Phase 2 HELIOS Interim Data in Wolfram Syndrome

April 10, 2024

Our mission is to one day end the suffering caused by neurodegenerative diseases.

Every day, we strive for better therapies.
On Today’s Call
Statements contained in this presentation regarding matters that are not historical facts are “forward-looking statements” within the meaning of the Private Securities Litigation Reform Act of 1995, as amended. Because such statements are subject to risks and uncertainties, actual results may differ materially from those expressed or implied by such forward-looking statements. Such statements include, but are not limited to, statements regarding the potential of AMX0035 as a treatment for neurodegenerative diseases, including Wolfram syndrome (WS) and expectations around the timing of full results for the HELIOS trial of AMX0035 in WS; expectations about the market size for WS; expectations around interactions with regulatory authorities on potential development plans for AMX0035 in WS; and expectations regarding our longer-term strategy. Any forward-looking statements in this presentation are based on management’s current expectations of future events and are subject to a number of risks and uncertainties that could cause actual results to differ materially and adversely from those set forth in or implied by such forward-looking statements. Risks that contribute to the uncertain nature of the forward-looking statements include: the success, cost, and timing of Amylyx’ program development activities, including ongoing and planned clinical trials, Amylyx’ ability to execute on its research, development, and regulatory strategy, regulatory developments, Amylyx’ ability to fund operations, and the impact that the COVID-19 pandemic may have on Amylyx’ operations, as well as the risks and uncertainties set forth in Amylyx’ United States Securities and Exchange Commission (SEC) filings, including Amylyx’ Annual Report on Form 10-K for the year ended December 31, 2023, and subsequent filings with the SEC. All forward-looking statements contained in this presentation speak only as of the date on which they were made. Amylyx undertakes no obligation to update such statements to reflect events that occur or circumstances that exist after the date on which they were made.
Wolfram Syndrome Treatment Landscape

Dr. Fumihiko “Fumi” Urano, MD, PhD
Samuel E. Schechter Professor of Medicine
Washington University, St. Louis, USA
Primary Investigator for Phase 2 HELIOS Trial
Disclosures: Fumihiko Urano, MD, PhD

Patents licensed:
• Amarantus Bioscience
• Opris Biotechnologies

Technologies licensed:
• Novus Biologicals
• Sana Biotechnology

Patents:
• US 9,891,231
  SOLUBLE MANF IN PANCREATIC
  BETA CELL DISORDERS
• US 10,441,574
• US 10,695,324
  TREATMENT FOR WOLFRAM
  SYNDROME AND OTHER
  ER STRESS DISORDERS

Research support:
• NIH
• Prilenia
• Amylyx Pharmaceuticals

Board Member:
• Healthbeat

Founder and President:
• CURE4WOLFRAM, INC

Scientific Advisory Board:
• Emerald Biotherapeutics, INC
• Opris Biotechnologies, INC

Off-label use:
• Dantrolene sodium
• Liraglutide
• Valproic acid
Objectives

1. Summarize two types of Wolfram syndrome and related disorders.
2. Share lessons and stories from past and current Wolfram Syndrome clinical studies, including both achievements and obstacles.
3. Emphasize the need for cooperation with patient organizations and industry partners to support the development of new therapies.
Wolfram Syndrome

- Diabetes Mellitus (median age 6) – Insulin, GLP-1R agonists, metformin
  - Optic nerve atrophy (median age 11)
- Deafness (median age 14) – Hearing aids, cochlear implants
- Diabetes Insipidus (median age 13) - DDAVP
  - Neurodegeneration (begin to appear during the later years of adolescence)

- Causative Genes: WFS1 and CISD2 (autosomal Recessive)
Two Types of Wolfram Syndrome

<table>
<thead>
<tr>
<th>Type</th>
<th>Gene</th>
<th>Inheritance</th>
</tr>
</thead>
<tbody>
<tr>
<td>WFS Type 1</td>
<td>WFS1</td>
<td>Autosomal Recessive</td>
</tr>
<tr>
<td>WFS Type 2</td>
<td>CISD2</td>
<td>Autosomal Recessive</td>
</tr>
</tbody>
</table>

• Most patients have Wolfram Type 1
• Prevalence: 1 in 250,000-700,000
• Patients have two mutated copies of WFS1 or CISD2 gene (autosomal recessive)
Wolfram Syndrome Type 1: A spectrum disorder

Evan Lee, WUSTL MSTP  Megha Verma SLU Med  Nila Palaniappan UMKC Med

- Females: Milder symptoms than males
- Two or one missense WFS1 variants: Milder symptoms (60% of our cohort)
- Two frameshift/nonsense WFS1 variants: More severe symptoms (40%: a classic form)
- WFS1 c.1672C>T (p.Arg558Cys), a common variant in Ashkenazi-Jewish population associated with mild manifestations
- Prevalence could be 1:70,000 (5000 pts in the US)
Wolfram syndrome and WFS1-related disorders

- 1 pathogenic dominant WFS1
- 2 pathogenic WFS1 or CISD2
- 1 pathogenic dominant WFS1

Diabetes
Hearing loss
Optic Nerve Atrophy & Hearing loss
Cataract

Wolfram Syndrome
- Diabetes Mellitus
- Diabetes Insipidus
- Optic Nerve Atrophy
- Hearing loss
- Neurodegeneration

WFS1-related disorders:
- Neonatal diabetes
- Congenital cataracts/glaucoma
- Sensorineural deafness
- Hypotonia
- Developmental delay/Intellectual disability

https://wolframsyndrome.wustl.edu/
**Neurogenic Bladder**
- Urodynamic testing
- Anti-cholinergic medications
- Botox injections
- Antibiotics
- Neural stimulator
- Catheterization

