CY6463, a CNS-penetrant sGC stimulator, elicits benefits in preclinical models of mitochondrial complex 1 deficiency

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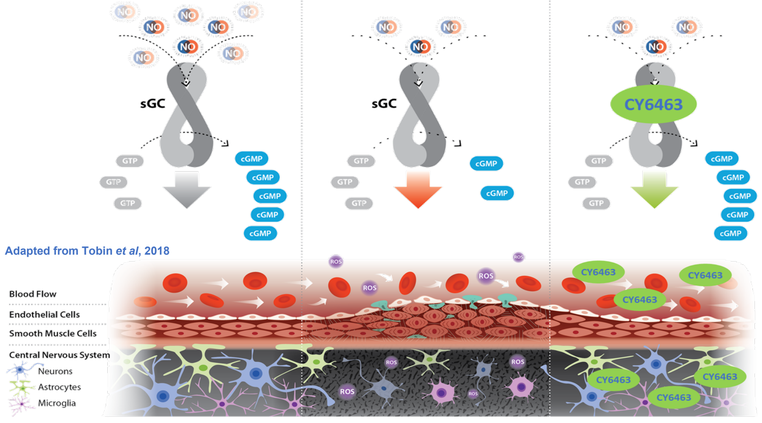
Introduction

- Nitric oxide (NO) is a gasotransmitter that stimulates soluble guanylate cyclase (sGC) to produce cyclic guanosine 3',5'-monophosphate (cGMP).
- Impaired NO-sGC-cGMP signaling has been implicated in the pathogenesis of mitochondrial diseases, including stroke-like episodes (SLEs), and dysregulated cerebral blood flow.
- Modulation of the NO-sGC-cGMP pathway has been reported to increase mitochondrial biogenesis and function and improve neuronal function and cognition, all of which are impacted in patients with mitochondrial disease.
- The consensus guidelines from the Mitochondrial Medicine Society recommend acute arginine administration to improve clinical symptoms associated with SLEs in patients with Mitochondrial Encephalomyopathy with Lactic Acidosis and Stroke-like episodes (MELAS).
- CY6463 is a CNS-penetrant sGC stimulator currently in clinical development.
- We studied CY6463 in cells from patients with Leber hereditary optic neuropathy (LHON) or Leigh syndrome.
- In addition, we explored the effect of CY6463 on astrogliosis in a mouse model of mitochondrial complex-1 deficiency.



Methods

Effect of CY6463 on ATP levels in mitochondrial disease patient cells

- Lymphoblast cells from healthy subjects, (GM00333), Leigh patients (GM13740), and LHON patients (GM11605 and GM10742) were purchased from the Coriell Institute and cultured according to vendor's recommendations.
- ATP levels in the cells were measured using ATPlite kit (PerkinElmer Cat #6016943) according to manufacturer's protocol.
- The effects of CY6463 were analyzed by one-way analysis of variance (ANOVA), followed by uncorrected Fisher's LSD vs the vehicle condition *: p<0.05; **: p<0.01; ***: p<0.001; ****: p<0.0001.
- Note: Healthy, Leigh, and LHON patient cells that do not have a basal ATP deficit did not increase ATP in response to CY6463.

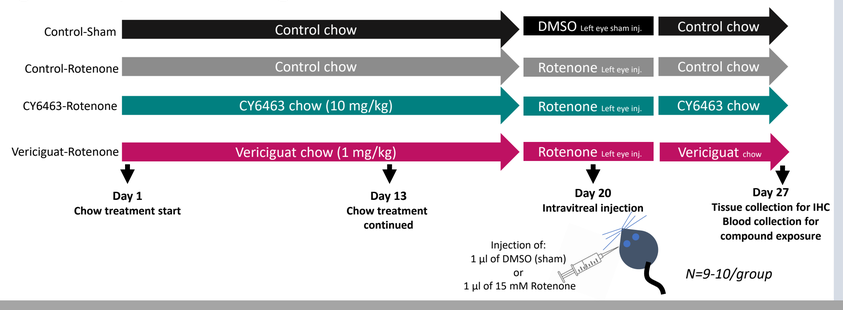
Assessment of CY6463 on gene expression in mitochondrial disease patient cells

- Healthy lymphoblast cells (GM 00333), LHON patient lymphoblasts (GM11605) and Leigh patient lymphoblasts (GM13740) were studied. Raw sequence data was assessed for quality and ribosomal content. Alignments to the human genome were performed using STAR and only unique alignments to the genome were retained. Differentially expressed genes (DEGs) between conditions and subsequent pathway analysis were conducted using DESeq2 package with adjusted p-values < 0.05.

