an endoplasmic reticulum (ER) transmembrane protein

involved in contacts between ER and mitochondria termed mitochondria associated-ER membranes (MAMs). Inherited mutations usually reduce the protein's stability, altering its homeostasis and ultimately reducing ER to mitochondria Ca^{2+} transfer resulting in mitochondrial dysfunction and cell death. We previously demonstrated that improving MAMs functioning by overexpressing NCS1, a wolframin partner, is efficient in correcting the cellular and behavioral alterations in our preclinical models of the pathology.

Based on these data, we focused our research on another crucial protein of the MAMs physiology, the sigma-1 receptor (S1R), an endoplasmic reticulum resident protein involved in Ca^{2+} transfer. Very interestingly, S1R could be activated by small active molecules to foster the Ca^{2+} transfer between ER and mitochondria. Therefore, we demonstrated that activation of S1R with the prototypic agonist PRE-084, restored Ca^{2+} transfer and mitochondrial respiration *in vitro*, corrected the associated increased autophagy and mitophagy, and was able to alleviate the behavioral symptoms observed in the genetic animal models of the disease, *i.e.* hyperlocomotion in *wfs1ab*^{KO} zebrafish and memory deficits and anxiety in *Wfs1*$^{\Delta Exon8}$ mice. Our findings provide a new therapeutic strategy for Wolfram syndrome patients, by efficiently boosting MAM function using the ligand operated S1R chaperone.

Points noted:

- NCS-1 (neuronal calcium sensor -1) represents a relevant target for treating WS.
- Activation of S1R is beneficial in WS – (e.g. improved memory and increased Ca^{2+} in WS models).
- S1R targeting is therefore relevant for WS patients.
- NCS-1 modulators / stabilizers were not investigated.

9. Helios – A Phase II Study of Safety and Efficacy of AMX0035 in Wolfram Syndrome Dr. Leinders – Amylyx Pharmaceuticals

Summary: Update on the clinical trial design of Helios, and the rationale for using AMX0035 as potential treatment for Wolfram Syndrome.

Points noted:

- AMX0035 is a combination therapy of two compounds– TUDCA (tauroursodeoxycholic acid) and sodium phenylbutyrate (PB).
- Approved in the US for ALS, not currently approved in the EU.
- Investigational drug for WS. Received orphan drug status in US.
- Shown to decrease neuronal death, mitigate ER stress and mitochondrial dysfunction.
- Excellent safety and tolerability profile shown in previous studies (Ph III in ALS). Taste issues have been reported previously (AMX0035 = sachet).
- Currently conducting a collaboration with Dr Urano (preclinical and clinical).
- As part of this collaboration, an open label Ph II study has started in US to recruit 12 patients to assess safety (using standard endpoints), tolerability, various measurements (e.g. beta cell function), and exploratory biomarkers.

- Study will monitor C-peptide levels (as a surrogate endpoint) over 24-week period. Patients will therefore need to be relatively healthy to be able to participate (It was noted that not many patients will meet the inclusion criteria required for C-peptide levels). Based on learnings from the dantrolene trial, the target of 12 patients should be achieved.
- The study aims to assess whether there is value in treating WS patients as early as possible and if data are encouraging will expand to larger and longer studies.
- The study protocol includes a 4-hour mixed meal tolerance test (MMTT) (which it was noted is a big ask for patients and researchers). This test was specifically requested by the FDA. If data are encouraging, the aim is to propose to reduce MMTT in a future larger trial.
- If data are encouraging, this will help to spur development in a juvenile programme.
- First patient recruited to this study April 2023 (week prior to the Symposium).

10. iPSC-based modeling of Wolfram Syndrome and therapeutic genome editing approaches Prof Catherine Verfaillie - Stem Cell Institute, KU Leuven, Belgium

No abstract provided.

Points noted:

- Developed iPSC-based models of different cell types – neural cells, ganglion, astrocytes, and blood brain barrier endothelial cells.
- These cell lines can be shared with other WS researchers who would like to use them.
- Further evaluate (e.g. myelination, stain for different markers) and explore changes over time. Also compare with WS-derived models to explore differences.
- Base editing conducted using this system – look to see if / how this can be delivered in vivo.
- Translate findings to mouse model - exchange with Fumi (Dr Urano) mouse model with current iPSC system.

Rounding up of the day's sessions to create action points. Moderator – Fumihiko Urano, M.D

At the end of Day 1, Dr Urano shared the action list that was agreed at the 1st International Symposium in 2009.

