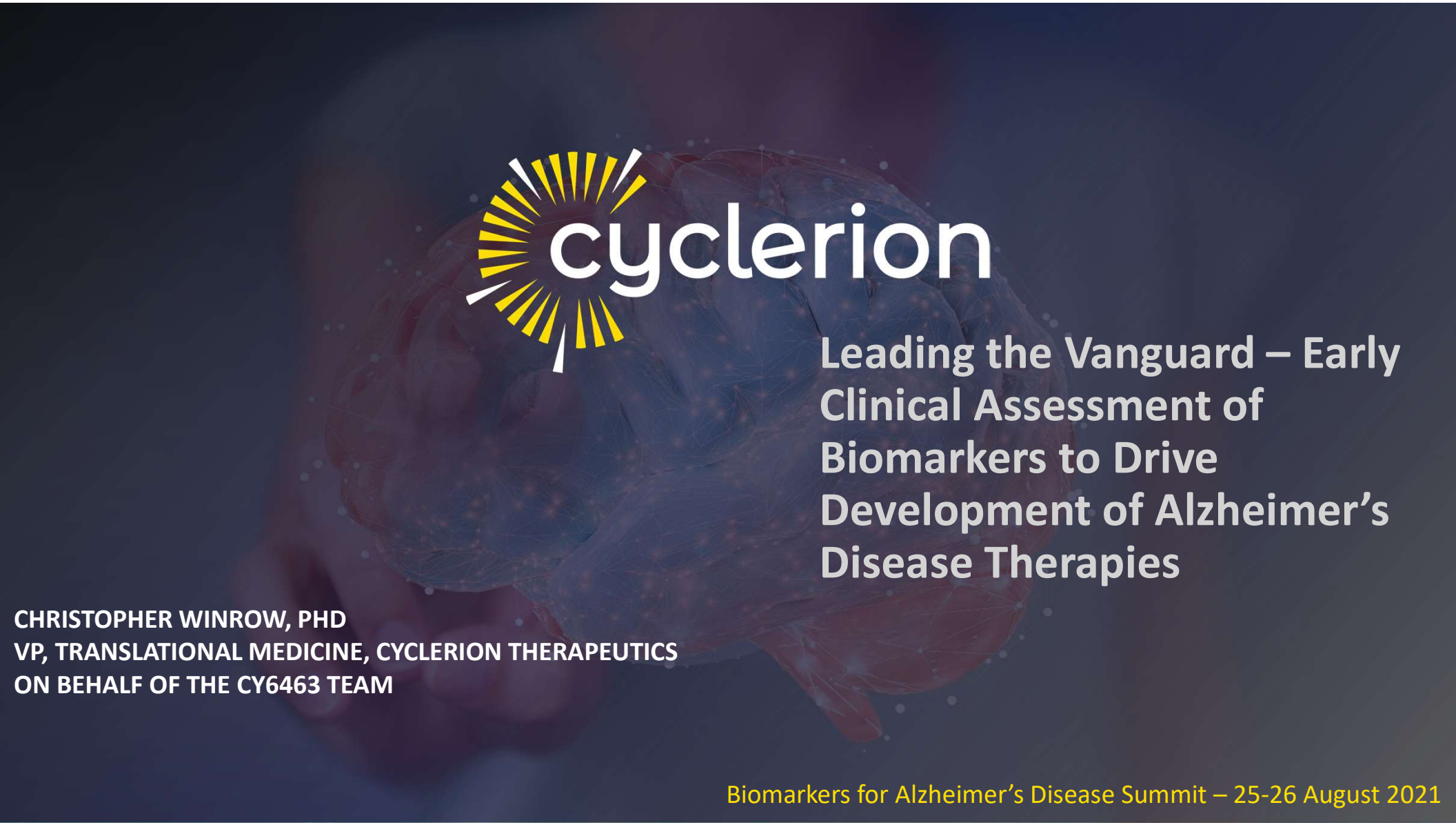


Leading the Vanguard – Early Clinical Assessment of Biomarkers to Drive Development of Alzheimer's Disease Therapies

CHRISTOPHER WINROW, PHD
VP, TRANSLATIONAL MEDICINE, CYCLERION THERAPEUTICS
ON BEHALF OF THE CY6463 TEAM

Biomarkers for Alzheimer's Disease Summit – 25-26 August 2021

Safe harbor statement

This document contains forward-looking statements within the meaning of Section 27A of the Securities Act of 1933, as amended, and Section 21E of the Securities Exchange Act of 1934, as amended.

Our forward-looking statements are based on current beliefs and expectations of our management team that involve risks, potential changes in circumstances, assumptions, and uncertainties, including statements about the anticipated timing of release of topline results of our clinical trials; the progression of our discovery programs into clinical development; and the business and operations of the Company. We may, in some cases use terms such as “predicts,” “believes,” “potential,” “continue,” “anticipates,” “estimates,” “expects,” “plans,” “intends,” “may,” “could,” “might,” “likely,” “will,” “should” or other words that convey uncertainty of the future events or outcomes to identify these forward-looking statements.

Each forward-looking statement is subject to risks and uncertainties that could cause actual results to differ materially from those expressed or implied in such statement. Applicable risks and uncertainties include those related to the possibility that any results of operations and financial condition of the Company reported are preliminary and subject to final audit and the risks listed under the heading “Risk Factors” and elsewhere in our 2020 Form 10-K filed on February 25, 2021, and our subsequent SEC filings including the Form 10-Q filed on April 30, 2021. Investors are cautioned not to place undue reliance on these forward-looking statements. These forward-looking statements (except as otherwise noted) speak only as of the date of this report, and the Company undertakes no obligation to update these forward-looking statements, except as required by law.

Early Clinical Assessment of Biomarkers to Drive Development of Alzheimer's Disease Therapies - ~~Se~~ssion Agenda

Time: 2:30 pm
Day: Day Two

Details:

- Building a translational strategy to identify robust preclinical signals
- Interrogating biomarkers by applying a translational pharmacology approach in Phase 1
- Capitalizing on objective assessments to guide clinical development in early patient studies

AD Biomarkers - integrating preclinical data to guide clinical strategies

- Range of opportunities for applying biomarkers in AD studies including:
  - Target engagement
  - Pharmacodynamics
  - Patient selection
  - Disease progression
- Modalities can include plasma, CSF, EEG and imaging biomarkers
- AD may be best addressed through targeting multiple aspects of disease
- Translation - explore relevant preclinical markers that can be evaluated clinically
- Build biomarker profiles in nonclinical studies and early in Phase 1 to inform subsequent clinical strategies in patients

The multi-faceted nature of Alzheimer's disease circa 1907

Alois Alzheimer (www.commons.wikimedia.org)

Auguste D. (www.commons.wikimedia.org)

The post-mortem showed an evenly atrophic brain without macroscopic focal degeneration. The larger vascular tissues show arteriosclerotic change.

The glia have developed numerous fibers, moreover, many glial cells show adipose saccules. There is no infiltration of the vessels, however, a growth appears on the endothelia, in some places also a proliferation of vessels.

