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cyclerion

CLINICAL DATA UPDATE FROM STUDY OF CY6463 IN MELAS

HOSTED BY UMDF
TUESDAY, JUNE 28, 2022
8:00 AM EDT

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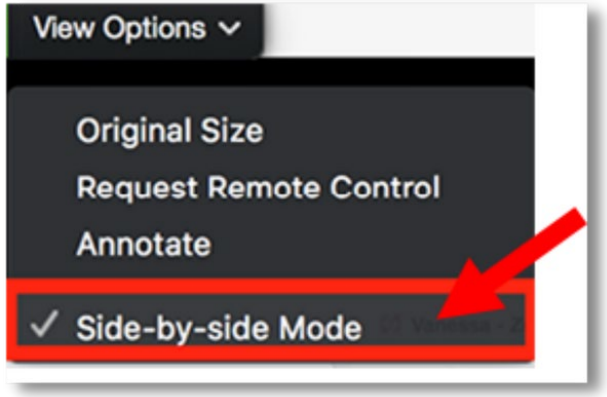

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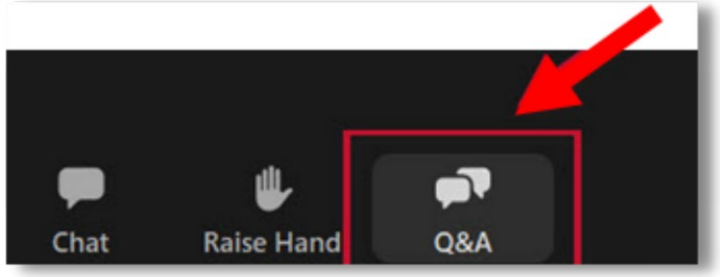
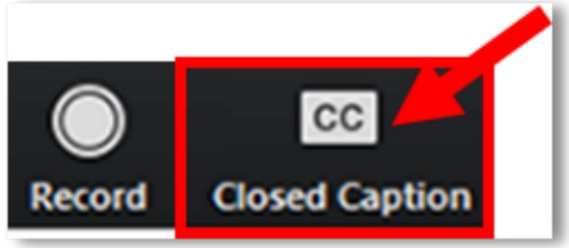


All attendees will be MUTED. Please submit your questions through the **Q&A BOX**.



CLOSED CAPTIONING is available. Turn it on or off as needed from the menu.

Speakers & Agenda

Philip Yeske, Ph.D.

Science & Alliance Officer, UMDF

Amel Karaa, M.D.

Director, The Mito Clinic, Harvard Medical School & Mass. General Hospital

Chris Winrow, Ph.D.

Vice President, Translational Medicine & Development Program Lead, Cyclerion

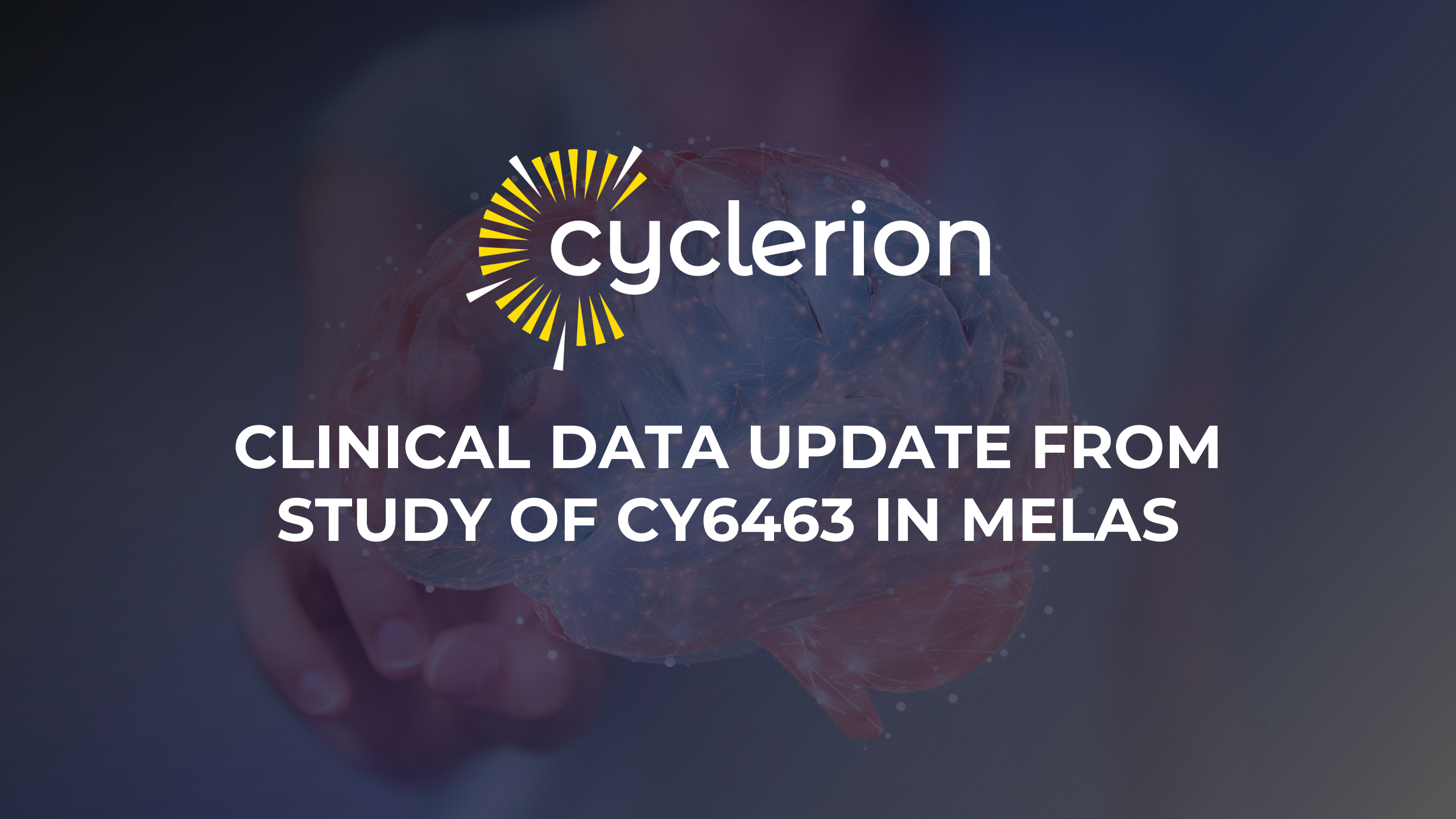
Chad Glasser, Pharm.D.

Director, Clinical Research, Cyclerion

Peter Hecht, Ph.D.