Effect of CY6463 on astrogliosis in a mouse model of mitochondrial complex-1 deficiency

- Mice received an IVT injection of either rotenone or DMSO into the left eye (Figure 2).
- Seven days after the IVT injection, both left and right eyes were collected, fixed, sectioned, and stained for glial fibrillary acidic protein to determine retinal astrogliosis and for 4′,6-diamidino-2-phenylindole (DAPI) to visualize cellular nuclei.
- Images were analyzed using the Halo software to quantify the percent positive staining within regions of interest in the outer nuclear layer area of the retina or to measure retinal thickness.

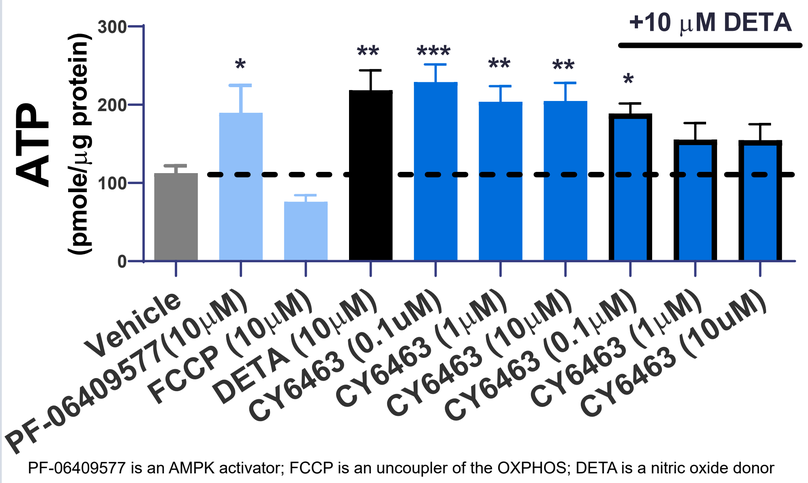
Figure 2: Experimental design



CY6463 increased the ATP levels in Leigh lymphoblasts with a baseline ATP deficit

Leigh patient lymphoblasts were incubated in different conditions → 24h → ATP and protein quantification

Leigh cells 1
GM13740 ATP6 8893T>G

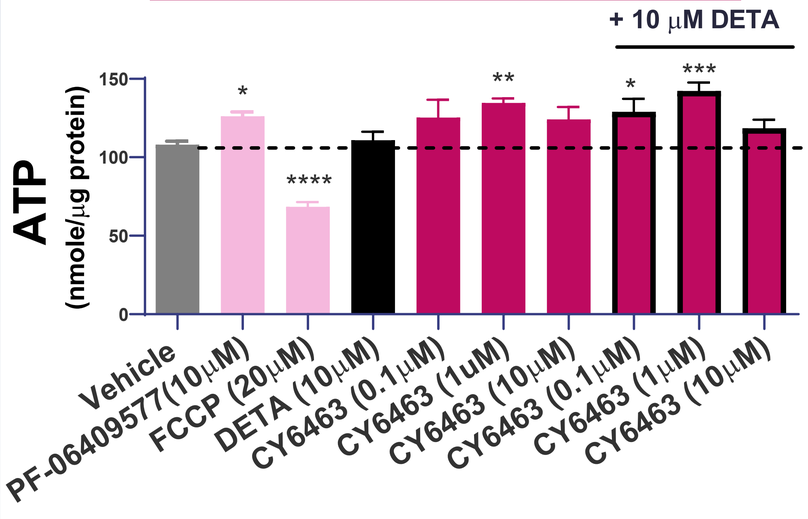


PF-06409577 is an AMPK activator; FCCP is an uncoupler of the OXPHOS; DETA is a nitric oxide donor