Action Items in 2009

1. **Patient registry**
2. **Longitudinal study**
3. **Biomarkers**
4. **Service clinic (multi-disciplinary)**
5. **Clinical guidelines**
6. **Animal and Cell models**
7. **Drug targets/Drug development**
8. **International Collaboration**
9. **Share data and reagents**
10. **Cure**

Extracted from presentation prepared by Dr Urano

It was noted all items developed in 2009 have been completed except one – "Cure". There was discussion on the action points for 2023 – which was continued at the end of Day 2.

Day 2 (18th April 2023)

11. Audiowolf : Description, contribution and interest of this protocol

Dr Christophe Orssaud - Functional Unit of Ophthalmology, Paris, France

Alongside the Treatwolfram protocol initiated by Pr T Barrett, a second new protocol has been initiated in France, called Audiowolf. These two protocols are similar in terms of their duration (3 years), the patients for whom they are intended (patients with Wolfram syndrome) and the molecule used (sodium valproate). They nevertheless differ by several points which make them complementary.

These differences relate first to the primary judgment criterion. This primary judgment criterion is auditory in Audiowolf and not visual. It is also a non-randomized protocol without double blinding. All the patients included receive the treatment at a dose adapted to their weight. Finally, it was initially designed as being monocentric (Paris), although it is currently undergoing extension to Spain (Almeria).

However, this Audiowolf protocol is not a competitor but rather complementary to Treatwolfram. Indeed, it makes it possible to include patients who did not meet the inclusion criteria of the Treatwolfram protocol due to too low visual acuity. On the other hand, the auditory, visual, neuro-radiological and biological tests are identical. Thus, after a separate processing of both protocols, these different data can be combined for common analyses. Finally, this protocol will make it possible to better analyze the auditory impairment of Wolfram syndrome. On the other hand, all patients receiving sodium valproate, an ancillary study was initiated to understand how this molecule may affect insulin regulation.

In practice, the inclusion criteria require, among other things, a hearing impairment of at least 5 dB at 8000 Hz. And this protocol can be delivered "turnkey" to teams that would like to propose it to their patients.

Points noted:

- Patients enrolled in this study must have some level of hearing loss (those with hearing implants can be included).
- Study first started in Nov 2021 – after TreatWolfram (to avoid patients needing to choose which study to participate in).
- Secondary objective of the study is to evaluate C-peptide to try to understand how sodium valproate acts on insulin secretion.
- A rare side effect of sodium valproate is hearing loss, which is typically bilateral and reversible. This will need to be considered should any patients experience severe hearing loss during the study (patient data likely to be excluded, at least initially).

12. Introduction from Camelot BioCapital Josh Sharan, , D.O (Cand.) Vice President of Camelot BioCapital

Points noted:

- Current collaboration with Dr Urano to conduct clinical trials.
- This collaboration includes a nasal formulation of dantrolene (secured through their partner Eagle Pharmaceuticals).
- As a company they are positioned to help – are very grateful to participate in these discussions with KOLs and patient support organisations.

13. Sodium Valproate efficacy and safety in WFS1 Spectrum Disorder (TREATWOLFRAM). Update on phase II pivotal international multicentre, double-masked, randomised controlled trial. Prof Timothy Barrett, Institute of Cancer and Genomic Sciences, University of Birmingham, Birmingham UK, on behalf of the TREATWOLFRAM investigators (Dr Renuka Dias, Dr Ben Wright (UK), Dr Gema Esteban Bueno (Spain), Prof Wojciech Mlynarski (Poland), Dr Christophe Orssaud, Prof Agathe Roubertie (France)

ABSTRACT:

Introduction

WFS1 spectrum disorder (WFS1-SD) comprises a classic form of progressive neurodegenerative disorder, formerly known as Wolfram syndrome, and non-classic forms including low-frequency sensorineural hearing loss. This is the commonest monogenic syndrome of diabetes and vision impairment, affecting 1:500,000 in the UK. Despite this, there are no licensed pharmacological therapies for WFS1-SD. Sodium valproate (VPA) is an anti-convulsant approved for use for the treatment of epilepsy and bipolar disorder. VPA has been shown to reduce Endoplasmic

Reticulum (ER) stress, is known to exert neuroprotective effects in models of neurodegeneration, and in the context of WFS1-SD, VPA is thought to mediate its effect via alteration of cell cycle kinetics, increase in p21cip1 expression levels or nuclear translocation and reduction in apoptosis, and increase in Wolfram protein expression. There is currently a lack of prospective controlled studies investigating therapeutic candidates in people with WFS1-SD.