Chief Executive Officer, Cyclerion

1. Welcome & UMDF Introduction
2. A Brief Overview of MELAS
3. Cyclerion CY6463 clinical data overview
   - CY6463 in MELAS
   - Clinical study design
   - Clinical data summary
4. Implications for CY6463 in MELAS
5. Perspective on CY6463
6. Q&A



CLINICAL DATA UPDATE FROM STUDY OF CY6463 IN MELAS

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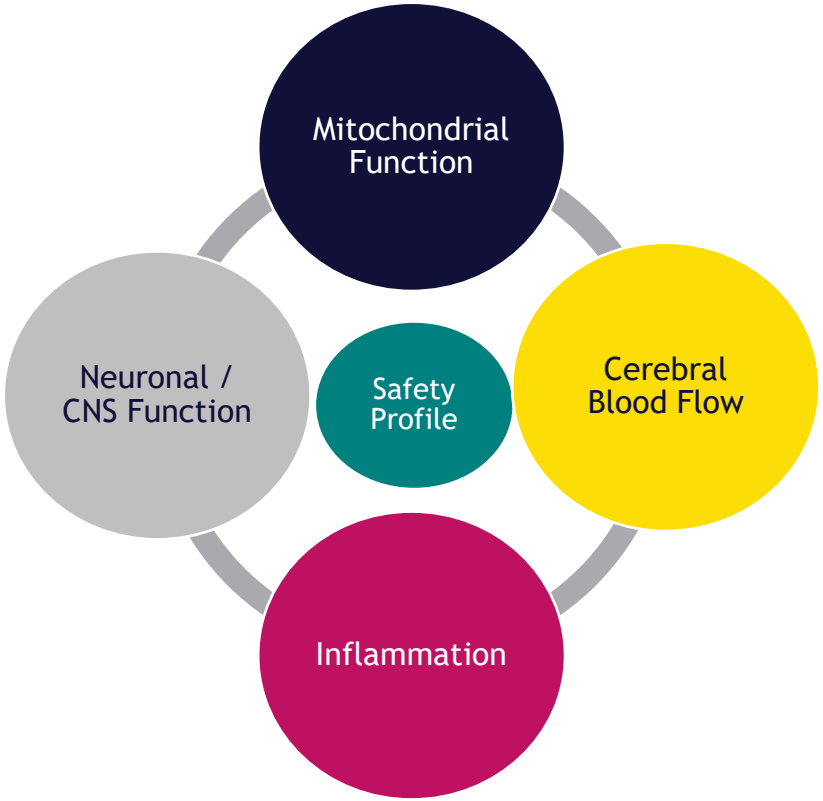
CY6463 has potential as breakthrough therapeutic for MELAS



Devastating, progressive orphan disease; No approved therapies

CY6463 demonstrated:

- **Improvements** observed across important biomarkers associated with MELAS :
  - Biomarkers associated with **mitochondrial function**
  - Broad panel of **inflammatory biomarkers**
  - **Cerebral blood flow (CBF)** across all brain regions
  - **Functional connectivity** between brain regions as measured by fMRI BOLD
- Well tolerated, no serious adverse events
- Oral, once-daily administration, CNS exposure

A Brief Overview of MELAS

AMEL KARAA, MD
DIRECTOR OF THE MITO CLINIC
MASSACHUSETTS GENERAL HOSPITAL
HARVARD MEDICAL SCHOOL

What is MELAS?

M: Mitochondrial

E: Encephalo-Myopathy

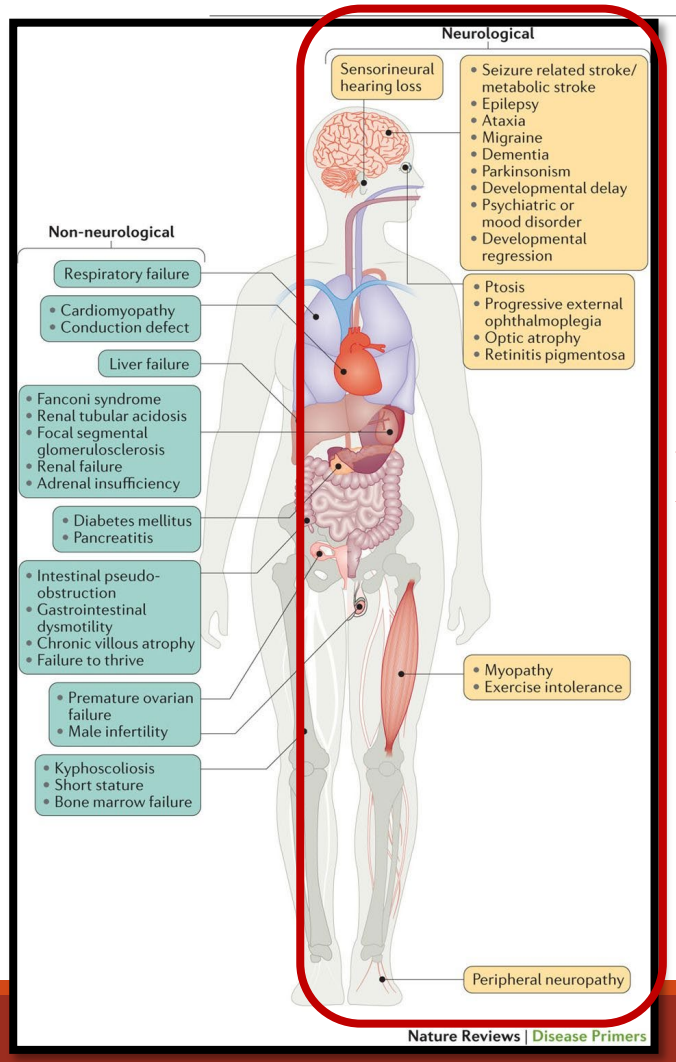
LA: Lactic Acidosis

S: Stroke-like Episodes

What is MELAS?

- The most common maternally inherited mitochondrial disease
  >80% caused by the m.3243A>G mutation (*MT-TL1*)
- MELAS affects any race and gender
- MELAS is a progressive disorder with a high morbidity & mortality rate