**Respiratory failure**
- Sleep test
- Positive pressure

**Choking**
- Swallow test
- Speech pathologist

[https://wolframsyndrome.wustl.edu/](https://wolframsyndrome.wustl.edu/)

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WolframSyndrome@wustl.edu
Phone: 314-747-7055

Christine Manning
BSN, RN

Cris Brown, BA

Caroline Raso

Stacy Hurst
BSN, RN, CDE
Consensus Clinical Guidelines

- Genetics/Diagnosis
- Endocrinology
- Neurology
- Psychiatry
- Urology
- Ophthalmology
Wolfram Syndrome: Prototype Endoplasmic Reticulum (ER) Disorder

Loss of Function of WFS1

1. High levels of ER stress, Mitochondrial dysfunction
2. Lower levels of ER calcium, higher levels of cytoplasmic calcium
The Spectrum of ER dysfunction

COMMON MILD

Aging

Type 2 Diabetes

WFS1-related disorders (Wolfram-like)

Congenital Nephrotic syndrome

Achromatopsia (ATF6)

RARE SEVERE

Wolfram syndrome (WFS1)

Pelizaeus-Merzbacher Disease (PLP1)

Marinesco-Sjögren Syndrome (SIL1)

FENIB (SERPIN1)

Walcott-Rallison Syndrome (PERK)
Molecular Mechanisms of Wolfram Syndrome

- Mutant WFS1 gene
- Mutant WFS1 mRNA
- Mutant WFS1 protein
- Dysregulated ER Ca\(^{2+}\) homeostasis
- ER stress
- Mitochondrial dysfunction
- Cell death

Clinical manifestations

Enhancement of residual functions (Ongoing)
AMX0035 (Amylyx)

Gene therapy / Regenerative therapy (3-10 years)
- Transfer normal WFS1 gene by AAV
- Correct pathogenic WFS1 variants (Drs. Lu, Verfaillie, Moon)
- Transfer a regenerative factor MANF (Opris Bio)
- Transplant iPSC-derived tissues

Pharmacological compensation (Ongoing)
- Dantrolene sodium
- ER calcium stabilizers
- GLP-1 R agonists
- Sigma 1 R agonist - Pridopidine (Prilenia)
- Valproic Acid (Prof Barrett, U-Birmingham)
- Ibudilast (Prof Ehrlich, Yale)

Marked in Red: URANO team at Washington U

ER: Endoplasmic Reticulum
Wolfram syndrome - Dantrolene Sodium Clinical Trial Progress

Preclinical study completed

2014

Orpha Drug Designation #15-4745 (Feb 2016)

2016

Orphan Drug Designation EU3/16/1800 for (Dec 2016)

2017

The trial started in pediatric and adult patients (Jan 2017)

IND 133439 approval (Jan 2017)

19 patients completed phase 1b/2a study (August 2019)

2019

Washington University in St. Louis

Institute of Clinical and Translational Sciences

NNI

National Institutes of Health

EUROPEAN MEDICINES AGENCY

SCIENCE MEDICINES HEALTH
A phase Ib/IIa clinical trial of dantrolene sodium in patients with Wolfram syndrome

Damien Abreu,1,2 Stephen I. Stone,3 Toni S. Pearson,4 Robert C. Bucelli,4 Ashley N. Simpson,5 Stacy Hurst,1 Cris M. Brown,1 Kelly Kries,1 Chinyere Onwumere,1 Hongjie Gu,6 James Hoekel,7 Lawrence Tychsen,7 Gregory P. Van Stavern,7 Neil H. White,3 Bess A. Marshall,3 Tamara Hershey,8 and Fumihiko Urano1,9

1Division of Endocrinology, Metabolism, and Lipid Research, Department of Medicine, 2Medical Scientist Training Program, 3Division of Endocrinology and Diabetes, Department of Pediatrics, 4Department of Neurology, 5Center for Clinical Studies, 6Division of Biostatistics, 7Department of Ophthalmology & Visual Sciences, 8Departments of Psychiatry and Radiology, and 9Department of Pathology & Immunology, Washington University School of Medicine, St. Louis, Missouri, USA.
Lessons Learned from the Dantrolene Repurposing Trial

- **Cost-effective**: Less expensive than developing new drugs
- **Faster development**: Can speed up the drug development process since the drug has already undergone clinical trials for other indications and has been tested for safety.
- **Outcome measures**: 30-min MMTT is not sufficient, MMTT could improve, Visual acuity could improve.
- **Challenge**: Not designed for Wolfram
- **Challenge**: Limited patent protection: Limited patent protection, which can limit financial incentives for pharmaceutical companies to invest in repurposing efforts.
Molecular Mechanisms of Wolfram Syndrome

- Mutant WFS1 gene
- Mutant WFS1 mRNA
- Mutant WFS1 protein
- Dysregulated ER Ca²⁺ homeostasis
- ER stress
- Mitochondrial dysfunction
- Cell death
- Clinical manifestations

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AMX0035 (Amylyx)

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Marked in Red: URANO team at Washington U

ER: Endoplasmic Reticulum
Clinical Trial of AMX0035
in adult patients with Wolfram syndrome

1. Started a collaboration with Amylyx (2017)
2. The U.S. FDA granted an orphan drug designation to AMX0035 for the treatment of Wolfram syndrome (October 2020).
3. IND (September 2022)
4. Washington University IRB Approval (February 2023)
5. Started a trial (April 2023) – First patient was dosed on April 12, 2023

Amylyx Pharmaceuticals Announces First Participant Dosed in Phase 2 Study of AMX0035 for the Treatment of Wolfram Syndrome

- Recently published preclinical data demonstrate initial proof-of-concept for the therapeutic development of AMX0035 (sodium phenylbutyrate and taurursodiol) in Wolfram syndrome