Methods

TREATWOLFRAM is a phase II, pivotal, international multi-centre, double-masked, placebo-controlled, randomised clinical trial designed to investigate whether a 36 month treatment with up to 800mg/day of VPA in children aged 6-12 years, or up to 1600mg/day for those aged 12 years of over, will slow the rate of change in visual acuity as assessed by corrected visual acuity in each eye, using standardised charts, in participants with WFS1-SD. Children aged 6 years or more and adults with genetically confirmed WFS1-SD and visual acuity with LogMAR score of 1.6 or better on an EDTRS chart, were assessed for eligibility at 6 recruitment centres in Europe. Patients who satisfied the eligibility criteria were randomly assigned (2:1) to receive twice daily tablets of either VPA or VPA-placebo (control). Analysing visual acuity outcomes using longitudinal hierarchical models, and using data on a cohort of 26 patients with Wolfram syndrome kindly provided by Prof Tamara Hershey, (significance level 0.05, power 0.90), and accounting for an estimated 15% withdrawal rate, we estimated a target sample size of 70 patients, so that a minimum of 21 participants were randomised to each treatment group. The primary outcome measure will be centrally assessed using an intention-to-treat analysis of the proportion of evaluable participants achieving a decline in the rate of progression of vision loss between baseline and after 36 months of treatment. Visual acuity progression will be defined as the change in LogMAR units over time as measured on EDTRS charts.

Ethics and dissemination

The protocol was designed with assistance from the EU Committee for Medicinal Products for Human Use (CHMP) and approved by The West of Scotland Research Ethics Service (18/WS/0020) and The Medicines and Healthcare Products Regulatory Agency (EudraCT 2017-001215-37; ISRCTN 10176118). Recruitment into TREATWOLFRAM started in January 2019 and ended in October 2021, with 63 patients randomised. The treatment follow-up of TREATWOLFRAM patients is currently ongoing and due to finish in October 2024. The findings of this trial will be disseminated through peer-reviewed publications.

Trial registration: Clinicaltrials.gov NCT03717909

Points noted:

- Visual acuity is the primary endpoint of TreatWolfram. A number of secondary endpoints are also included.
- Sodium valproate targets the final part of the treatment pathway. This drug has decades of use, has been shown to be neuroprotective and as it is off-patent – there is freedom to operate in clinical trials and ongoing licensing.

- TreatWolfram received 2014 orphan drug designation by EMA and FDA. Regulators may not require a gold standard double blind randomised controlled trial today.
- The trial was delayed by a range of issues – as a result some patients were unable to participate as their vision had deteriorated while awaiting trial commencement.
- If starting the study today, Milan would be included as a study site (this group has been developing a clinical trial centre).
- University of Birmingham now has an established clinical trial unit for delivering pivotal trials in rare disease and a network of trial sites that would like to be included in future WS clinical trials in Europe.
- The study clearly highlighted that Brexit has been damaging for rare disease research.
- Better biomarkers are needed that can demonstrate efficacy in around 6 months that are relevant for patients. Researchers need to be able to identify responders to potential treatments in a much shorter timeframe.
- With better biomarkers, new clinical trials could be designed to report in a shorter timeframe (and should therefore be less expensive). This could help attract more commercial partners into WS clinical research.
- It was noted patient samples will be available to help assist in developing biomarkers, including placebo data from clinical trials.

14. Exploring dual-incretin agonists as a novel therapeutic modality for Wolfram Syndrome Dr Mario Plaas, University of Tartu, Estonia

The abstract will be available following publication of the data presented.

15. Novel Therapies for Wolfram Syndrome Fumihiko Urano, MD, PhD, Samuel E. Schechter Professor of Medicine, Washington University School of Medicine, St. Louis, USA

Abstract: Wolfram syndrome is a rare genetic spectrum disorder characterized by insulin-dependent diabetes, optic nerve atrophy, and progressive neurodegeneration, and ranges from mild to severe clinical symptoms. There is currently no treatment to delay, halt, or reverse the progression of Wolfram syndrome, raising the urgency for innovative therapeutics for this disease. Here, we summarize our vision and progress on developing novel treatments and achieving a cure for Wolfram syndrome. Our approach entails utilizing oral pharmacotherapy aimed at modulating endoplasmic reticulum and mitochondrial functions to impede the progression of the disease, followed by the implementation of gene therapy to arrest its advancement, ultimately culminating in the application of regenerative therapy using a unique neurotrophic factor, MANF, and iPSC-derived tissues for the repair of any damaged tissues, especially retinal ganglion cells and brain cells. Our proposed approach has the potential to not only alleviate the symptoms of Wolfram syndrome, but it may also lead to a potential cure for medical conditions commonly seen in Wolfram syndrome, including diabetes, vision loss, and neurodegeneration. This is due to the innovative

nature of our strategy, which targets the root cause of Wolfram syndrome, thereby offering a universal solution for a wide range of human chronic disorders.