The burden of MELAS



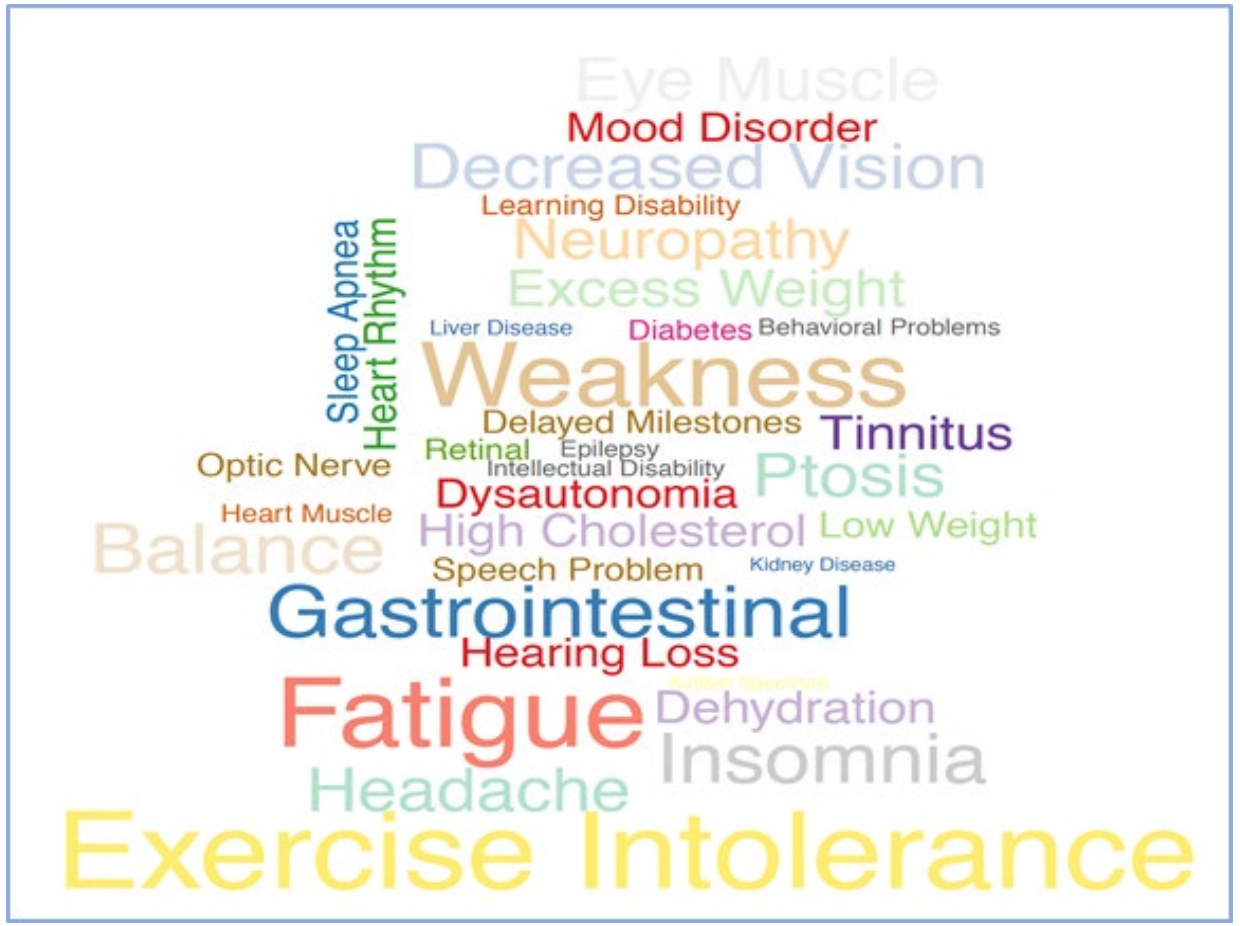
Frequency	Manifestations
≥25%	• Seizures • Recurrent headaches • Stroke-like episodes • Cortical vision loss • Muscle weakness • Recurrent vomiting • Short stature
10%-24%	• Altered consciousness • Impaired mentation • Hearing impairment • Diabetes mellitus (type 1 or 2)
<10%	• Developmental delay • Fever

https://www.ncbi.nlm.nih.gov/books/NBK1233/

	Onset 23.4 ± 15.6 years[a] (%)	Last evaluation 36.0 ± 20.7 years (%)
Hearing loss	**39 (31.0)**	**73 (57.9)**
Generalized seizures	**23 (18.3)**	**47 (37.3)**
Diabetes	**22 (17.5)**	**51 (40.5)**
Ptosis/ophthalmoparesis	**16 (12.7)**	**35 (27.8)**
Migraine	**16 (12.7)**	**35 (27.8)**
Stroke-like episodes	**14 (11.1)**	**51 (40.5)**
Muscle weakness	**14 (11.1)**	**50 (39.7)**
Exercise intolerance	**14 (11.1)**	**41 (32.5)**
Heart disease	**11 (8.7)**	**40 (31.7)**
Cognitive involvement	**11 (8.7)**	**31 (24.6)**
Failure to thrive/short st.	11 (8.7)	21 (16.7)
Increased CK	7 (5.6)	25 (19.8)
Muscle pain	7 (5.6)	13 (10.3)
Muscle wasting	6 (4.8)	29 (23.0)
Vomiting	5 (4.0)	7 (5.6)
Gastrointestinal dysmotil.	4 (3.2)	16 (12.7)
Neuropathy	4 (3.2)	13 (10.3)
Ataxia	3 (2.4)	25 (19.8)
Hypotonia	3 (2.4)	15 (11.9)
Retinopathy	3 (1.8)	13 (10.3)
Myoclonus	3 (2.4)	8 (6.3)
Hypothyroidism	2 (1.6)	5 (4.0)
Psychiatric involvement	1 (0.8)	8 (6.3)
Optic neuropathy	1 (0.8)	6 (4.8)
Hypogonadism	1 (0.8)	5 (4.0)
Pyramidal signs	–	14 (11.1)
Respiratory impairment	–	6 (4.8)
Status epilepticus	–	5 (4.0)