A range of contributors to the pathogenesis of Alzheimer's disease has emerged including:

-Tau, β-amyloid
-neuroinflammation
-metabolism/bioenergetics
-neuronal/synaptic function
-central clearance mechanisms
-endothelial function
-cerebrovascular regulation

Essential to incorporate this recognition into AD biomarker and therapeutic strategies

Über eine eigenartige Erkrankung der Hirnrinde
(About a peculiar disease of the cerebral cortex)
- Alois Alzheimer, 1907

Stelzmann et al., 1995 Clin. Anatomy; Alzheimer A. 1907 Allgemeine Zeitschrift fur Psychiatrie und Psychisch-gerichtliche Medizin; HP Haack www.commons.wikimedia.org

An example - early implementation of neuroimaging biomarkers

Approaches for identification of neuroimaging biomarker for AD risk

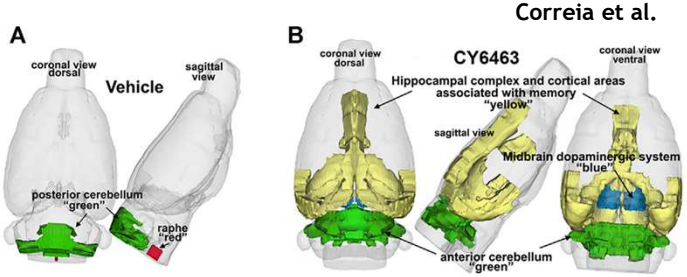
Talwar et al.

Modality	Principle	Use	Clinical utility in AD
MRI (T1 weighted)	Technique that involves a magnetic field and radio waves to create detailed images of the tissues using signals from ^{1}H (proton) nuclei in water and fat over thousands of voxels	Structural visualization of gray matter, white matter and cerebrospinal fluid	Gray matter atrophy beginning in the medial temporal lobe and progressing to the temporal neocortex, parietal cortex and frontal cortex
rs-fMRI	Used to study the brain's functional organization based on the BOLD signal fluctuation	Measures spontaneous fluctuations in the blood oxygen level-dependent (BOLD) signal	Decreased functional hippocampal connectivity to the prefrontal cortex and cingulate cortex
DTI	MRI-based neuroimaging technique that relies on signals from water protons and enables in vivo quantification of differences in molecular diffusion at the cellular level	Used to visualize the location, orientation, and anisotropy of the brain's white matter tracts	Anatomical distribution of white matter microstructural damage in the early stages of AD
PET	Nuclear medicine functional imaging technique that uses radiation and radiotracer	Measure regional brain glucose metabolism; amyloid deposition in the brain	Regional brain glucose hypometabolism; Amyloid detection in AD brain
MRS	Uses spectra in a small number of voxels reflecting small metabolite molecules differentiated by their chemical shifts (δ)	Detects the chemical composition of the scanned tissue	Regional metabolite concentration in AD brain

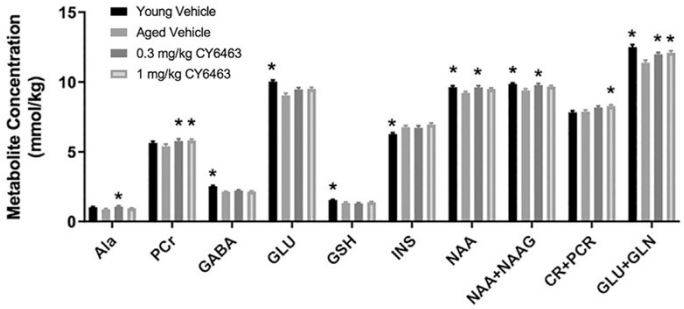
MRI magnetic resonance imaging, *rs-fMRI* resting stage functional magnetic resonance imaging, *DTI* diffusion tensor imaging, *BOLD* blood oxygenation level dependent, *PET* positron emission tomography, *MRS* magnetic resonance spectroscopy, *AD* Alzheimer's disease

fMRI-BOLD (rat)

Correia et al.

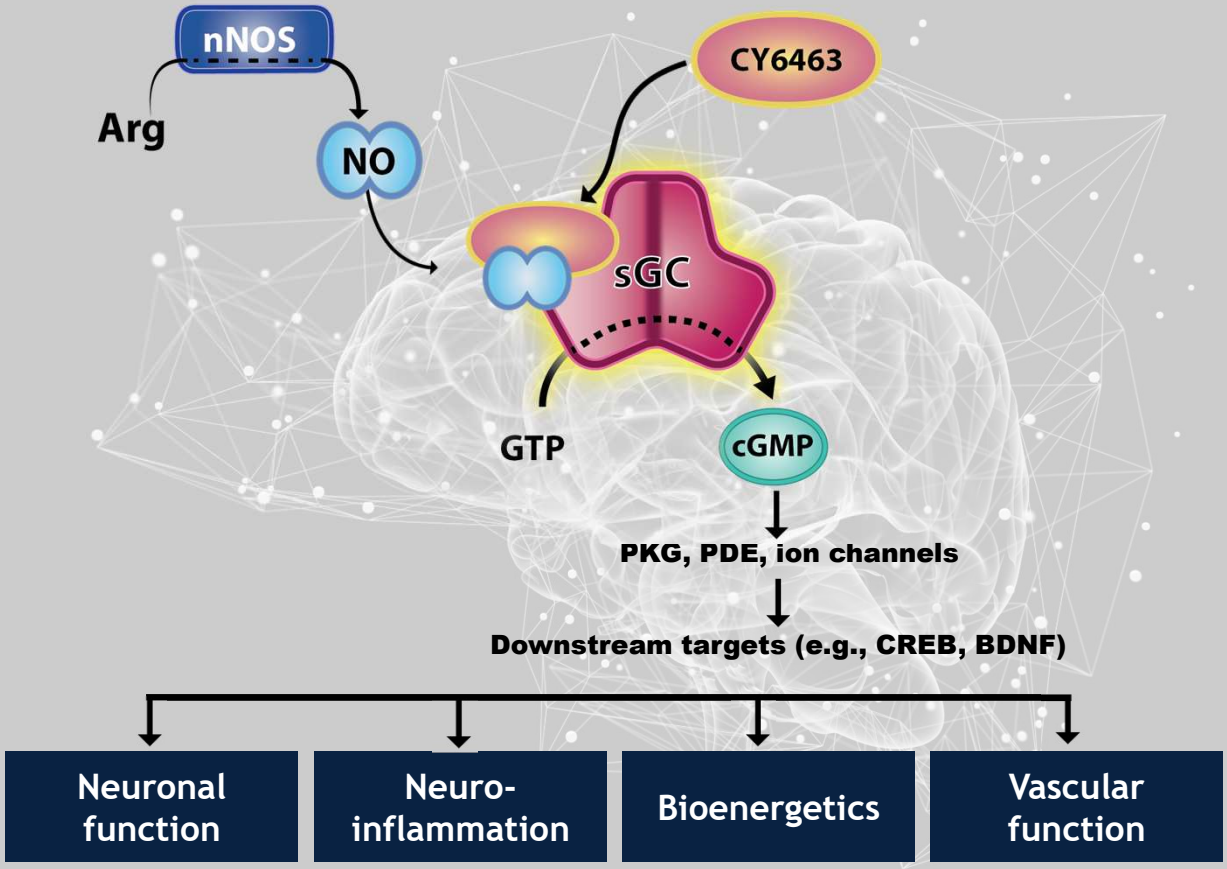


MRS (rat)



Talwar 2021 Clin Neuroradiol. Jul 23. doi: 10.1007/s00062-021-01057-7; Correia et al., 2021 Front. Pharmacol. 2021 May 24;12:656561. doi: 10.3389/fphar.2021.656561

Critical role of the NO-sGC-cGMP signaling pathway in the CNS



CY6463

- First-in-class, CNS-penetrant positive allosteric modulator of sGC
- Amplifies endogenous NO-sGC-cGMP signaling to attenuate central aspects of neurological disease pathophysiology

Preclinical data show multifaceted impact of modulating the NO-sGC-cGMP pathway

CY6463 completed Phase 1 SAD/MAD/FE and Translational Pharmacology studies.

