ON A MISSION TO DEVELOP TREATMENTS THAT RESTORE COGNITIVE FUNCTION

PIPELINE UPDATE WEBINAR

APRIL 27, 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 2019 Form 10-K filed on March 12, 2020, and our subsequent SEC filings, including the Form 10-Qs filed on May 4, 2020, August 3, 2020 and November 5, 2020. 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.

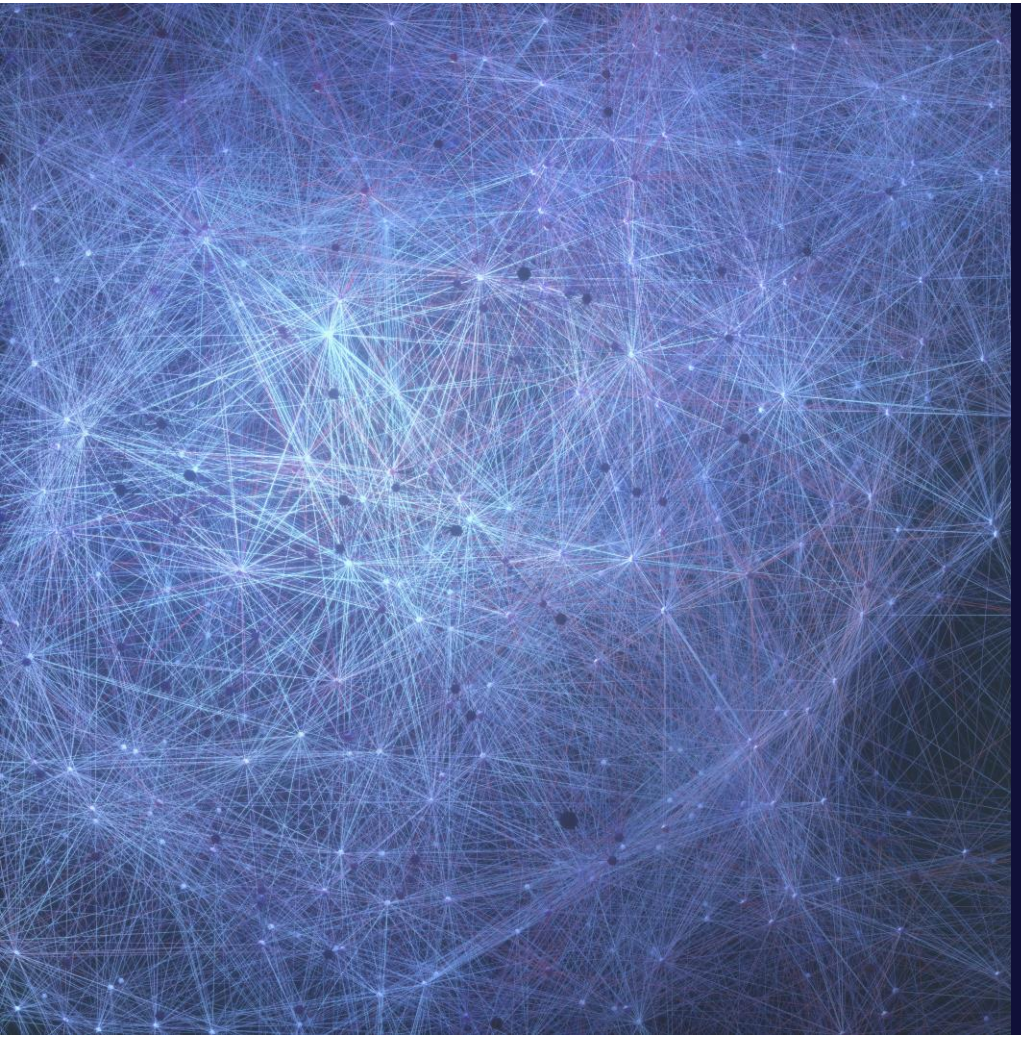
On a mission to develop treatments that restore cognitive function

Tapping into a fundamental CNS signaling pathway with CY6463, a first-in-class, CNS-penetrant sGC stimulator

Executing biomarker-guided development strategy in well-defined populations with cognitive impairment

Tackling the enormous burden and breadth of cognitive impairment through an innovative portfolio of indications and molecules