Journal of Neurology 2013 (261):504-510

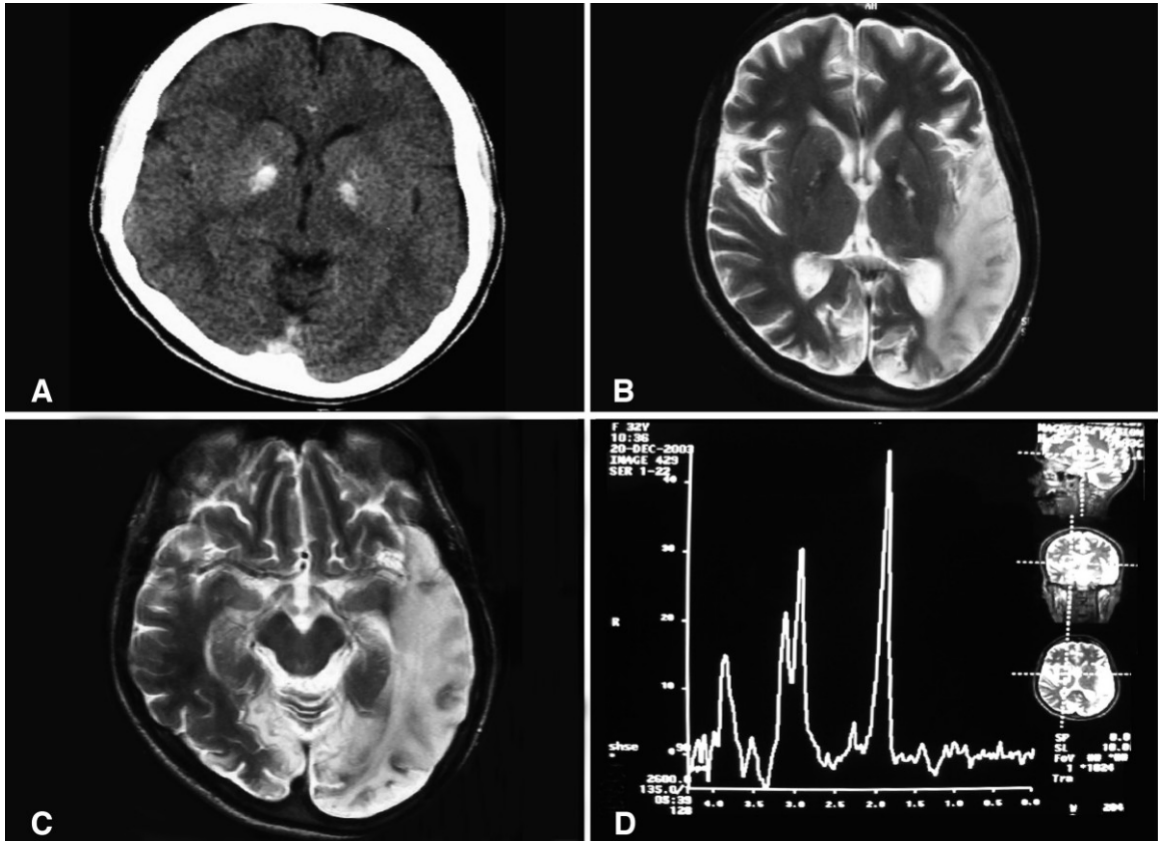
The burden of MELAS: Chronic

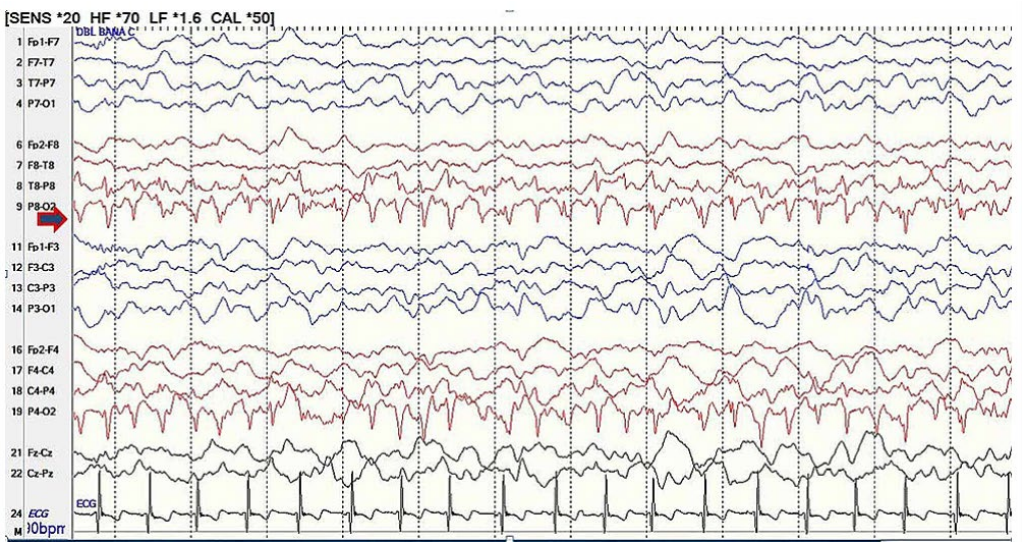


On average, a mito patient has 16 different symptoms

Zolkipli-Cunningham Z et al. PLoS One. 2018 May

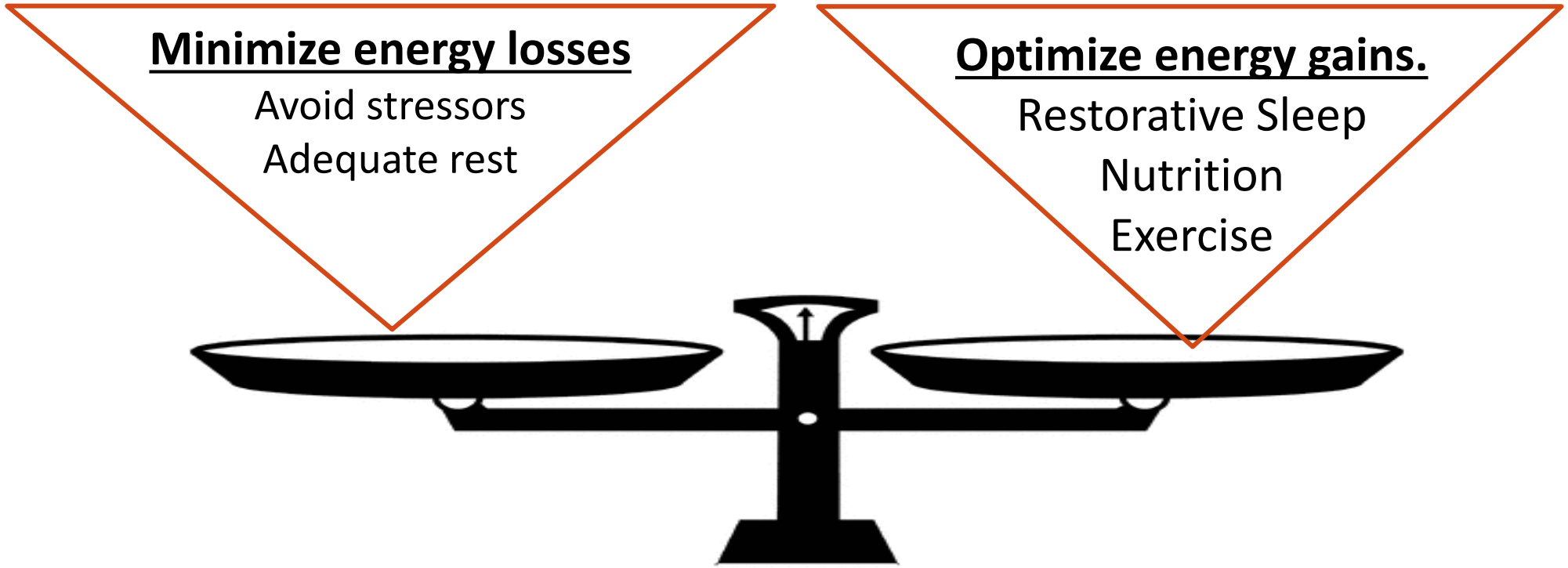
The burden of MELAS: Acute





Journal of Medical Case Reports 2009 3:77
Cureus 13(1): e12908. doi:10.7759/cureus.12908

MELAS: Current treatment options



MELAS: Current treatment options

Symptomatic Care

- Dietary Supplements (The Mito Cocktail)
- L-arginine (PO and IV)
- L- Citrulline, Taurine
- Organ specific management

Supportive Care

- Early Intervention
- Social worker, case manager
- Support Groups, etc.
- Palliative Care Team