Identified safe and well-tolerated dose levels with steady-state CNS exposure in therapeutic target range with QD PK.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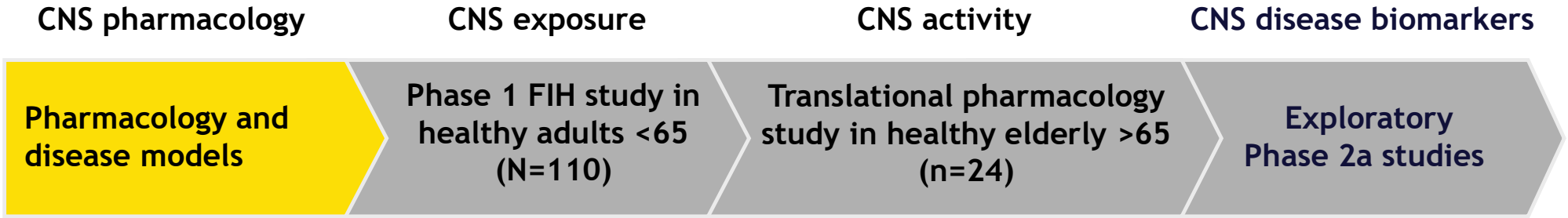
PRECLINICAL
DATA

Biomarkers have been integrated into CY6463 clinical development

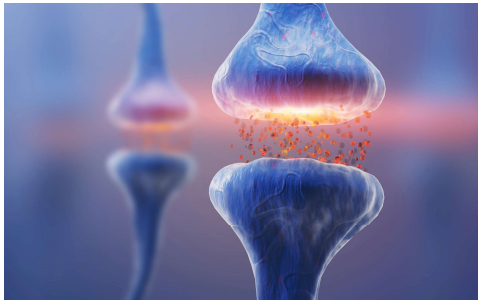


- CNS-exposure
- drug-like properties
- pharmacological profile consistent with known role of pathway in CNS
- biomarker identification

CY6463 showed effects in preclinical studies across multiple neurophysiological domains

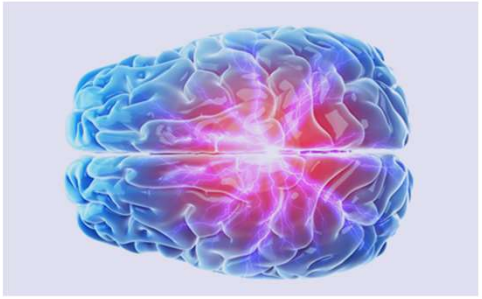
Neuronal Function

Enhanced memory & spine density in aged animals; increased LTP in neurodegenerative models; affected qEEG



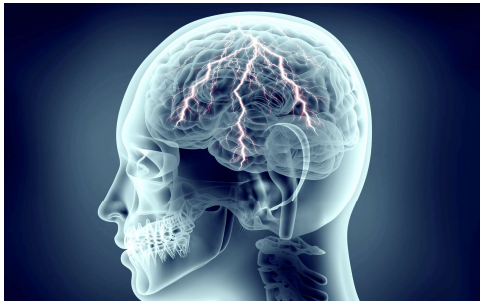
Neuro-inflammation

Decreased markers of LPS-induced neuroinflammation (ICAM1, VCAM1, IL6) *in vitro*



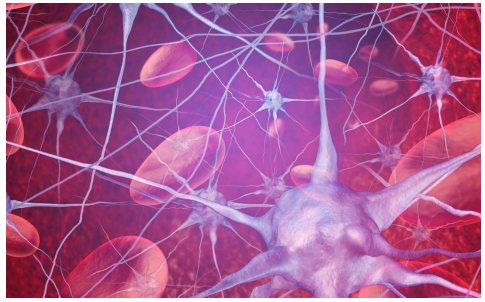
Cellular Bioenergetics

Increased ATP and restored gene expression in cells from patients with mitochondrial diseases



Cerebral Blood Flow

Increased blood flow in areas associated with memory and arousal by fMRI BOLD imaging

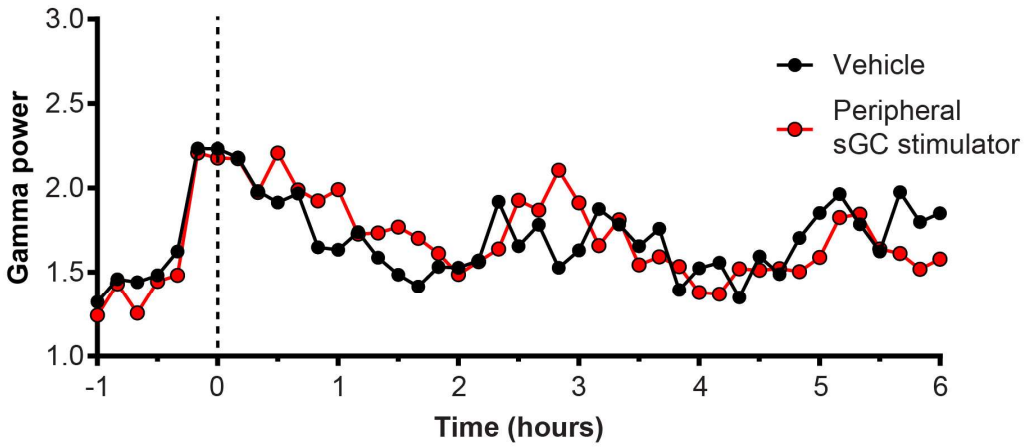


CY6463 increased qEEG gamma power

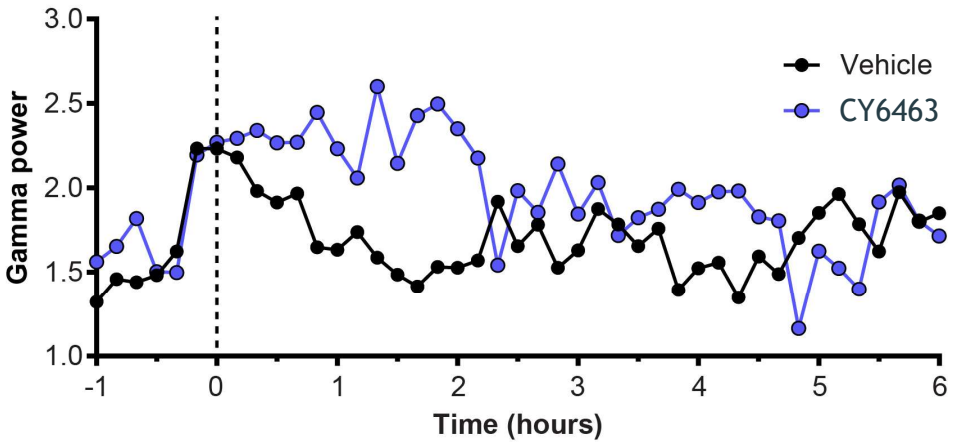
Dose dependence and alignment with pharmacokinetics and exposure