April 13, 2023 09:00 AM Eastern Daylight Time
Pre-clinical Efficacy: AMX0035 suppresses cell death in Wolfram iPSC-derived Neuronal Progenitor Cells

Vehicle PBA TUDCA AMX0035

**
P = 0.1231

Relative Caspase 3/7 activity (Normalized with Cell Viability)

Vehicle PBA TUDCA AMX0035

***
P = 0.0735

Relative Caspase 3/7 activity (Normalized by cell viability)

Vehicle PBA TUDCA AMX0035

****

Relative Caspase 3/7 activity (Normalized with Cell Viability)

Vehicle PBA TUDCA AMX0035
Pre-Clinical Efficacy: AMX0035 restores mitochondrial function in Wolfram iPSC-derived NPCs

Mito stress test (Seahorse machine) W024 NPCs

OCR is increased

Mitochondrial function is improved
Multidimensional analysis and therapeutic development using patient iPSC-derived disease models of Wolfram syndrome

Rie Asada Kitamura,1 Kristina G. Maxwell,1,2 Wenjuan Ye,3 Kelly Kries,1 Cris M. Brown,1 Punn Augsornworawat,1,2 Yoel Hirsch,4 Martin M. Johansson,4 Tzvi Weiden,5 Joseph Ekstein,4 Joshua Cohen,6 Justin Klee,6 Kent Leslie,6 Anton Simeonov,3 Mark J. Henderson,3 Jeffrey R. Millman,1,2 and Fumihiro Urano1,7

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Measure Efficacy and Safety

BETA CELLS (Diabetes)

EYE (Visual Acuity)

Neurological Functions
**Beta Cells**

![Diagram of Beta Cells]

**C-peptide** (Insulin from patient's own pancreas)

![Graph showing C-peptide levels over time]

**Eye**

- **Snellen:**
  - 1: 20/200
  - 2: 20/100
  - 3: 20/70
  - 4: 20/50
  - 5: 20/40
  - 6: 20/30
  - 7: 20/25
  - 8: 20/20

- **LogMar:** 1.0

- **OCT**

![OCT image]
C-peptide in Wolfram syndrome

C-peptide in Monogenic Diabetes

Fig. 1 - C-peptide response during MMTT (mixed meal tolerance test) according affected gene

1000 pmol/L = 3.02 ng/mL

Stankute et al. 2021
### LogMar Snellen Test

<table>
<thead>
<tr>
<th>LogMar</th>
<th>Snellen</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>20/20</td>
</tr>
<tr>
<td>1.0</td>
<td>20/200</td>
</tr>
</tbody>
</table>

### OCT (Optical Coherence Tomography)

![OCT Image]

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**James Hoekel OD & Larry Tychsen MD**

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**Greg Van Stavern MD**

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### Graphs

**A**

- **Visual Acuity vs. Age:**
  - Red: Rapid
  - Black: Slow

**B**

- **Average RNFL Thickness vs. Age:**
  - Red: Rapid
  - Black: Slow
Trial Team

Bess Marshall, MD (Endocrinology, Medical Director)
Stacy Hurst, RN, CDE (Lead Nurse Coordinator)
Paulina Cruz Bravo, MD (Endocrinology)
Alexis McKee, MD (Endocrinology)
Amy Viehoever, MD, PhD (Neurology)
Saumel Ahmadi, MD, PhD (Neurology)
Greg Van Stavern, MD (Ophthalmology)
Tamara Hershey, PhD (Neuropsychiatry)
Jennifer Powers Carson, PhD (Core Lab)
Cris Brown (Research Lab)
Gabriel Skinner (Research Lab)
Caroline Raso (Coordinator)
Joshua Chen (Coordinator)
Nila Palaniappan (Coordinator)
Mary Jane Clifton (Coordinator)
Kathryn Bohnert (Coordinator)
Fumihiko Urano, MD, PhD (PI, Medical Genetics)
Amylyx Pharmaceuticals (Sponsor)
Significance of Working with Patient Organizations

• Raising awareness for Wolfram syndrome.
• Patient Organizations facilitate collaboration between academic researchers and industry.
• Collaborative research between researchers, clinicians, and patients is necessary to ensure patient-centered outcomes.
What a great day today meeting Dr. Fumihipko Urano's Lab! The Snow Foundation presented a check for a research project and Raquel and I had lunch with his team. We learned about all the great research projects that are currently taking place. Extremely informative and very inspiring! Keep up the good work Urano Lab!

We're honored to have hosted such an important conversation!

I had an amazing interview session with Beth and Ellie White at PBS in Denver! Together, we discussed Wolfram syndrome and our shared mission to raise awareness and work towards finding a cure! @rmpbs @JeremyDanMoore @DanaKnowles123 @littleelliebean @EllieWhiteFound @PBS
IT TAKES A VILLAGE TO ACHIEVE A CURE FOR WOLFRAM SYNDROME

- Wolfram Syndrome Clinic
- Entrepreneurship: CURE4WOLFRAM, Emerald Biotherapeutics, Amylyx Pharmaceuticals, Prilenia
- Therapeutic and Diagnostic Development
- Philanthropies/Grants (i.e. $) by Wolfram Syndrome Clinic
- Improvement of Clinical Care
- Raise Awareness
- Patient Organizations

Rachel Hartmann
WashU
Mentors
Jane Workman
Edward Berk
Marjorie Feinstein
Junichi Hata, MD, PhD
Akihiro Umezawa, MD, PhD
David Ron, MD
Michael Green, MD, PhD
Julie Neidich, MD
Aldo Rossini, MD

https://wolframsyndrome.wustl.edu/

Junko Urano, MD
1936-2024

AMX0035 Wolfram Syndrome Program & Phase 2 HELIOS Interim Data

Dr. Camille L. Bedrosian
Chief Medical Officer, Amylyx
Wolfram Syndrome Is a Rare and Fatal Genetic Disorder\textsuperscript{1,5}