Conclusion

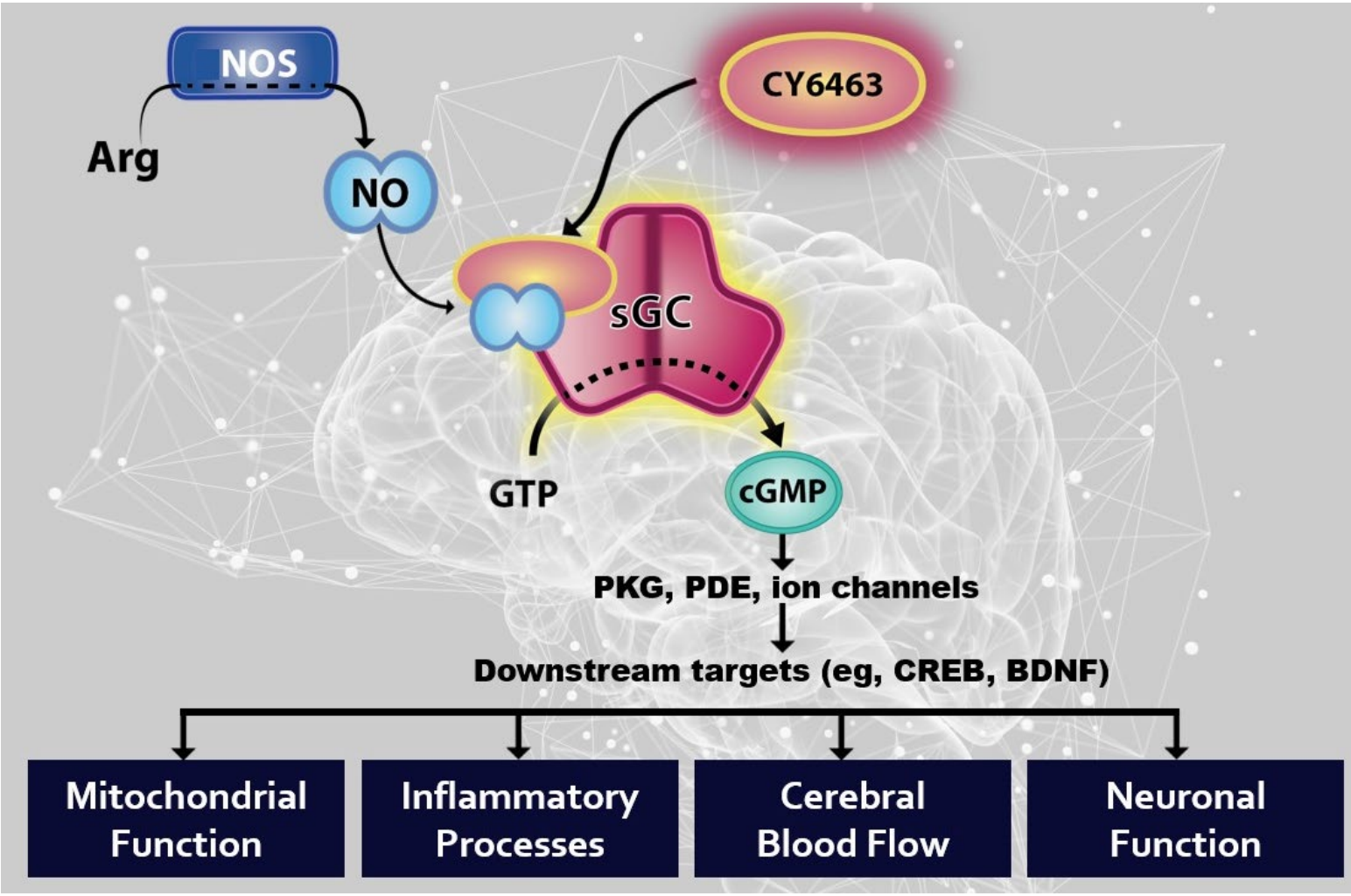
- Heterogeneous disease
- Affects many members of the same family
- High morbidity and poor prognosis
- No specific treatment available

→ **Major unmet needs**

CY6463 IN MELAS

Chris Winrow, Ph.D.
Vice President, Translational Medicine & Development
Program Lead, Cyclerion

Strong therapeutic rationale for stimulating NO-sGC-cGMP pathway to treat mitochondrial disease



- CY6463, a brain penetrant sGC PAM, amplifies endogenous NO signaling
- NO deficiency in mitochondrial disease linked to multiple disease domains
- Use of NO precursors (arginine or citrulline) recommended by Mitochondrial Medicine Society
- Preclinical data demonstrate effects across multiple aspects of disease pathophysiology

Correia et al., 2021 Front. Pharmacol.; Almannai and El-Hattab 2021 Front. Mol. Neurosci.; Parikh et al., 2017 Genetics in Med.; Nisoli et al., 2003 Science; Garthwaite et al., 2018 Brain Neurosci Advances; Hollas et al., 2019 Nitric Oxide

CY6463 CLINICAL STUDY DESIGN

Chad Glasser, Pharm. D.
Director, Clinical Research, Cyclerion

Open-label, 29-day study of CY6463 in MELAS patients to assess safety, PK, PD and impact on important domains of mitochondrial disease

Study population N=8	Genetically confirmed with history of CNS symptoms such as strokes, seizure, headaches Stable medications including NO precursors (e.g., arginine and citrulline) permitted
Safety	Safety and tolerability profile with 15-mg QD dosing Safety on top of NO precursors and other stable medications
PK	Plasma and, when available, cerebrospinal fluid (CSF) concentrations of CY6463
CNS/PD	**Objective measures of key domains of mitochondrial disease** • Mitochondrial function • Inflammatory processes • Cerebral blood flow • Neuronal function **Patient-reported outcomes (PROs)** • Patient's Global Impression of Change (PGIC) • PROMIS Cognitive Function battery • Modified Fatigue Impact Scale (MFIS)

Enrolled broad range of genetically confirmed patients with history of CNS symptoms consistent with MELAS

Baseline characteristics (N=8)		
Demographics and medical history	**Age**	19 to 54 years
	Sex	5 women and 3 men
	Disease symptoms	All had history of 1 or more CNS symptoms (e.g., stroke-like episodes, seizures, headaches), Fatigue, exercise intolerance
	Concomitant therapy	6 of 8 were on stable doses of NO precursors (arginine, citrulline)
Biomarkers	**Mitochondrial Function**	
	Plasma lactate	1.7-5.6 mmol/L (normal range: <2mmol/L)
	GDF-15	0.49-4.1 ng/mL (normal range: 0.14-0.46 ng/mL)
	FGF-21	0.055-0.72 ng/mL (normal range: <0.44 ng/mL)
	Inflammation	6-19 elevated of 40 evaluated
Patient-reported assessments	**MFIS (fatigue, 0-84)**	3-66
	PROMIS (cognition, 160-0)	148-77

MFIS, Modified Fatigue Impact Scale; PROMIS, Patient-reported Outcomes Measurement Information System Item Bank v2.0-Cognitive Function

CY6463 CLINICAL DATA SUMMARY

Chad Glasser, Pharm. D.
Director, Clinical Research, Cyclerion

Strong safety/tolerability and once-daily profile demonstrated in participants with MELAS

CY6463 well tolerated with and without NO precursors (L-arginine and L-citrulline)