Neuronal Function | Neuroinflammation | Cellular Bioenergetics | Cerebral Blood Flow

Peripherally restricted sGC stimulator



CY6463 CNS penetrant sGC stimulator



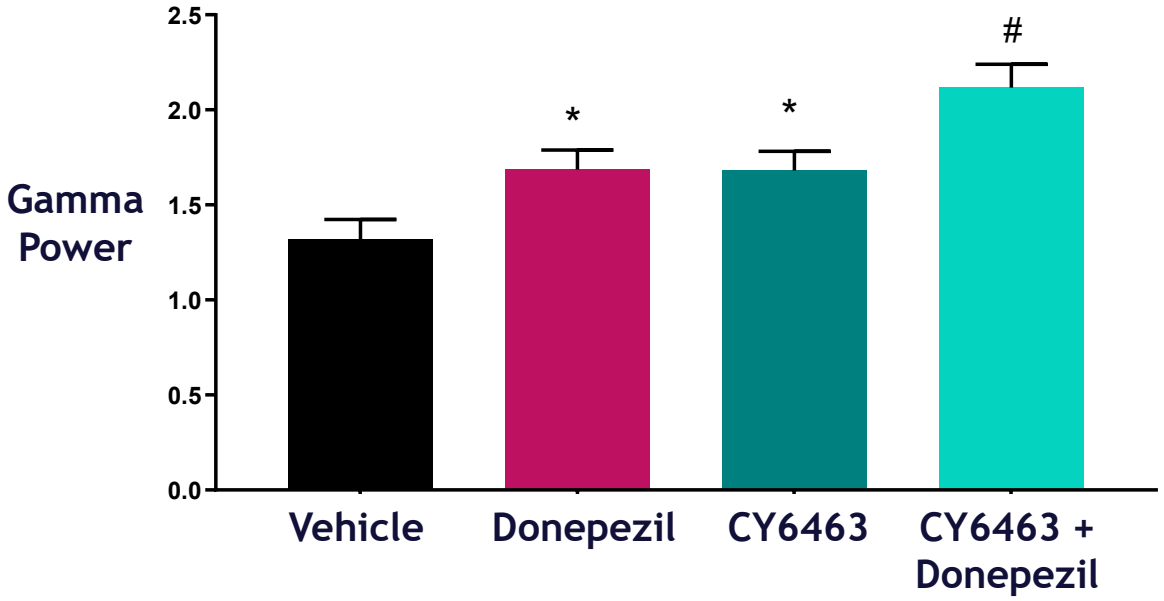
Male rats administered IW-6463 (1 mg/kg) or a peripheral sGC stimulator (3 mg/kg) with telemetry EEG monitoring

Correia et al., 2021 Front. Pharmacol. 2021 May 24;12:656561. doi: 10.3389/fphar.2021.656561

CY6463 and donepezil act independently to enhance qEEG signal

Combination elicited additive increase in gamma band power in healthy rats

Neuronal Function | Neuroinflammation | Cellular Bioenergetics | Cerebral Blood Flow



CY6463 may offer opportunity to enhance attention and cognitive performance alone and on top of standard of care

*p<0.05 vs Veh

# p<0.05 CY6463 vs CY6463 +Donepezil

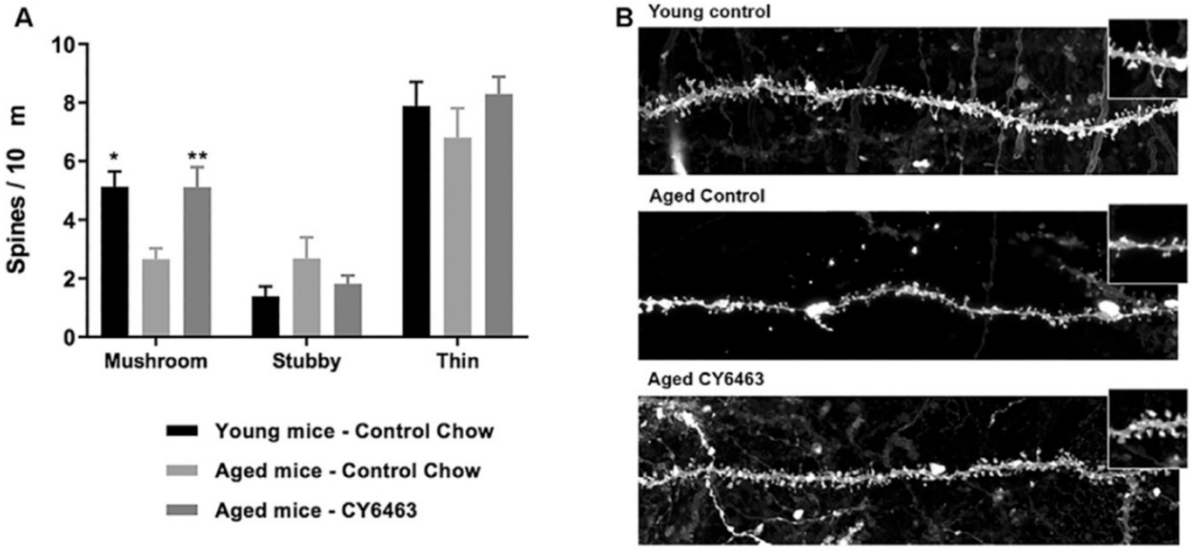
Healthy rats orally administered CY6463 (10mg/kg), Donepezil (1mg/kg), or a combination. Graph displays 1-2h post-dose, mean ± SEM

Correia et al., 2021 Front. Pharmacol. 2021 May 24;12:656561. doi: 10.3389/fphar.2021.656561

CY6463 impacted synaptic morphology in aged animals

Enhanced hippocampal spine density in aged animals treated with CY6463

Neuronal Function | Neuroinflammation | Cellular Bioenergetics | Cerebral Blood Flow



Restoration of spine density has potential to provide neuroprotective effects and improve synaptic function in neurodegenerative diseases