Characterized by childhood-onset diabetes mellitus, optic nerve atrophy, deafness, diabetes insipidus, and neurodegeneration, eventually resulting in premature death\textsuperscript{1-5}

Loss of Vision
As cells in the optic nerve die, individuals gradually go blind

Diabetes Insipidus
Because of a faulty pituitary gland, the kidneys produce too much urine

Balance and Coordination Difficulty
The disease attacks the cerebellum, hampering the ability to control movement

Deafness
People with Wolfram syndrome usually begin to lose their hearing in their teens

Difficulty Breathing
By damaging the brain stem, the disease can disrupt respiration; death occurs at a mean age of 30 years (range 25-49 years), mainly from respiratory failure

Diabetes Mellitus
Insulin-producing beta cells die, hindering the body’s use of sugar as energy

There are currently no disease-modifying therapies for Wolfram syndrome; treatment strategies focus on use of life-sustaining medications, clinical monitoring, and symptom management\textsuperscript{6,7}


Wolfram Syndrome is a Prototypical Endoplasmic Reticulum Stress Disorder

Pathogenic Mutations in $WFS1^{2,3}$

Endoplasmic Reticulum (ER) Stress

Impaired Mitochondrial Dynamics

Dysfunction and apoptosis of cells, including pancreatic beta cells and neurons$^{2,3}$

References:
Recent Studies Suggest Wolfram Syndrome May be More Common than Previously Estimated¹

Older literature estimates anywhere between ~500 to ~3,400 people living with Wolfram syndrome in the U.S.²,³

<table>
<thead>
<tr>
<th>Studies Pre-Dating Molecular Genetic Testing</th>
<th>Prevalence Estimate</th>
<th>Extrapolated U.S. Prevalence Estimate¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1977 Publication Extrapolating Wolfram Prevalence Based on Frequency in Juvenile Diabetes in North America²</td>
<td>1:100,000 Individuals</td>
<td>~3,400 cases</td>
</tr>
<tr>
<td>1995 Prevalence Study in the U.K.³</td>
<td>1:770,000 Individuals</td>
<td>~500 cases</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Studies Evaluating Genetic Causes of Diabetes</th>
<th>Prevalence Estimate</th>
<th>Extrapolated US Prevalence Estimate¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>2023 Diabetes Study Evaluating Monogenic Diabetes in France¹</td>
<td>WFS1 mutations found in 3% of monogenic diabetes cases (monogenic diabetes = ~1% of diabetes cases in U.S.)</td>
<td>~11,000 casesb</td>
</tr>
</tbody>
</table>

¹All U.S. prevalence extrapolations assume a U.S. population of 341,814,420.

²Extrapolations to U.S. prevalence from diabetes population are illustrative only to show potential trends in higher prevalence and should not be considered exact numbers. Extrapolation for monogenic diabetes assumes 38.4 million cases of diabetes in U.S.; 1% of those cases are monogenic⁵ = 384,000 people with monogenic diabetes in U.S.

³Older literature estimates anywhere between ~500 to ~3,400 people living with Wolfram syndrome in the U.S.
Enhanced Awareness and Testing Will Likely Result in Increased Prevalence Estimates as Has Been Observed in Other Rare Diseases

• As seen in other rare diseases, the prevalence of Wolfram syndrome may be underestimated due to underdiagnosis or misdiagnosis; several examples in rare disease highlight that prevalence estimates increase with improvements in disease awareness and diagnostic methods1-5

> In HELIOS participants:
  - Median age of diabetes onset (range)
    • 9 years old (3-32)
  - Median age at diagnosis (range)
    • 20 years old (8-35)

<table>
<thead>
<tr>
<th>Example</th>
<th>Prevalence Trends</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paroxysmal Nocturnal Hemoglobinuria (PNH)</td>
<td>PNH prevalence increased with introduction of high sensitivity diagnostic test, increased awareness, and effective treatments</td>
</tr>
</tbody>
</table>
| PNH Prevalence Over Time      | 1991-2006: 1.59:100,000 (U.K.)¹  
  2004-2018: 3.81:100 000 (U.K.)²  
  2016-2017: 12-13:100,000 (U.S.)³ |
| Hypophosphatasia (HPP)       | In a study from Spain, prevalence doubled when a new diagnostic algorithm introduced⁴ |
| Huntington’s Disease         | Increase in Huntington’s disease global prevalence attributed to enhanced availability of molecular testing, earlier diagnosis, increased life expectancy, and de novo mutations⁵ |
| Huntington’s Disease Prevalence Over Time⁵ | 1985-2010: 2.71:100,000  
  2010-2022: 4.88:100,000 |

AMX0035 Targets ER Stress and Mitochondrial Dysfunction, Critical Pathways in Wolfram Syndrome Pathophysiology

Pathogenic Mutations in WFS1

Endoplasmic Reticulum Stress

Impaired Mitochondrial Dynamics

Dysfunction and apoptosis of cells, including pancreatic beta cells and neurons

Sodium phenylbutyrate is a chemical chaperone shown to stabilize protein conformation during folding, decrease trafficking of mutant proteins, and restore normal insulin secretion in Wolfram mutant cells.