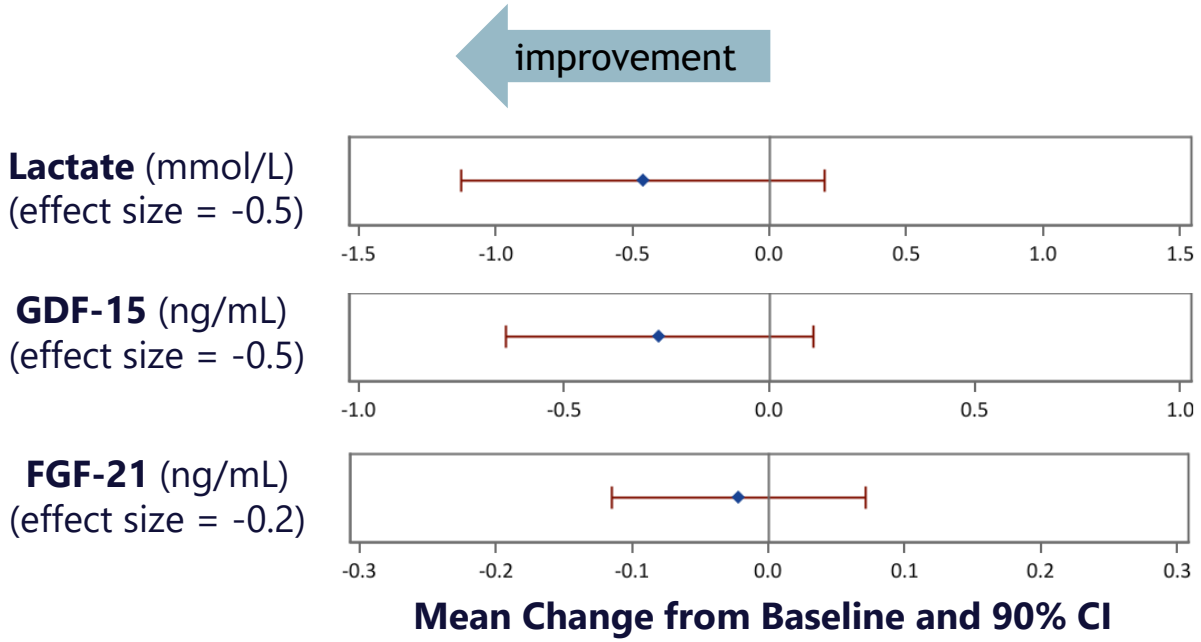
- Mostly mild adverse events (AEs), no severe adverse events (SAEs)
- No SAEs, no discontinuations due to AEs
- Most common AE was headache, all but 1 mild
- No signals on clinical labs, vital signs, ECGs, or suicidal rating scale

Once-daily dosing with consistent pharmacokinetics

- Pharmacokinetics (AUCtau, Cmax, and Ctrough) in MELAS participants consistent with PK studies in healthy volunteers
- Confirmed CNS exposure with CSF:plasma ratio consistent with that observed in healthy volunteers

ECG, electrocardiography; CSF, cerebral spinal fluid; AUC_{tau}, area under the concentration-time curve during a dosing interval; C_{max}, maximum observed concentration; C_{trough}, pre-dose concentration

CY6463 improved biomarkers of mitochondrial function that are elevated in MELAS



Mean Change from Baseline and 90% CI

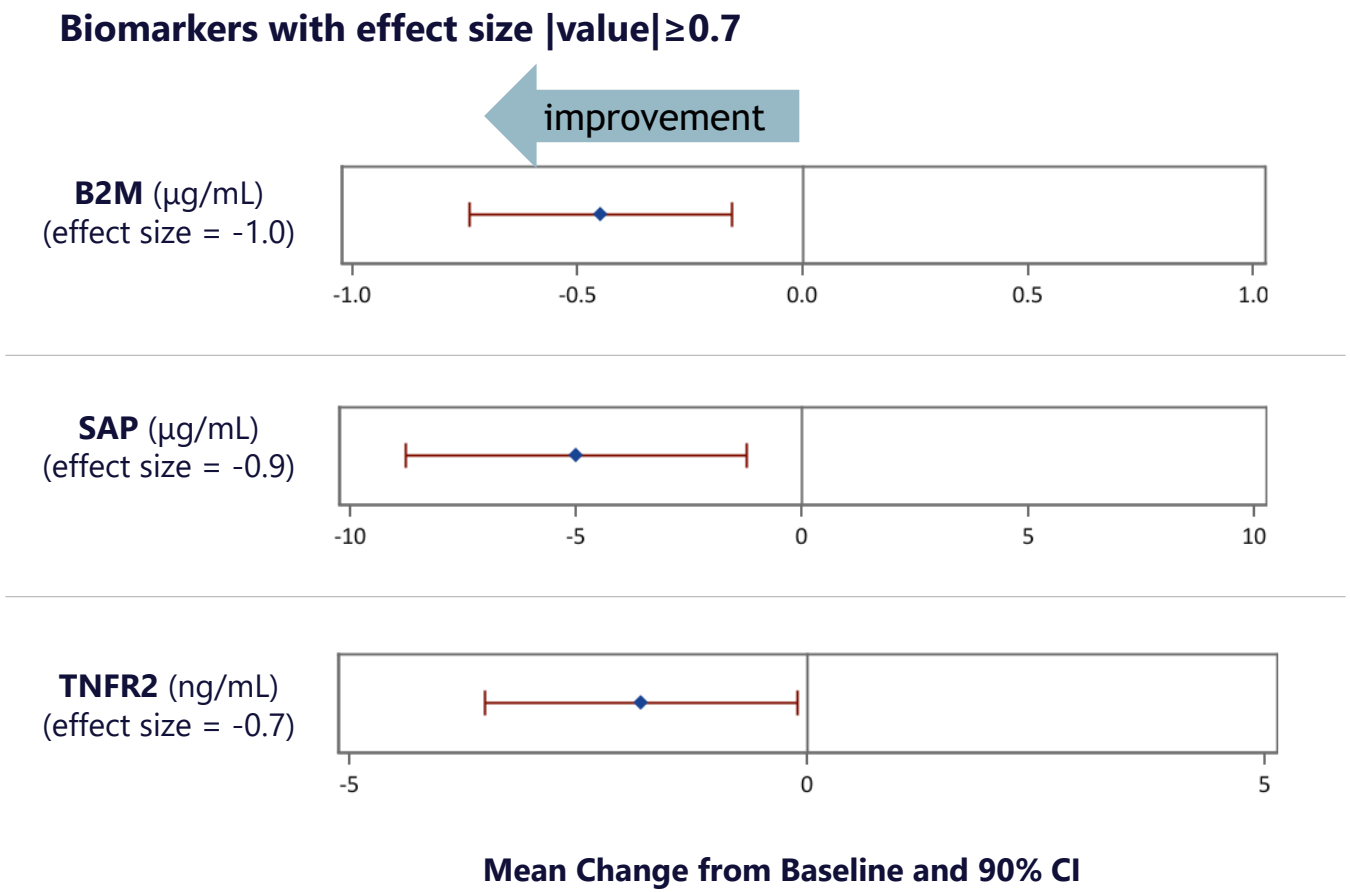
- Blood biomarkers linked to mitochondrial dysfunction were elevated at baseline across participants (mean)
- Improvement after 29-day dosing was correlated with CY6463 plasma concentration

Correlations between changes in biomarkers and CY6463 plasma concentrations on Day 29 (r)