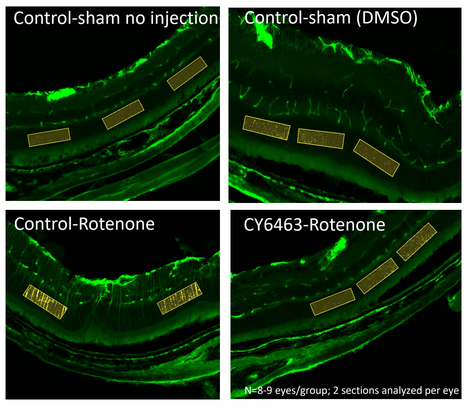
CY6463 increased the ATP levels in LHON lymphoblasts with a baseline ATP deficit

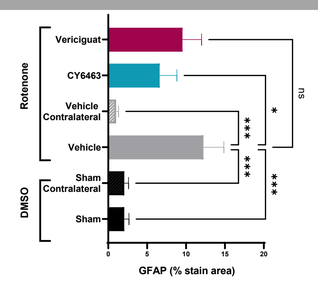
LHON patient lymphoblasts were incubated in different conditions → 24h → ATP and protein quantification

LHON cells 1
GM11605 MTND1 3460G>A



CY6463 prevented increase in GFAP staining induced by complex-1 disruption

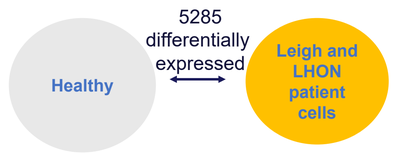




The effects of CY6463 were analyzed by one-way analysis of variance (ANOVA), followed by uncorrected Fisher's LSD vs the vehicle-rotenone group. The symbols for statistical significance are *: p<0.05; ***: p <0.001.

CY6463 increased the expression of down-regulated mito genes in patient cells

Healthy, Leigh, and LHON patient lymphoblasts were incubated in different conditions → 24h → Analysis of gene expression by RNA sequence



2436 genes are down-regulated

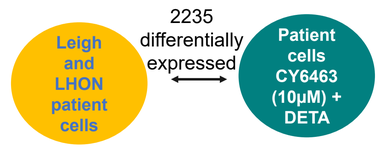
2849 genes are up-regulated

Down-regulated genes

Cellular component	FDR
mitochondrion	5.25E-33
mitochondrial part	2.228E-24
mitochondrial envelope	3.836E-20
mitochondrial membrane	1.36E-17
organelle envelope	8.084E-15

Up-regulated genes

Cellular component	FDR
Golgi apparatus	6.986E-18
endoplasmic reticulum part	6.986E-18
endoplasmic reticulum membrane	2.031E-15
nuclear outer membrane-endoplasmic reticulum membrane network	3.213E-15