Taurursodiol stabilizes the mitochondrial membrane by reducing the translocation of cell death regulator Bax, improving mitochondrial function and energy production and increasing cell apoptotic threshold.
Wolfram Syndrome Pathophysiology is Well Characterized with Pre-Clinical Models that Allow Us to Evaluate Different Etiological Aspects

**Pre-Clinical Models**

**Patient iPSC-Derived Disease Models**
- Patient-derived induced pluripotent stem cells (iPSCs) with homozygous mutations of the *WFS1* gene, can be used to generate pancreatic islet (beta) and neural progenitor cells
- Cells exhibit hallmark features of Wolfram syndrome (e.g., decreased WFS1 expression, organelle function, viability, and insulin section)

**Mouse Models**
- *Wfs-1*-knockout mice develop progressive glucose intolerance during adolescence and do not exhibit increases in serum insulin in response to glucose stimulation
- Considered a valid mouse model of Wolfram syndrome

**Clinical Manifestations**
- Mutant WFS1 gene
- Mutant WFS1 mRNA
- Mutant and ↓ Wildtype (WT) WFS1 Protein
- Dysregulated ER Ca$^{2+}$ Homeostasis
- ER Stress & Mitochondrial Dysfunction
- Pancreatic β Cell Death
- Neuronal Cell Death
AMX0035 Rescues Wolfram Syndrome Phenotype In Patient-Derived Beta Cell Models

Wolfram Syndrome Etiology Schematic*

Mutant WFS1 gene
Mutant WFS1 mRNA
Mutant and WT WFS1 Protein
Dysregulated ER Ca²⁺ Homeostasis
ER Stress Mitochondrial Dysfunction
Pancreatic β Cell Death
Neuronal Cell Death
Clinical Manifestations

Increased WFS-1 Protein Levels in Patient-Derived Beta Cells (P<0.05)

Rescued WFS1-Mutant Islet Cell Viability in Patient-Derived Beta Cells (P<0.001)

Improved WFS1 Mutant Insulin Secretion in Two Patient-Derived Cell Lines (P<0.05)

W024 and W121 indicate cell lines from specific patients
*P<0.05 by unpaired t test compared with Ctrl; ***P<0.001 and ****P<0.0001 by unpaired t test compared with Ctrl; #P<0.05 and ##P<0.01 by 2-way unpaired t test; ^Normalized by cell viability

WFS1 Protein Expression
Fold Change (Relative to Ctrl)

AMX0035

Insulin Secretion (µIU/10⁶ cells)

Glucose Levels

AMX0035 Rescues Wolfram Syndrome Phenotype In Patient-Derived Neuronal Cell Models

AMX0035 Prevented Cell Death (P<0.0001) In Three Different Patient-Derived Neuronal Cell Models

AMX0035 Significantly Delayed Onset of Diabetic Phenotypes in Wfs1-deficient mice

AMX0035-Treated Mice Showed Better Glycemic Control (P<0.001) than Untreated After 1 Month with Minimal to No Diabetes Progression Based on Glucose Tolerance Test (GTT)

**P < 0.01 and ***P < 0.001 by 1-way ANOVA; #P < 0.01 and ###P < 0.0001 by 1-way ANOVA compared with WT: Baseline; ††††P < 0.0001 by 1-way ANOVA compared with WT: 1 month)

AMX0035 has been Extensively Studied in Wolfram Syndrome Models including Patient-Derived Cells and Mouse Model

- Clear Improvement in Insulin Secretion in Patient-Derived Beta Cells
- Clear Improvement in Cell Viability in Patient-Derived Beta Cells
- Clear Improvement in Cell Viability in Patient-Derived Neuronal Cells
- Highly Statistically Significant Delay in Glycemic Progression in Wolfram Mice

Measurement of Progression in Wolfram Syndrome

In memory of Lauren, a beautiful daughter and passionate Wolfram syndrome warrior.
Progression Measurements in Wolfram Syndrome Typically Focus on Disease Manifestations with Greatest Prevalence and Impact

**Clinical Manifestations of Wolfram Syndrome**

- **Central Nervous System**
  - Hearing loss
  - Balance & coordination problems
  - Irregular breathing
  - Loss of sense of smell
  - Loss of gag reflex
  - Muscle spasms
  - Seizures
  - Behavior problems
  - Depression

- **Gastrointestinal System**
  - Ulcers
    - (Wolfram syndrome type 2)
  - Bleeding

- **Peripheral Nervous System**
  - Peripheral neuropathy

- **Ophthalmologic System**
  - Vision loss

- **Endocrine System**
  - Insulin shortage
    - (diabetes mellitus)
  - Pituitary gland dysfunction
    - (diabetes insipidus)

- **Urogenital System**
  - Reduced testosterone
    - (males)
  - Duct obstruction
  - Disrupted urination
  - Incontinence
  - High-capacity atonal bladder

**Key Focus Areas for Wolfram Syndrome Progression Measurement**

- **Diabetes Mellitus**
- **Vision**

**Other Measures of Wolfram Syndrome Progression**

- **Overall Wolfram Syndrome Symptom Burden**

---

Measures of Diabetes Mellitus Progression: C-Peptide

The pancreas produces insulin over a series of steps: 1

- Preproinsulin is cleaved into proinsulin which is cleaved into insulin and C-peptide

C-peptide levels are often used as a surrogate marker of pancreatic function and glycemic control as C-peptide 2, 3

- Is secreted in a 1:1 ratio with insulin and degraded at a slower rate
- Is not cleared by the liver and can be measured in the blood
- Is produced endogenously and is not confounded by external insulin use
- Has been shown to predict future diabetic complications and glycemic control

C-peptide levels have been used as the primary outcome in several major diabetes trials and multiple publications consider it to be a validated surrogate marker 4, 5

C-Peptide Declines in Wolfram Syndrome—HELIOS was Designed to Measure a Slowing in Decline

- C-peptide levels are an objective laboratory measure assessed during a mixed meal tolerance test (MMTT), a physiological stimulation test using a standardized liquid meal ingested in the morning followed by timed measurements over the subsequent pre-determined time period\(^1\)

- 4-hour MMTT is expected to have less variance and be a better measure
  - Amylyx is the first to conduct 4-hour MMTT in Wolfram syndrome