Biomarker parameters	Fibroblast growth factor 21	Growth differentiation factor 15	Lactate	Trough CY6463 plasma concentration
Fibroblast growth factor 21	1.00			
Growth differentiation factor 15	0.86	1.00		
Lactate	0.74	0.87	1.00	
Trough CY6463 plasma concentration	-0.75	-0.68	-0.41	1.00

Darker greens are correlations ≥0.8 (very strong)
Lighter greens are correlations ≥0.6 but <0.8 (strong)

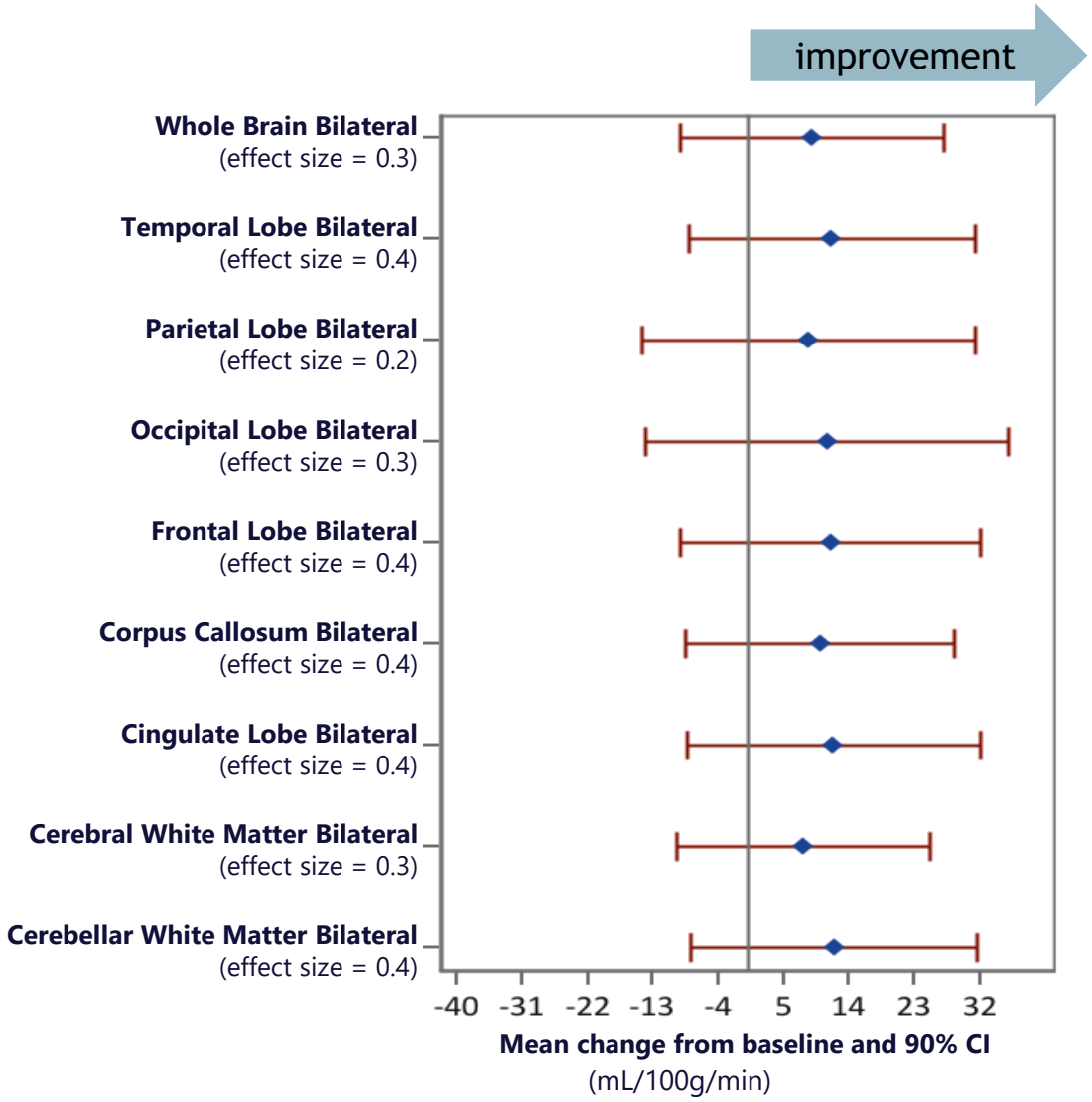
CY6463 improved a broad range of inflammatory biomarkers



- Central and peripheral inflammatory processes are upregulated in patients with mitochondrial dysfunction
- CY6463 had impacts on ~65% of 40 inflammatory biomarkers measured in blood, with effect sizes |value|≥0.3

B2M: Beta-2-Microglobulin; SAP: Serum Amyloid P-Component; TNFR2: Tumor Necrosis Factor Receptor 2

CY6463 increased cerebral blood flow across all regions analyzed



- Vascular and neuronal injury caused by NO deficiency and mitochondrial failure lead to reductions in cerebral blood flow
- Dysregulated cerebral blood flow is linked to stroke-like episodes and CNS symptoms
- Improvements after 29 days of dosing strongly correlated with clinical improvement as assessed by the Patient Global Impression of Change (PGIC) scale (r value of -0.84)

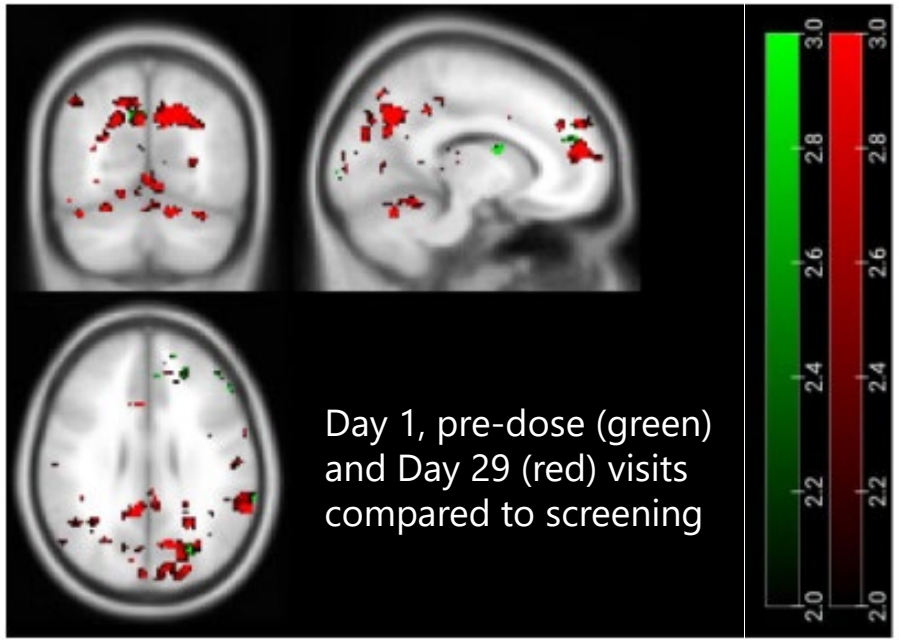
CY6463 increased functional connectivity and visual-evoked BOLD signal, which is reduced in MELAS

Task-based fMRI (visual activation) shows occipital region activation by CY6463

- fMRI BOLD response to visual stimulus is markedly reduced in symptomatic MELAS compared to controls (Rodan et al 2020)
- CY6463 increased activation of occipital brain regions in response to the visual stimulus, with greater activation at Day 29 compared to screening
- Minimal change observed between screening and pre-dose Day 1

Enhanced connectivity also observed at rest in networks involved in executive function and sensorimotor processing

Visual-evoked fMRI-BOLD signal by visit



Whole-brain voxelwise statistical parametric maps (SPM) of task-based fMRI visual activation at day 1, pre-dose (green) and day 29 (red) visits compared to screening.
Maps thresholded a t = 2.0 for exploratory visualization.