999 genes are down-regulated

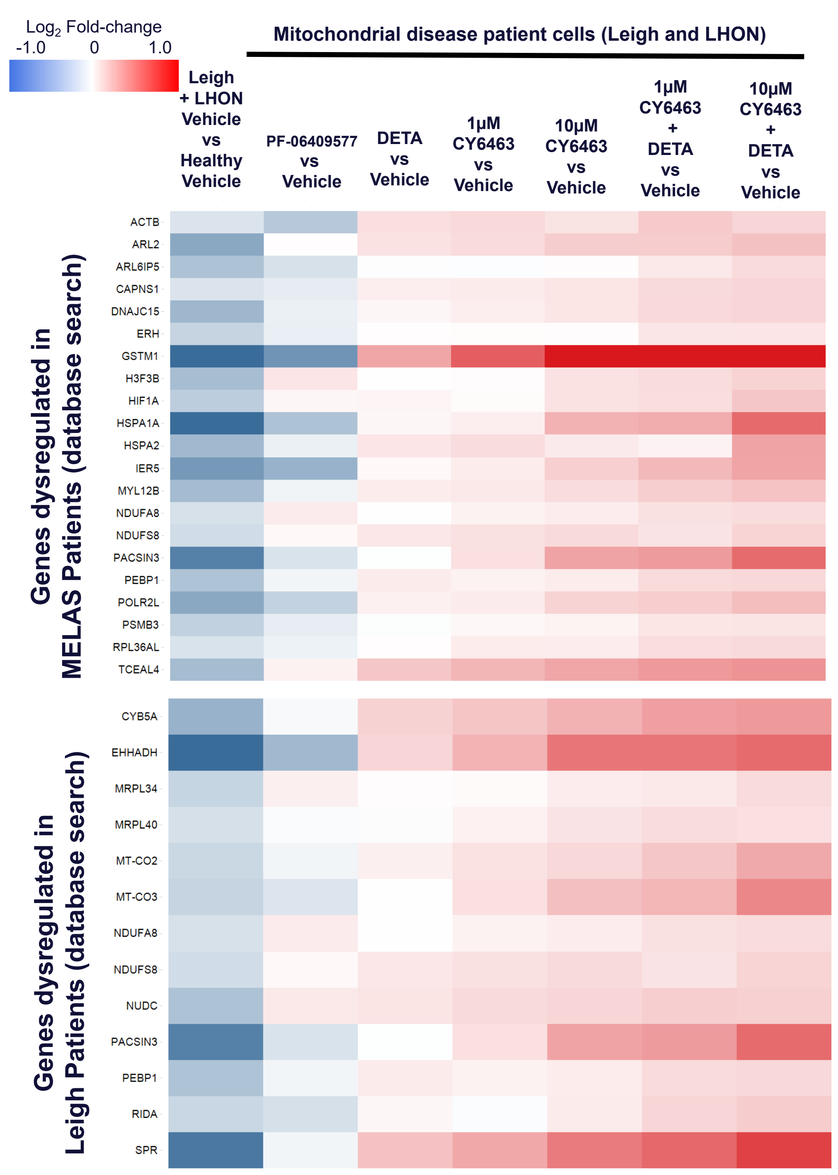
1236 genes are up-regulated

Up-regulated genes after CY6463 + DETA treatment

Cellular component	FDR
mitochondrion	5.824E-9
organelle envelope	1.051E-8
envelope	1.051E-8
mitochondrial envelope	1.070E-8
mitochondrial part	6.796E-8

Genes down regulated in MELAS and Leigh patients were upregulated by CY6463 treatment in Leigh and LHON cells

Healthy, Leigh and LHON patient lymphoblasts were incubated in different conditions → 24h → Analysis of gene expression by RNA sequence → Analyze a subset of genes known to be dysregulated in MELAS and Leigh syndrome



- **Expression of mitochondrial genes associated with MELAS (top) and Leigh syndrome (bottom) is suppressed in mitochondrial disease patients' cells (Leigh and LHON).**
- **CY6463 restored the expression of these genes in a dose-dependent manner.**
- **PF-06409577 exhibited minimal effects.**

Conclusions

- **CY6463 increased ATP in mitochondrial disease patient cells (Leigh and LHON lymphoblasts) with an ATP deficit, indicating that CY6463 may improve cellular energetics.**
- **Expression levels of mitochondrial genes were lower in Leigh and LHON lymphoblasts than in healthy cells.**
- **Treatment with CY6463 restored expression of these affected genes, including a subset of genes that are associated with MELAS and Leigh syndrome, suggesting that CY6463 may alleviate mitochondrial dysfunction in cells from patients with mitochondrial disease.**
- **In an in vivo model of mitochondrial complex-1 inhibition, protein staining of glial fibrillary acidic protein (GFAP), a marker of astrogliosis that results from tissue damage and inflammation, was higher in the retina of mice challenged with a single intravitreal (IVT) injection of the mitochondrial complex-1 inhibitor rotenone than in vehicle-treated mice. Retinal astrogliosis was less pronounced in mice treated with CY6463 for 3 weeks prior to rotenone IVT injection than in vehicle-treated mice.**

References: Tobin JV, et al (2018). Pharmacological Characterization of IW-1973, a Novel Soluble Guanylate Cyclase Stimulator with Extensive Tissue Distribution, Antihypertensive, Anti-Inflammatory, and Antifibrotic Effects in Preclinical Models of Diseases. J Pharmacol Exp Ther. 2018 Jun;365(3):664-675; PMID: 29643251

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