- The 90-minute MMTT has been shown to be a highly sensitive and specific measure of peak insulin secretion and AUC C-peptide and is considered to be a useful alternative to a full 2-hour MMTT\(^2\)

- A recent natural history study demonstrated decline in C-peptide (as measured by 30-minute MMTT) after diabetes mellitus onset in Wolfram syndrome\(^3\)
  - **In the first ~2 years:** Average decline of 0.37 ng/mL per year of diabetes mellitus
  - **After the first ~2 years:** Average decline of 0.13 ng/mL per year of diabetes mellitus

---

Measures of Diabetes Mellitus Progression: HbA1c

• The level of glycosylated hemoglobin (HbA1c) provides a measure of the glycemic control of diabetes during the preceding 2-3 months

• HbA1c is inversely correlated to C-peptide; improved metabolic function is associated with higher C-peptide and lower HbA1c

  – In Wolfram syndrome, HbA1c levels may remain stable, if blood glucose is well-controlled with available diabetes mellitus treatments, however it may get more difficult for levels to remain stable as the disease progresses

WS Natural History Expectations: HbA1c gets more challenging to control over time

Measures of Vision: Best Corrected Visual Acuity

• Measurement of visual acuity for both eyes after correction, using the Snellen chart

• LogMar values range from 0 (perfect vision) to +2 (near blindness)*

• In a recent 10-year analysis of 38 individuals with Wolfram syndrome, visual acuity declined over time in all participants with a mean slope of 0.059 logMar/year (95% CI: 0.07 to 0.05 logMar/year)

  > A subset of individuals (26%) had rapid decline in visual acuity; mean rate of decline was 0.16 logMar/year (SD 0.05)

*Values of -0.1 and -0.2 are also possible representing better than perfect vision
Measures of Overall Symptom Burden: PGI-C and CGI-C

**PGI-C**: Patient-reported global impression of change
- Participants evaluate the change in their WS-related symptoms since initiation of study drug
- Asked by the investigator or qualified designee to rate their change in status using a 7-point scale

**CGI-C**: Clinician-reported global impression of change
- The CGI-C rates improvement by the same 7-point scale

While not specific to WS, these measures have been used across multiple disease states to provide a holistic assessment of treatment benefit

**PGI-C and CGI-C 7-Point Scale**
- 1 – Very much improved
- 2 – Much improved
- 3 – Minimally improved
- 4 – No change
- 5 – Minimally worse
- 6 – Much worse
- 7 – Very much worse

Responses used to define responders in HELIOS as Wolfram syndrome is a universally progressive disease
Interim Efficacy and Safety Results of AMX0035 in Wolfram Syndrome
HELIOS Study Design\textsuperscript{1,2}

**Primary Objectives:**

- To assess the safety and tolerability of AMX0035 administered orally for up to 96 weeks
- To evaluate the effect of AMX0035 on residual beta cell function over 24 weeks by monitoring C-peptide levels

---

**Screening Period (4 wk)**

- Aged $\geq 17$ years
- Definite diagnosis of Wolfram syndrome\textsuperscript{a}
- Stimulated C-peptide level of $\geq 0.2$ ng/mL at screening

**Open-Label Treatment Period (48 wk)**

- Insulin-dependent diabetes mellitus due to Wolfram syndrome
- Must be willing to wear a CGM device for the study duration
- No GLP-1 Agonist Use

**AMX0035 (n=12)**

**Treatment Extension Phase (48 wk)**

**Follow-up Period (4 wk)**

---

**Key Trial Entry Criteria\textsuperscript{1,2}**

- Insulin-dependent diabetes mellitus due to Wolfram syndrome
- Must be willing to wear a CGM device for the study duration
- No GLP-1 Agonist Use

---

CGM, continuous glucose monitoring.

\textsuperscript{a}Documented functionally relevant recessive mutations on both alleles of the \textit{WFS1} gene based on historical test results (if available) or from a qualified laboratory at screening.


\textsuperscript{2}Data on File. Amylyx Pharmaceuticals Inc. 2024.
HELIOS Study Design

Primary Objectives:
• To assess the safety and tolerability of AMX0035 administered orally for up to 96 weeks
• To evaluate the effect of AMX0035 on residual beta cell function over 24 weeks by monitoring C-peptide levels

Data shared today are from an interim analysis performed as of March 5, 2024, when 8 participants had completed their Week 24 assessments
Interim Analysis Has Complete Efficacy for 8 Participants at Week 24 and Demographic and Available Safety Data for All 12 Enrolled

Today’s Data

- Demographics
- Efficacy
- Safety

8 Participants with Data Fully Cleaned Through Week 24

4 Recently Enrolled Participants, Have Not Yet Reached Week 24

12 Individuals Enrolled With No Dropouts or Discontinuations Thus Far
### Patient Baseline Characteristics

#### Median Age:
- 25 years (range: 18-39)

- **Male:** 2 (17%)
- **Female:** 10 (83%)

#### Median Duration of WS:
- 5 years (range: 1 to 24)

#### Median Age at Diagnosis
- 20 (range: 8 to 35)

#### Median Age of Onset, Years (Range)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Median Age (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes Mellitus</td>
<td>9 (3 to 32)</td>
</tr>
<tr>
<td>Diabetes Insipidus*</td>
<td>11 (7 to 24)</td>
</tr>
<tr>
<td>Vision Loss</td>
<td>11.5 (5 to 29)</td>
</tr>
<tr>
<td>Hearing Loss</td>
<td>16 (7 to 33)</td>
</tr>
</tbody>
</table>