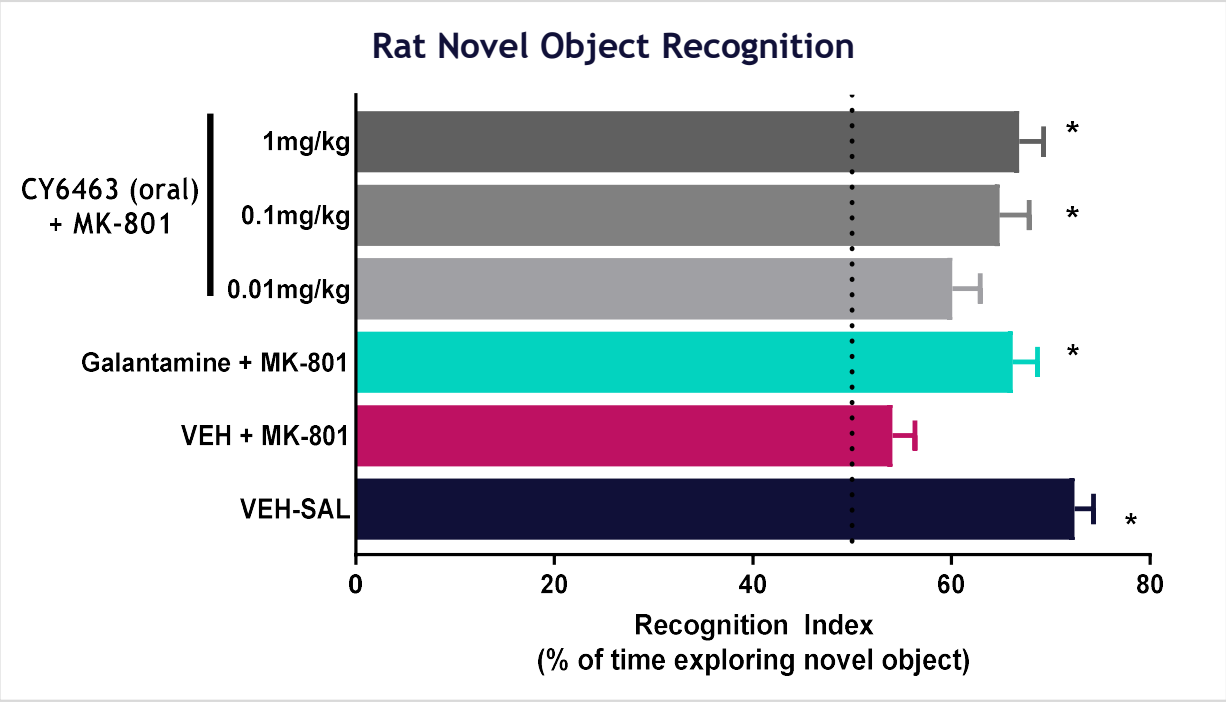
Correia et al., 2021 Front. Pharmacol. 2021 May 24;12:656561. doi: 10.3389/fphar.2021.656561

**p<0.05 vs. Aged*

3-month old (young) or 16-month old (aged) healthy mice at study initiation
Aged mice treated for 4 months with 1 mg/kg CY6463

CY6463 improved cognitive function in pharmacologically impaired rats

Neuronal Function | Neuroinflammation | Cellular Bioenergetics | Cerebral Blood Flow



CY6463 acts downstream of NMDA receptor to reverse deficit induced by NMDA antagonist (MK-801)

*p<0.05 vs. VEH + MK-801 rats

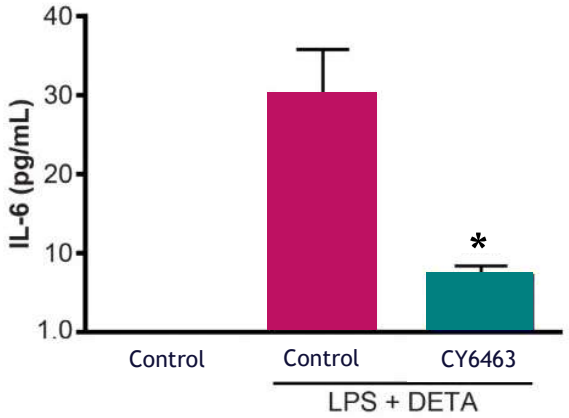
Correia et al., 2021 Front. Pharmacol. 2021 May 24;12:656561. doi: 10.3389/fphar.2021.656561

CY6463 reduced neuroinflammatory markers

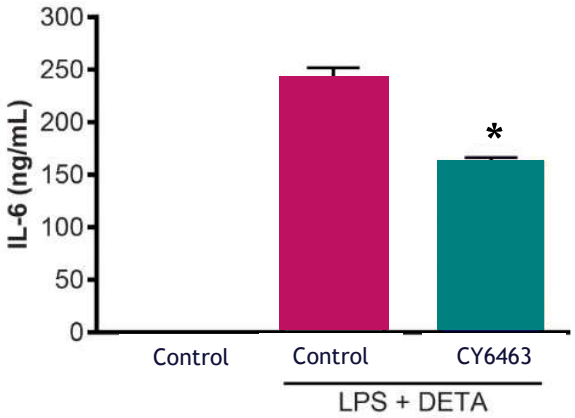
Inhibited in vitro LPS-induction of biomarkers of neuroinflammation

Neuronal Function | **Neuroinflammation** | Cellular Bioenergetics | Cerebral Blood Flow

Neuroinflammation in rat brain 3D microtissues



Neuroinflammation in mouse microglial cells



*p<0.05 vs. control LPS-treated wells

CY6463 (10 µM) and DETA (30 µM) were incubated with SIM-A9 cells or rat brain 3D microtissues for 30 minutes before LPS (100 ng/ml) incubation and further incubated for 18-20h at 37ºC before IL-6 quantification in the media

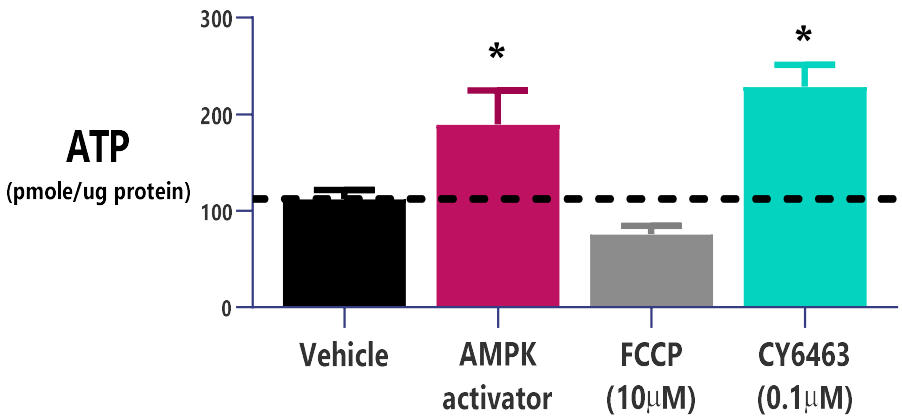
Correia et al., 2021 Front. Pharmacol. 2021 May 24;12:656561. doi: 10.3389/fphar.2021.656561

CY6463 enhanced cellular bioenergetics

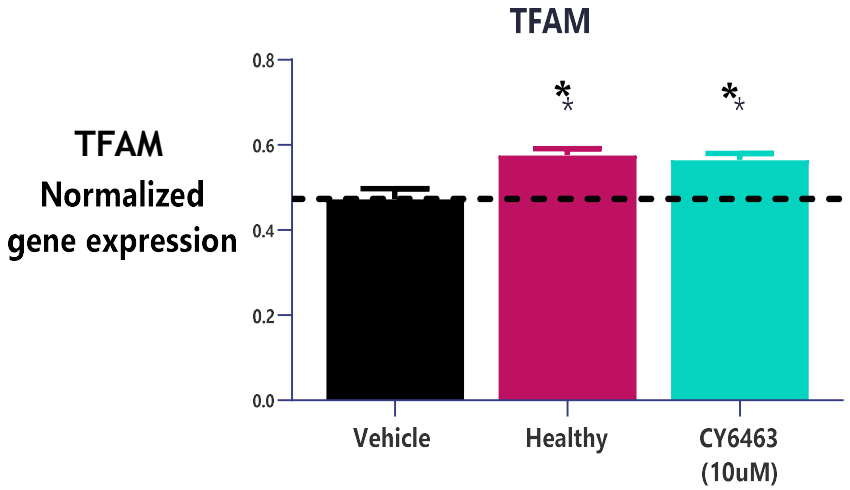
Increased ATP and altered gene expression in cells from patients with mitochondrial diseases