Points noted:

- Dr Urano is working with many collaborators. The team have medical records for +250 patients (2009-present) and long-term data for +50 patients (2011-present).
- There is an increased understanding of this rare disease.
- Look to identify patient cohorts for clinical trials and the best outcome measures.
- Previous dantrolene clinical study showed improved beta cell function in a subset of patients. Patients with greatest increase tended to have visual acuity and less severe disease.
- AMX-0035 – preclinical studies have confirmed that this combination therapy performs better than a single agent (e.g. reduced cell death and improved mitochondrial function). First clinical trial initiated in April 2023 with C-peptide as the primary outcome measure.
- S1R agonist (Pridopidine) is being tested in pre-clinical models (through collaboration with Prilenia).
- Working with an industry partner on gene therapies and gene editing with Prof Catherine Verfaillie. Willing to share protocol for iPSCs models with other researchers.
- Shared WS mutant rodent (rat and mice) models with other researchers and is willing to share with additional researchers who would like them.
- For entrepreneurship, need industry partners, which are also needed in Europe (current industry collaborators are US companies).

16. Pharmacological targets to correct deficient ER-mitochondrial Ca^{2+} homeostasis in the neuronal models of Wolfram Syndrome Liiv M[1], Vaarmann A[1], Kuum M[1], Gupta-Blixt R[1], Janickova L[1], Hodurova Z[1], Cagalinec M[1], Zeb A[1], Choubey V[1], Hickey MA[1], Safiulina D[1], Yi-Long H[2], Gogichaisvili N[1], Mandel M[1], Plaas M[1], Vasar E[1], Loncke J[3], Vervliet T[3], Tsai T-F[2], Bultynck G[3], Veksler V[4], Kaasik A[1].
[1]Departments of Pharmacology and Physiology, University of Tartu, Estonia.
[2]Department of Life Sciences, National Yang Ming Chiao Tung University, Taiwan
[3]Laboratory of Molecular and Cellular Signaling, KU Leuven, Belgium.
[4]INSERM UMR-S 1180, University Paris-Saclay, France.

Abstract: Wolfram syndrome (WS) is a rare genetic disease caused by mutations in the WFS1 or CISD2 gene. A primary defect in WS involves poor endoplasmic reticulum (ER) Ca^{2+} handling, but how this disturbance leads to the disease is not known. Although the clinical symptoms of WS resemble mitochondrial diseases and WFS1 or CISD2 deficiency leads to mitochondrial abnormalities, no causal link has been established between the ER defects, mitochondrial dysfunction and cell metabolic disturbances. The current study, performed in primary isolated neurons, the

most affected and disease-relevant cells, involving both WS genes explains how the disturbed ER Ca^{2+} handling compromises mitochondrial function and in turn affects neuronal health. Loss of ER Ca^{2+} content in the axons of the WFS1 or CISD2 deficient neurons is associated with lower IP_3R3-mediated Ca^{2+} transfer from ER to mitochondria leading to decreased mitochondrial Ca^{2+} uptake. In turn, reduction in mitochondrial Ca^{2+} content inhibits mitochondrial ATP production leading to an increased axoplasmic NADH/NAD^+ ratio. The resulting bioenergetic deficit and reductive stress further compromise the neurons health. Our work also identifies pharmacological targets and compounds that potentially may restore Ca^{2+} homeostasis, enhance mitochondrial function and improve neuronal function.

Points noted:

- Compounds affecting ER and mitochondrial Ca^{2+} could improve mitochondrial function and neuronal health.
- WS serves as an important disease prototype for ER and mitochondrial dysfunction.
- Hope to publish findings soon.

Rounding up of the day's sessions to create action points & closing comments. Fumihiko Urano, M.D.

Following discussion on Day 1, Dr Urano shared a summary of action items for the next few years, which he subsequently circulated to participants by email (20th April 2023). Dr Urano welcomes feedback and comments from participants on this action list.

In addition, as noted in his 20th April email, following the International Symposium, Dr Urano has reached out to the National Organization of Rare Diseases to discuss the development of clinical guidelines and has initiated conversations with several biotechnology companies and research collaborators regarding the novel biomarker discovery program.

The action list developed by Dr Urano has been included in the following pages of these meeting notes for completeness. Please contact Dr Urano to share your thoughts and comments.