#### Diabetes Medications at Baseline

<table>
<thead>
<tr>
<th>Medication</th>
<th>Frequency (count)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin Lispro</td>
<td>8</td>
</tr>
<tr>
<td>Insulin Glargine</td>
<td>5</td>
</tr>
<tr>
<td>Metformin</td>
<td>3</td>
</tr>
<tr>
<td>Insulin Aspart</td>
<td>2</td>
</tr>
<tr>
<td>Glucagon</td>
<td>1</td>
</tr>
<tr>
<td>Insulin Degludec</td>
<td>1</td>
</tr>
<tr>
<td>Insulin Lispro-aabc</td>
<td>1</td>
</tr>
</tbody>
</table>

* N=4

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Data on File. Amylyx Pharmaceuticals Inc. 2024.
### HELIOS Endpoints

**Primary Efficacy**
- Change from baseline in C-peptide (ΔC-peptide, AUC c-peptide)

**Secondary Efficacy**
- Change in baseline best-corrected visual acuity on the LogMar scale using the Snellen chart
- Change from baseline in exogenous insulin dose
- Change from baseline in overall time in target glucose range (70–180 mg/dL)
- Change from baseline in HbA1c level

**Exploratory**

<table>
<thead>
<tr>
<th>General</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wolfram United Rating Scale</td>
</tr>
<tr>
<td>Clinician-reported Global Impression of Change</td>
</tr>
<tr>
<td>Patient-reported Global Impression of Change</td>
</tr>
<tr>
<td>Most bothersome symptom</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Visual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual Functioning Questionnaire–25</td>
</tr>
<tr>
<td>Optical Coherence Tomography measurements</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Additional Pancreatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic measurements, including fasting glucose, fasting proinsulin, AUC C-peptide/ AUC glucose, delta proinsulin</td>
</tr>
<tr>
<td>Change from Week 96 to Week 100 in C-peptide levels</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Neurological</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood biomarker (panel) levels of neurodegeneration and neuroinflammation</td>
</tr>
<tr>
<td>Scale for the Assessment of Rating Ataxia</td>
</tr>
</tbody>
</table>

**Interim Analysis Results Focus on Diabetes and Vision Assessments in 8 participants (Week 24)**
- Final Week 24 data will report all 12 participants and include additional assessments

AUC, area under the curve; MMTT, mixed-meal tolerance test.

Primary Endpoint: Improvement in C-Peptide Response at Weeks 12 and 24

Overall increase in mean C-peptide (Total Production/Area Under Curve) when decrease expected

Partial Reversal in C-peptide Phenotype Observed at Weeks 12 and 24 Compared to Screening
Primary Endpoint: Improvement in C-Peptide Response at Weeks 12 and 24

Overall increase in mean C-peptide (Total Production/Area Under Curve) when decrease expected

C-Peptide AUC Mean Change from Baseline to Weeks 12 and 24 (N=8)

Partial Reversal in C-peptide Phenotype Observed at Weeks 12 and 24 Compared to Screening

Direction of Expected Change from Baseline with Wolfram Syndrome Progression

Data on File. Amylyx Pharmaceuticals Inc. 2024.
Primary Endpoint: \( \Delta \) C-Peptide Change from Baseline Improved

Overall increase in numerical response at 90-, 120-minutes and Peak, when decrease expected

\[ \Delta \text{C-Peptide Mean Change from Baseline at Weeks 12 and 24 (N=8)} \]

Data on File. Amylyx Pharmaceuticals Inc. 2024.

Improvement in Average Beta Cell Responsiveness at 12 and 24 Weeks Compared to Screening

5 of 8 participants demonstrated increased \( \Delta \text{C-Peptide change at peak from baseline} \)

Change in C-Peptide (or \( \Delta \text{C-Peptide} \)) = (C-peptide at Specific Timepoint in MMTT [e.g., 90 minutes]) – (C-peptide at 0 minutes)

Change in \( \Delta \text{C-Peptide} \) (or \( \Delta \Delta \text{C-Peptide} \)) = (\( \Delta \text{C-Peptide} \) over MMTT at Timepoint of Interest [e.g., 24 weeks]) – (\( \Delta \text{C-Peptide} \) over MMTT at Baseline)
Primary Endpoint: Time to Peak C-Peptide Improved with AMX0035

Shorter time to peak C-peptide suggesting more rapid beta-cell response to glucose challenge

7 of 8 Participants Demonstrated Improved Pancreatic Function (at Least 30 min Shorter Time to Peak C-Peptide) at Week 24 Compared to Screening

Time to Peak C-Peptide By Participant
Secondary Endpoint: HbA1c

HbA1c Mean Change from Baseline to Week 24 (N=8)

HbA1c Mean Change from Baseline to Week 24 (N=8)

<table>
<thead>
<tr>
<th>HbA1c Change from Baseline (%)</th>
<th>HbA1c Mean Change from Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1.0%</td>
<td>-0.26% (SE: 0.15%)</td>
</tr>
<tr>
<td>-0.5%</td>
<td></td>
</tr>
<tr>
<td>0.0%</td>
<td></td>
</tr>
<tr>
<td>0.5%</td>
<td></td>
</tr>
<tr>
<td>1.0%</td>
<td></td>
</tr>
</tbody>
</table>

Change from Baseline: -0.26% (SE: 0.15%)

Improved Glycemic Control as Measured by HbA1c at Week 24 Compared to Screening

6 of 8 participants demonstrated reduced HbA1c from Screening to Week 24
**Secondary Endpoint: Overall Time in Target Glucose Range**

**Time in Target Glucose Range Change from Baseline to Week 24 (N=8)**

- **Change from Baseline:** +7.1% (SE: 4.7%)

**Direction of Expected Change with Wolfram Syndrome Progression**

- Improved
- Worsened

**Mean Time in Target Glucose Range by Participant**

- **Met goal** time in target range at baseline: 2
- **Met goal** time in target range at Week 24: 5