Neuronal Function | Neuroinflammation | **Cellular Bioenergetics** | Cerebral Blood Flow

Mitochondrial disease patient cells



Mitochondrial disease patient cells



*p<0.05 vs. vehicle-treated wells

GM13740 Leigh Syndrome patient cells obtained from the Coriell Institute were treated for 24h before ATP quantification

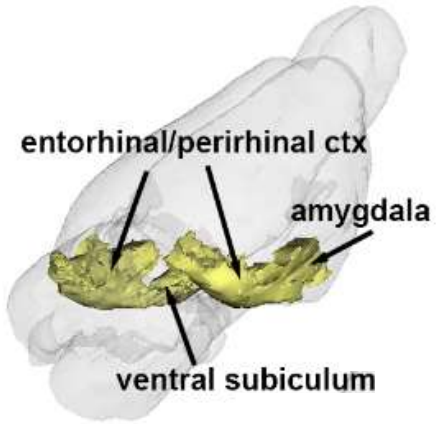
TFAM: mitochondrial transcriptional factor A, a key activator of mitochondrial transcription as well as a participant in mitochondrial genome replication.

CY6463 affected cerebral blood flow

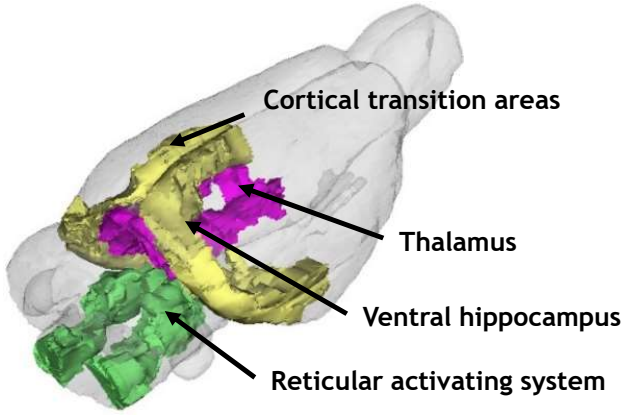
Increased blood flow in areas associated with memory and arousal by fMRI BOLD imaging

Neuronal Function | Neuroinflammation | Cellular Bioenergetics | **Cerebral Blood Flow**

Peripherally restricted sGC stimulator



CNS-penetrant sGC stimulator CY6463



Healthy awake male rats treated with 0.3 mg/kg iv; image quantification 20-30 minutes post-dose

Correia et al., 2021 Front. Pharmacol. 2021 May 24;12:656561. doi: 10.3389/fphar.2021.656561

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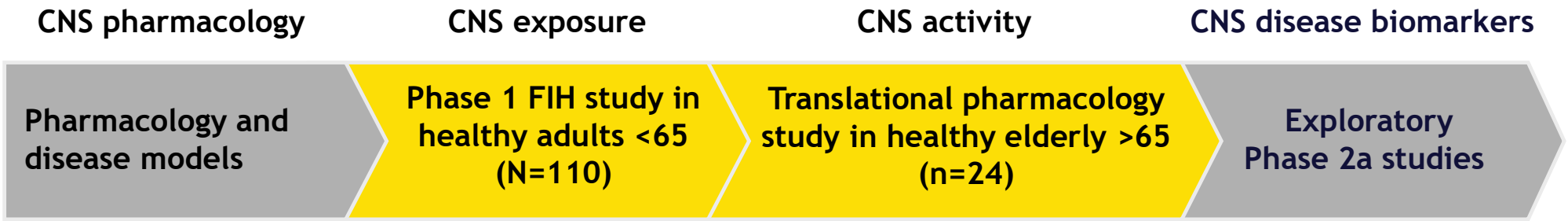
CLINICAL DEVELOPMENT

Biomarkers have been integrated into CY6463 clinical development



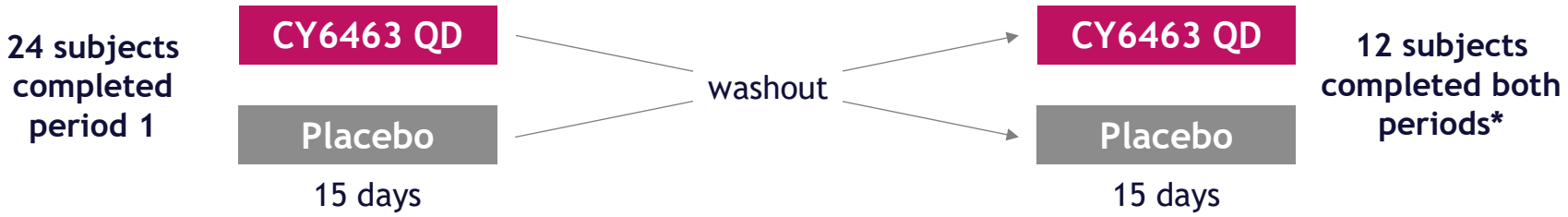
- CNS-exposure
- drug-like properties
- pharmacological profile consistent with known role of pathway in CNS
- biomarker identification

- SAD/MAD/FE
- safe/tolerated dose range
- once-daily PK
- CNS target engagement

- crossover
- safe/tolerated dose
- confirmed PK
- PD biomarkers
- mechanistic biomarkers

Phase 1b translational pharmacology study designed to evaluate CNS activity

Healthy elderly population (≥65 years)



Objectives

- Safety and tolerability
- Pharmacokinetics
- Target engagement
- CNS activity

CY6463 showed rapid and persistent effects on multiple independent biomarkers associated with ~~co~~gnition

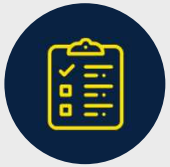
In a 15-day study in 24 healthy elderly subjects CY6463 demonstrated:

increased alpha and gamma power

improved N200 latency

faster saccadic eye movement (SEM) reaction time

reduction in neuroinflammatory biomarkers



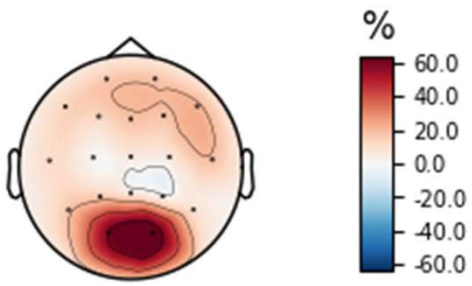
- Rapid onset (<15 days)
- Effect increased with age
- Biomarkers linked to AD and aging