Prepared by Fumihiko URANO, MD, PhD, on April 19th, 2023

Addressing Challenges and Improving Outcomes for Wolfram Syndrome Patients

International Wolfram Syndrome Consortium

A. Action Items in 2023

1. Develop Clinical Guidelines for Wolfram Syndrome Management

- Adapt existing format for rare diseases and finalize guidelines.
- Publish a downloadable version on patient organizations' websites.
- Incorporate a gene panel-based screening strategy.
- Collaborate with patient organizations to disseminate guidelines to targeted specialists.

2. Compile a List of International Clinical Trial Sites for Wolfram Syndrome

- Identify motivated and experienced physicians to conduct trials.
- Ensure a diverse and representative selection of trial sites.

3. Explore Novel Biomarkers for Wolfram Syndrome

- Conduct research to identify new biomarkers related to disease progression.
- Implement blood draw protocols at multiple centers every six months to monitor biomarker levels.

4. Connect Patient Registries

- Develop a plan to connect existing patient registries for Wolfram syndrome.
- Collaborate with patient organizations to gather and share patient data.

5. Create an Innovative Clinical Trial Protocol for Novel Therapies, including Gene Therapy

- Develop a clinical trial protocol for novel therapies, including gene therapy.
- Identify potential partners for collaboration on the clinical trial.

B. Action Items in 2009

1. Patient registry
2. Longitudinal study
3. Biomarkers
4. Service clinic (multi-disciplinary)
5. Clinical guidelines
6. Animal and Cell models
7. Drug targets/Drug development
8. International Collaboration
9. Share data and reagents
10. Cure

Prepared by Fumihiko URANO, MD, PhD, on April 19th, 2023

C. Three pillars

1. Improving Patient Outcomes:

To enhance patient outcomes for those with Wolfram syndrome, it is crucial to develop comprehensive clinical guidelines that address early diagnosis, treatment options, and interventions for improving quality of life (QOL). This entails standardizing diagnostic criteria, establishing best practices for management, and exploring innovative therapies such as gene therapy and stem cell treatments. Additionally, promoting multidisciplinary care and offering psychological support can significantly improve patients' QOL.

2. Engaging the Medical Community:

Raising awareness and fostering collaboration among the broader medical community is vital in tackling Wolfram syndrome. This can be achieved by hosting conferences, workshops, and webinars that provide up-to-date information and promote the exchange of knowledge. Encouraging research and funding opportunities, as well as facilitating collaborations between clinicians, researchers, and patient advocacy groups, can contribute to advancements in understanding and treating this rare disease.

3. Advocating for Public Policy Changes:

To benefit Wolfram syndrome patients, advocacy for public policy changes should focus on improving access to care, promoting research funding, and supporting affected families. This may include pushing for insurance coverage of specialized treatments, establishing centers of excellence, and implementing early screening programs. Working with policymakers and leveraging the power of patient advocacy groups can help in effecting these changes, ultimately benefiting those living with this rare condition.

Meeting attendee list

Name	Hospital/Institution
Stephanie Gebel	Snow Foundation
Tracy Lynch	WSUK
Dr Saad Naseer	Snow Foundation
Derik Liberatore	Be a Tiger Foundation
Dr Mario Plaas	University of Tatu
Dr Gina Isherwood	WSUK
Dr Allen Kaasik	University of Tatu
Dr Lieve Moons	KU Leuven
Dr Lies De Groef	KU Leuven
Prof. Catherine Verfaillie	KU Leuven
Dr Fumihiko Urano	Washington University Hospital
Dr Gema Esteban Bueno	Spanish Association Wolfram Syndrome
Dr Leticia Fernandez Amores	Spanish Association Wolfram Syndrome
Dr Xuehao Cui	University of Cambridge
Prof Timothy Barrett	University of Birmingham
Dr Malgorzata Zatyka	University of Birmingham
Dr Sovan Sarkar	University of Birmingham
Nolwen Le Floch	French WS Association
Dr Mathias Leinders	Amylx Pharmaceuticals
Dr Lahar Mehta	Amylx Pharmaceuticals
Dr Christophe Orssaud	Hôpital Européen Georges-Pompidou (HEGP)
Dr Benjamin Delprat	Molecular Mechanisms in Neurodegenerative Dementia (MMDN)
Dr. Leibowitz	Haddassah University Medical Centre
Dr Vania Broccoli	San Raffaele Hospital
Dr Niccolò Vanni	San Raffaele Hospital
Miguel Navarro Cabrero	
Dr Marc Peschanski	I-STEM
Ameen al Muslimani	ME Foundation
Randal Hand	Prilenia
Dr Laetitita Aubry	I-STEM
Josh Sharan	Camelot Biocapital