Additional analyses of imaging data ongoing
n=6 (fMRI data collected at one site were not analyzable)

Patient level data shows promising changes across key domains of mitochondrial disease after 29-day dosing with CY6463

	Endpoint domain	Participant→	A	B*	C	D	E	F	G*	H
Objective endpoints	Mitochondrial Function	Lactate	29%	-18%	-31%	22%	-46%	-22%	-17%	-7%
		GDF-15	0	0	14%	0	-39%	-18%	-16%	-15%
		FGF-21	0	68%	-22%	-8%	-46%	15%	-7%	-16%
	Inflammation	Overall panel	9 ↓ 24 ↑	8 ↓ 25 ↑	17 ↓ 17 ↑	27 ↓ 12 ↑	35 ↓ 4 ↑	29 ↓ 9 ↑	26 ↓ 13 ↑	26 ↓ 10 ↑
		B2M	5%	0	-15%	-33%	-16%	-20%	-22%	-23%
		SAP	0	17%	-19%	-33%	-44%	-10%	-6%	-30%
		TNFR2	9%	-3%	18%	-44%	-48%	-31%	-26%	-6%
	Cerebral blood flow		-10%^	-55%^	19%	42%	-4%	60%	36%	52%

GDF=growth differentiation factor; FGF=fibroblast growth factor; B2M=beta-2 microglobulin; SAP=serum amyloid P component; TNFR2=tumor necrosis factor receptor 2; CBF=cerebral blood flow; ; MFIS=Modified Fatigue Impact Scale; PROMIS=Patient-Reported Outcomes Measurement Information System (Cognitive Function Item Bank v2)

* Denotes 2 subjects not taking concomitant NO precursor;

^ Denotes value collected using 3DPCASL sequence and GE scanner; all other values collected using Siemens scanners and 2DPASL sequence

Improvement No change Decline

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		TNFR2	9%	-3%	18%	-44%	-48%	-31%	-26%	-6%
	Cerebral blood flow		-10%^	-55%^	19%	42%	-4%	60%	36%	52%
Subjective endpoints	Patient-reported outcomes	Perceived change	No Change	Much Worse	No Change	Minimally Improved	No Change	Much Improved	No Change	Very Much Improved
		MFIS (↓ =good)	Cognitive -5 Physical -1 Psychosocial -5 Total -11	Cognitive 24 Physical 5 Psychosocial 3 Total 32	Cognitive 2 Physical 11 Psychosocial 2 Total 15	Cognitive -4 Physical -11 Psychosocial -1 Total -16	Cognitive -13 Physical 1 Psychosocial-4 Total -16	Cognitive -1 Physical -3 Psychosocial -1 Total -5	Cognitive 7 Physical 3 Psychosocial 3 Total 13	Cognitive 5 Physical 6 Psychosocial 4 Total 15
		PROMIS (↑=good)	10%	-47%	-11%	9%	22%	-7%	-2%	-18%

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Improvement | No change | Decline

IMPLICATIONS FOR CY6463 IN MELAS

Amel Karaa, M.D.

Director, The Mito Clinic, Harvard Medical School &
Massachusetts General Hospital

PERSPECTIVE ON CY6463

Chris Winrow, Ph.D.
Vice President, Translational Medicine &
Development Program Lead, Cyclerion

Peter Hecht, Ph.D.
Chief Executive Officer, Cyclerion

MELAS Next steps

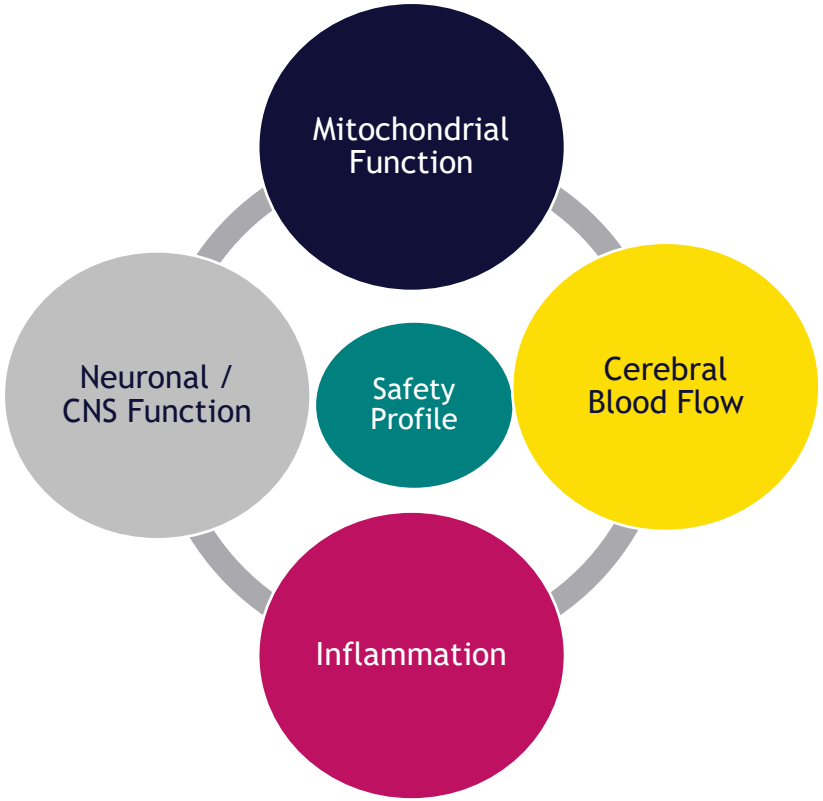
Now	2H 2022	2023
Engaging expert advisors and refining development plan	**FDA meeting to align on path to approval**	**Initiate registrational study***

* Pending alignment with FDA

Current development plan assumptions – subject to change!

- Preparing to meet with FDA to explore an expedited path to registration in MELAS
- Planning to seek Orphan Drug Designation
- Current thoughts on study design:
  - Treatment duration 3-6 months + open-label extension
  - Endpoints will include measures of cognition, fatigue and biomarkers associated with mitochondrial disease
- Sites to include global centers of excellence in mitochondrial disease

CY6463 has potential as breakthrough therapeutic for MELAS



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Q&A

Moderated by
Cheryl Gault,
Chief Operating Officer, Cyclerion