CY6463 altered qEEG: significant increase in alpha power in aged adults

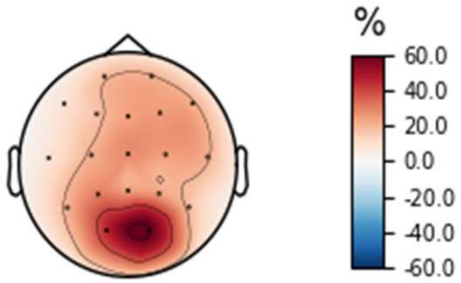
Significant increase in EEG alpha power

No effect of placebo

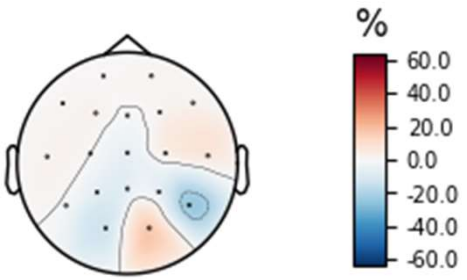
CY6463 vs. baseline



CY6463 vs. placebo

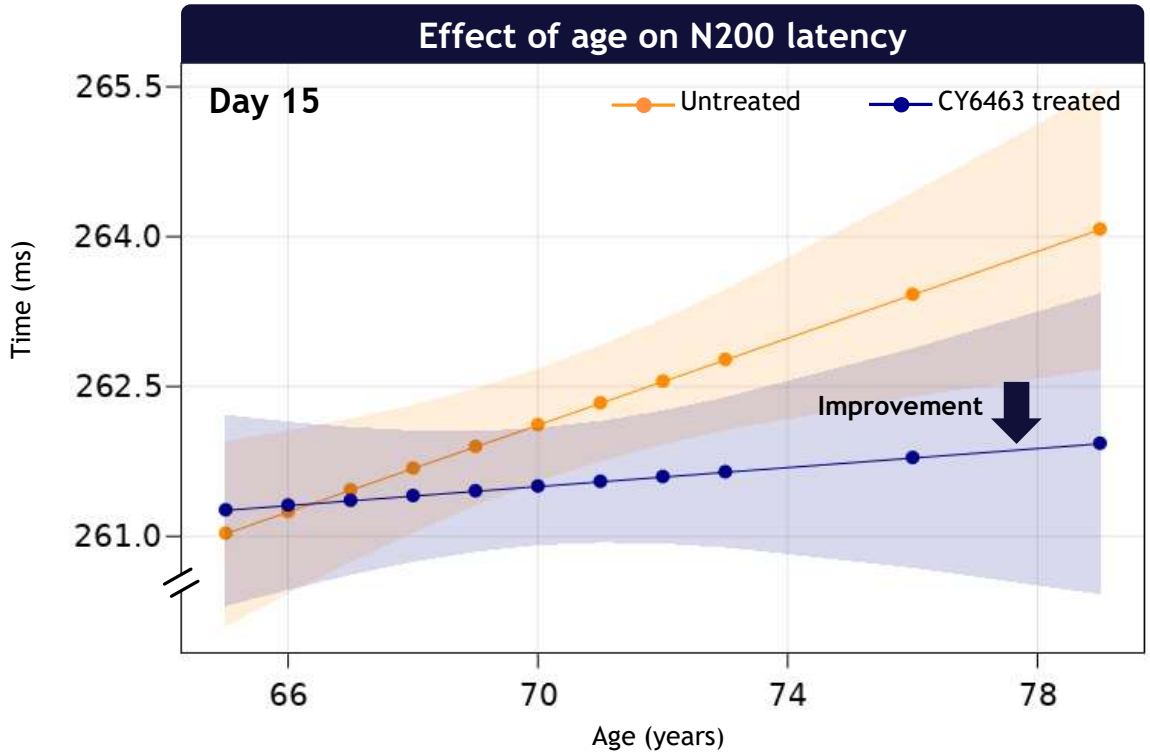


Placebo vs. baseline



change (%) in alpha power on day 15

CY6463 improved N200 latency and effect increased with age

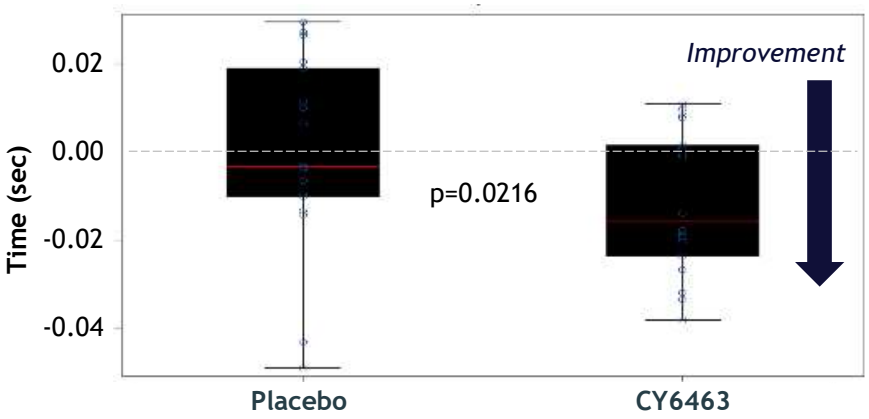


Overall decrease in N200 latency for CY6463 treated vs untreated on day 15 ($p<0.02$)

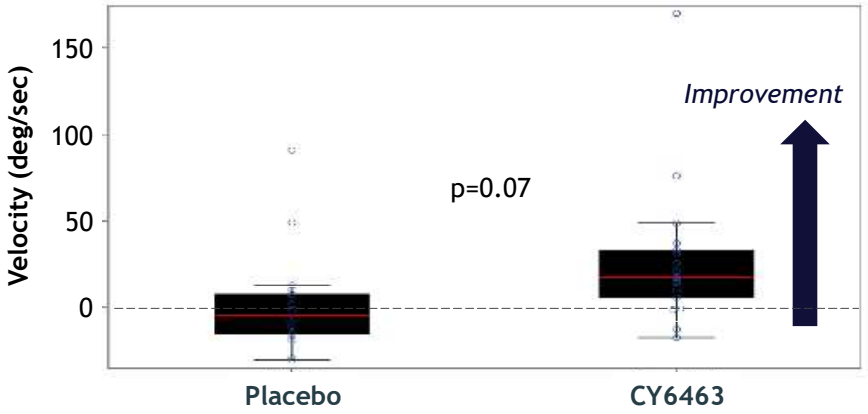
Effect more pronounced in older subjects