**Improved Glycemic Control as Assessed by Continuous Glucose Monitoring at Week 24 Compared to Screening**

- Of remaining 3 participants, 1 stable over time and 2 progressed

---

*Goal as defined by Recommendations from the International Consensus on Time in Range (Battelino T, et al. Diabetes Care. 2019;42(8):1593-1603.)*
Secondary Endpoint: Best Corrected Visual Acuity (BCVA)

BCVA, Best Eye Mean Change from Baseline to Week 24 (N=8)

-0.06 -0.04 -0.02 0.00 0.02 0.04 0.06 0.08 0.10 0.12 0.14 0.16
Best-Corrected Visual Acuity, Best Eye Change from Baseline (-LogMar)

Change from Baseline: +0.05 (SE: 0.09)

Direction of Expected Change with Wolfram Syndrome Progression

Change in BCVA, Best Eye by Participant

Better Vision

Worse Vision

BCVA, Best Eye (-LogMar)

Timepoint (Week)

0 24

5 of 8 demonstrated improved visual acuity in at least one eye from Screening to Week 24
Includes 1 participant blind at baseline who now has vision in one eye

Of remaining participants:
- 1 stable in one eye
- 1 progressed in both eyes
- 1 legally blind with no change

Trend Indicating Potential Visual Acuity Improvement at Week 24 Compared to Screening

Data on File. Amylyx Pharmaceuticals Inc. 2024.
Exploratory Endpoint: PGI-C and CGI-C

Patient-Reported Global Impression of Change (PGI-C) (N=8)

Clinician-Reported Global Impression of Change (CGI-C) (N=8)

100% of Participants Met Responder Criteria by Self and Clinician Assessment

75% of participants claim to improve on AMX0035; clinician reports 62.5% of patients improving
AMX0035 Safety and Tolerability in HELIOS (N=12)

- AMX0035 was **generally well tolerated**
  - Diarrhea was the most common TEAE (41.7%)
- No new safety signals were identified
- Nearly all participants reported ≥1 TEAE during the trial
  - Most did not lead to modification or interruption of AMX0035 dosing and **none** led to drug discontinuation

**Summary of Treatment Emergent Adverse Events (TEAEs)**

<table>
<thead>
<tr>
<th>Event</th>
<th>AMX0035 (N=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants with ≥1 TEAE— n (%)</td>
<td>11 (91.7%)</td>
</tr>
<tr>
<td>TEAE related to study drug — n (%)</td>
<td>7 (58.3%)</td>
</tr>
<tr>
<td>Treatment-emergent serious adverse events — n (%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Drug interrupted owing to TEAE — n (%)</td>
<td>2 (16.7%)</td>
</tr>
<tr>
<td>Dose reduced owing to TEAE — n (%)</td>
<td>1 (8.3%)</td>
</tr>
<tr>
<td>Drug discontinued owing to TEAE — n (%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

**N=8 with data through 24 weeks; For remaining 4, all available safety data as of March 5, 2024 used**
Summary of Interim Clinical Evidence: Potential of AMX0035 in Wolfram Syndrome

Expected Progression of Wolfram Syndrome

<table>
<thead>
<tr>
<th>Measure</th>
<th>Trend*</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-Peptide AUC</td>
<td>↓ Progressive Decline</td>
</tr>
<tr>
<td>Δ C-Peptide</td>
<td>↓ Progressive Decline</td>
</tr>
<tr>
<td>HbA1c</td>
<td>↓ Progressively More Difficult to Remain Stable</td>
</tr>
<tr>
<td>Time in Target Glucose Range</td>
<td>↓ Progressive Decline</td>
</tr>
<tr>
<td>Visual Acuity</td>
<td>↓ Progressive Decline</td>
</tr>
<tr>
<td>CGI-C and PGI-C</td>
<td>↓ Progressive Decline</td>
</tr>
</tbody>
</table>

*Baseline to Week 24 in N=8 participants

Data on File. Amylyx Pharmaceuticals Inc. 2024.
Thank you!

We extend our deepest gratitude to the HELIOS trial participants, their loved ones, Dr. Fumi Urano, the Washington University site team, and the entire Wolfram Syndrome community for their support of this trial.
Closing Remarks

Josh Cohen and Justin Klee
Co-CEOs, Amylyx
Key Takeaways

Strong Scientific Rationale

- Wolfram syndrome is a progressive, genetic disease caused by mutations in WFS1 that cause endoplasmic reticulum (ER) stress and impaired mitochondrial dynamics
- AMX0035 has been shown to simultaneously mitigate ER stress and mitochondrial dysfunction
- Preclinical data have demonstrated the efficacy of AMX0035 in cell lines, patient-derived cells, and mouse models

Compelling Data Support AMX0035’s Potential in Wolfram Syndrome

- Interim analysis demonstrated improvement in pancreatic function and glycemic control, as measured by C-peptide and other markers of glucose metabolism, rather than worsening typically expected with disease progression
- All participants met prespecified responder criteria demonstrating either improvement or stabilization of disease according to both patient- and clinician-reported scales
- Majority of participants reported some improvement in vision
- AMX0035 was generally well-tolerated in all participants

Urgent Unmet Need

- There are currently no disease-modifying therapies for Wolfram syndrome; treatment strategies focus on symptom management
- Wolfram syndrome impacts ~3,000 people in the U.S. and results in premature death
Orphan drug designation granted to AMX0035 for the treatment of Wolfram syndrome by U.S. FDA

Phase 2 HELIOS study of AMX0035 in Wolfram syndrome initiated in April 2023 and completed enrollment in Feb 2024

Continue to progress the Phase 2 HELIOS study and evaluate options for future development

Planning to engage with regulatory authorities to align on the development path

Topline data for all 12 participants at Week 24 are expected in the second half of 2024