CY6463 improved saccadic eye movement, an objective measure of CNS function

Decrease in saccadic reaction time



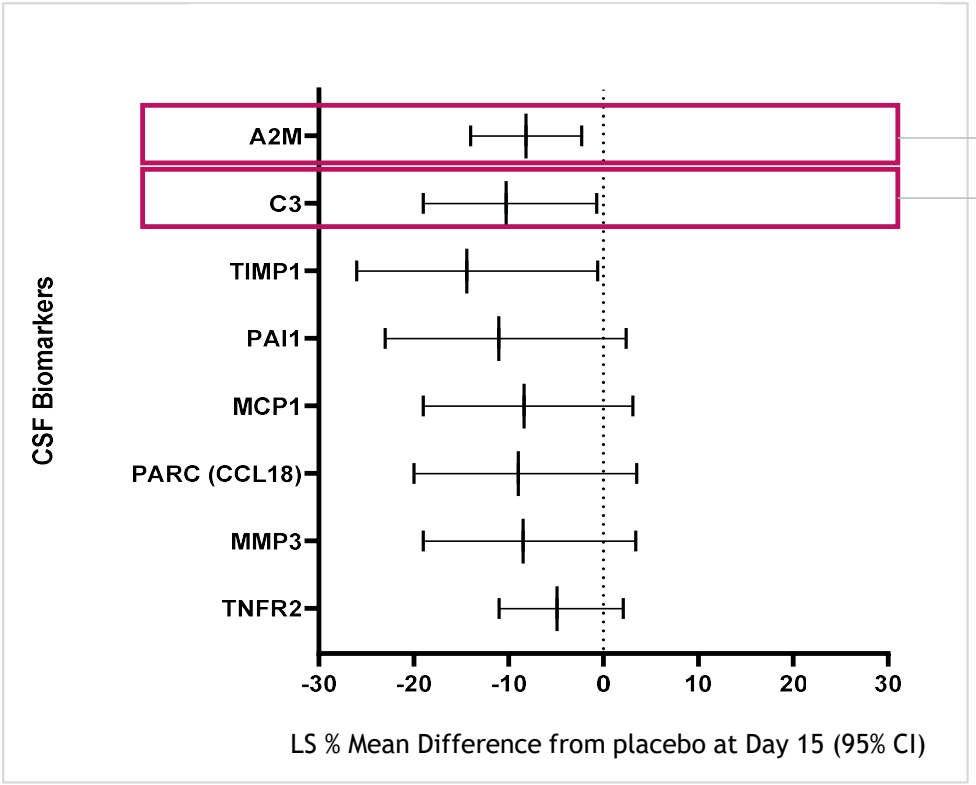
Increase in saccadic peak velocity



- Shorter saccadic reaction times and faster saccadic velocities indicate that CY6463 is improving CNS functional performance and affecting neurophysiology
- Cognitive enhancers (e.g., modafinil) also positively impact saccadic eye movements

Mean change from baseline on day 15 post-dose

CY6463 reduced neuroinflammatory biomarkers in aged adults

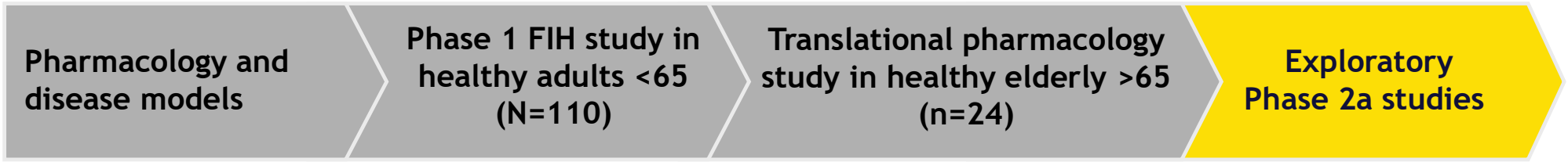


Alpha-2-macroglobulin (A2M) levels predict cognitive decline and development of AD; may lead to tau hyperphosphorylation

Complement C3 (C3) colocalizes with Aβ plaques and tau tangles; involved in synaptic remodeling and degeneration

A2M and C3 increases are associated with aging, Alzheimer's disease and other neurodegenerative diseases

Biomarkers have been integrated into CY6463 clinical development



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- SAD/MAD/FE
- safe/tolerated dose range
- once-daily PK
- CNS target engagement

- crossover
- safe/tolerated dose
- confirmed PK
- PD biomarkers
- mechanistic biomarkers

- defined populations
- patient biomarker data
- early impacts on disease

AD with vascular pathology (ADv) – a focused mixed dementia subset

Defined population well suited for treatment

DISEASE RATIONALE FOR PATIENT SELECTION

Pathophysiology

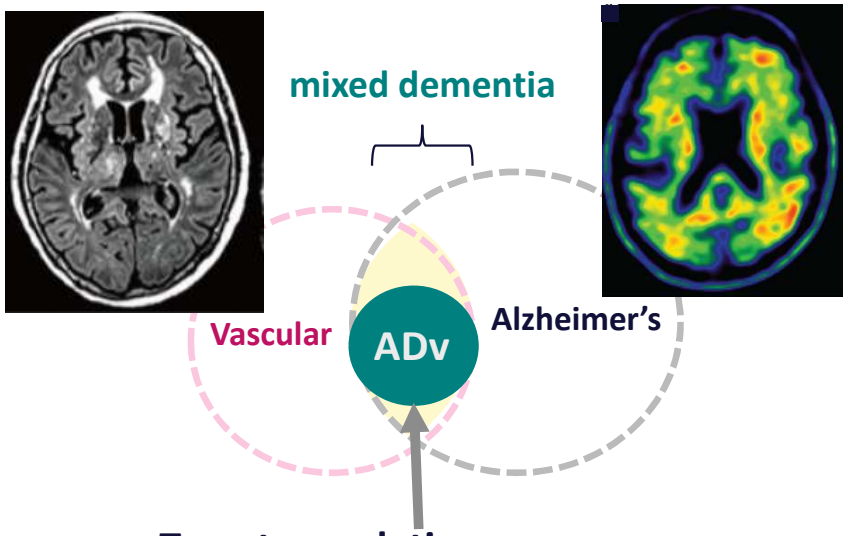
NO dysregulation, endothelial cell loss, impaired blood flow, vascular leakage, inflammation, neuronal dysfunction, and neuronal loss are major contributing factors to rapid disease progression

Standard of care

No approved therapies to treat vascular dementia.
AD therapies offer limited benefits.

Pharmacology

Our preclinical data suggest CY6463 has potential to improve cerebral blood flow, endothelial health, neuroinflammation, and cellular energetics as well as prevent neurodegeneration



Target population

ADv: an identifiable subset of mixed dementia patients with:

- AD pathology **AND**
- sub-cortical vascular disease **AND**
- CV risk factors

Rizzi et al. 2014 Biomed Res Int. 2014:908915; Roh and Lee 2014 J Stroke. 16(1): 18–26.; Alzheimer's Association; NCI Analysis

Phase 2a ADv clinical study initiated in mid-2021

Objectives	Exploratory, signal-seeking study to evaluate safety, tolerability, and pharmacodynamic effects (EEG, MRI, neuroinflammatory biomarkers, cognition)
Study design	• Once-daily CY6463 vs. placebo • 12 weeks • 30 participants • ClinicalTrials.gov identifier: NCT04798989
Patient targeting	• Confirmed AD pathology (PET, CSF) • 2+ cardiovascular risk factors • Mild-moderate subcortical small-vessel disease on MRI • Mini mental state exam score (20-26)
Collaborations	• Partially funded by the Alzheimer's Association's Part the Cloud-Gates Partnership • Collaborating with Dr. Andrew Budson at Boston University on a study to examine the relationship between ERP/EEG and cognitive measures in dementias

AAIC 2021 July Presentations: P-54463 Glasser et al., CY6463 Phase 2a ADv clinical trial design; P-54365 Marin et al., Peak alpha frequency and N200 latency as predictors of neuropsychological performance in a memory disorders clinic

Summary and conclusions

- A multifaceted approach offers more opportunities to simultaneously treat a range of AD pathologies
- Focus on translatable preclinical measures can enable efficient bridging into Phase 1
- Selecting a discrete and well-defined patient population key for early POC studies
- Critical evaluation of biomarkers early in clinical development can serve to inform patient studies
- Accumulating clinical data point to new understanding of AD contributors (e.g., vascular pathologies) and considerations for designing next-generation trials