Agenda



1. Welcome and overview
2. NO-sGC-cGMP is a fundamental CNS signaling pathway
3. Clinical development strategy: ADv, MELAS, CIAS
4. sGC pathway: role in CIAS
5. CY6463 Phase 1b study in CIAS
6. Next-generation sGC stimulator program
7. Q&A

Today's Speakers

Cyclerion Leadership

Peter Hecht, PhD

Chief Executive Officer

Andy Busch, PhD

Chief Scientific Officer

Chris Winrow, PhD

Head of Translational Medicine

Jennifer Chickering, PhD

Senior Director of Clinical Strategy

Neuropsychiatry KOL

Andreas Reif, MD

Department of Psychiatry, Psychosomatic Medicine and Psychotherapy University Hospital Frankfurt

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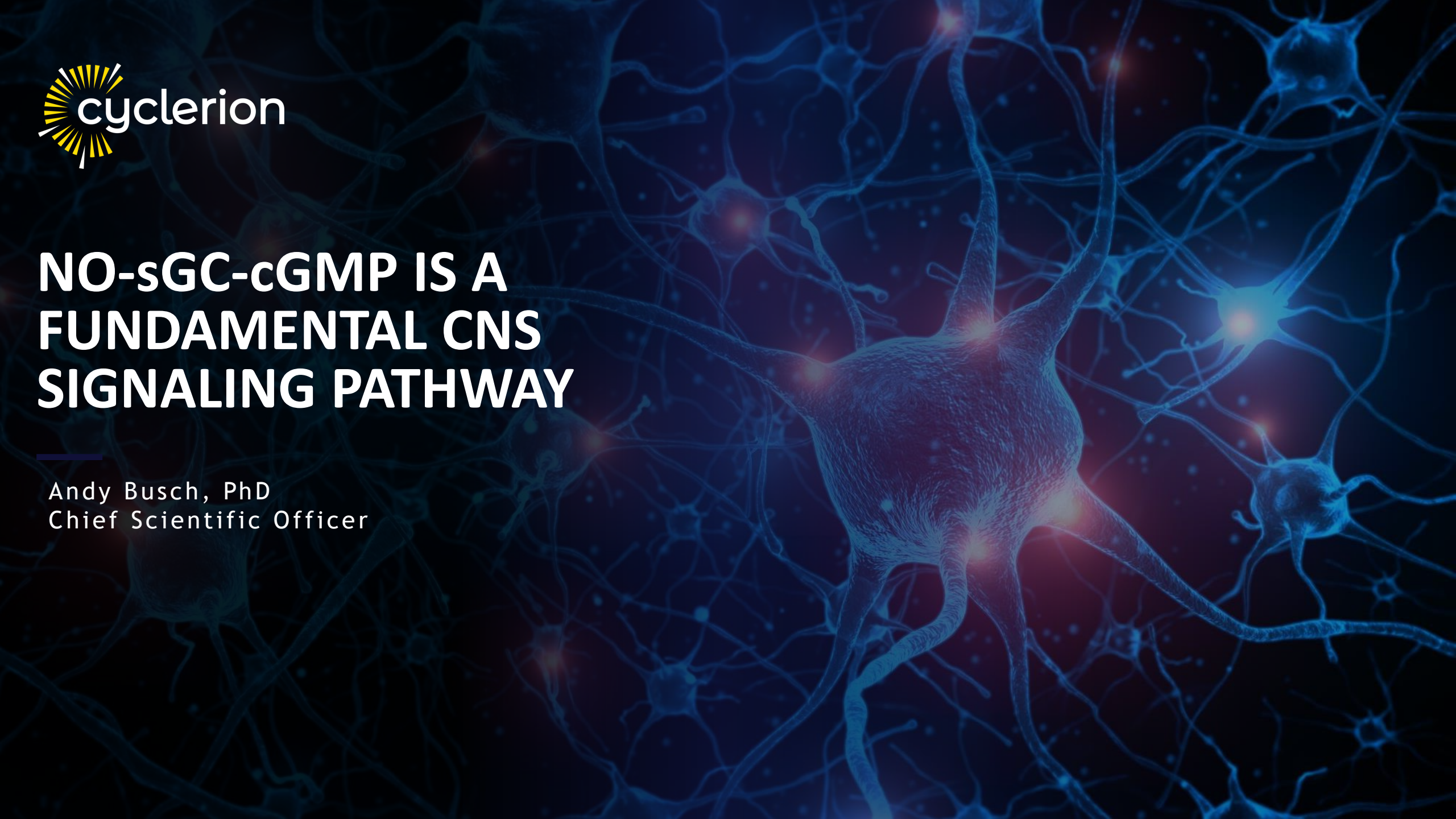
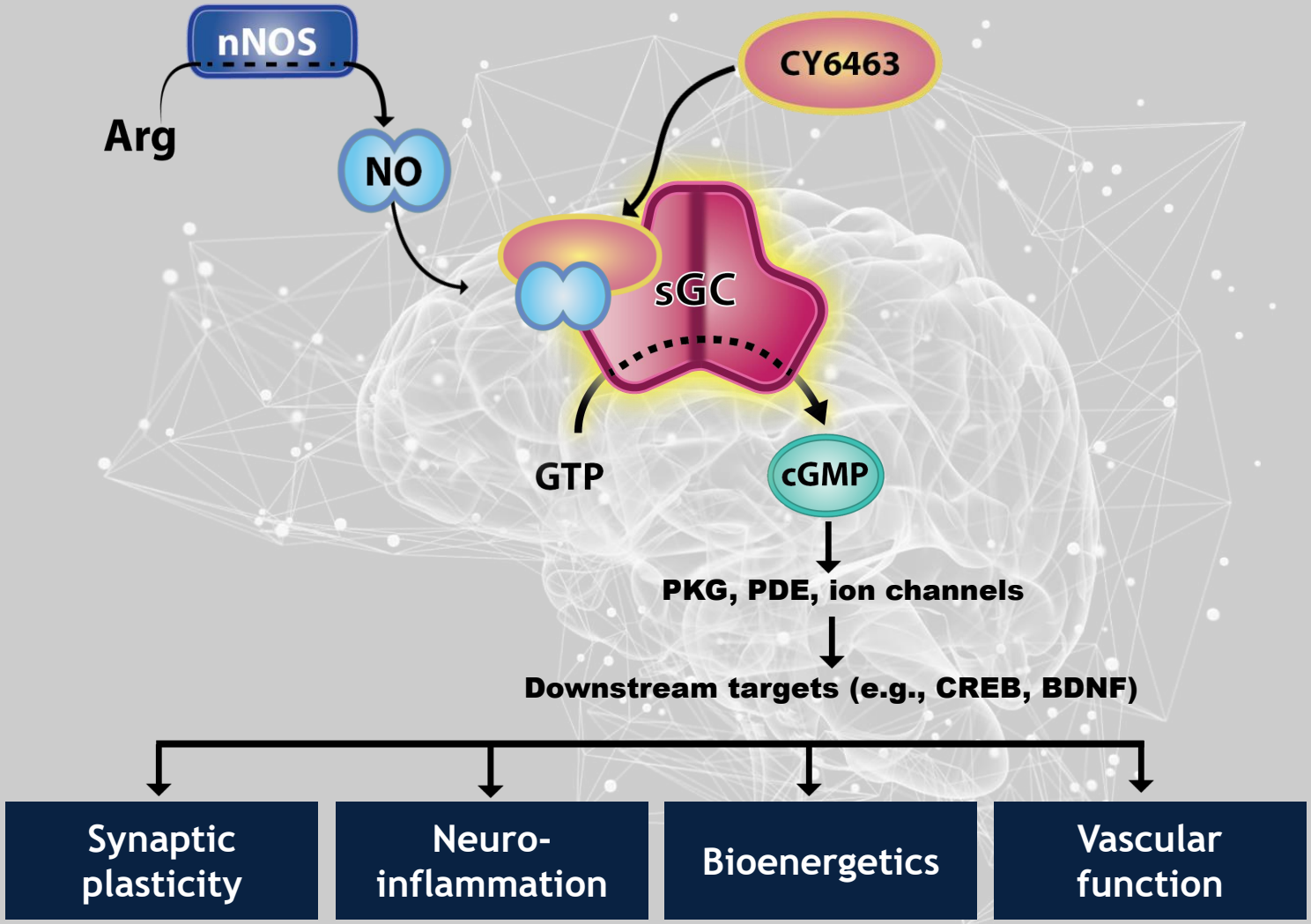


NO-sGC-cGMP IS A FUNDAMENTAL CNS SIGNALING PATHWAY

Andy Busch, PhD
Chief Scientific Officer

CY6463 amplifies the fundamental NO-sGC-cGMP signaling pathway



CY6463

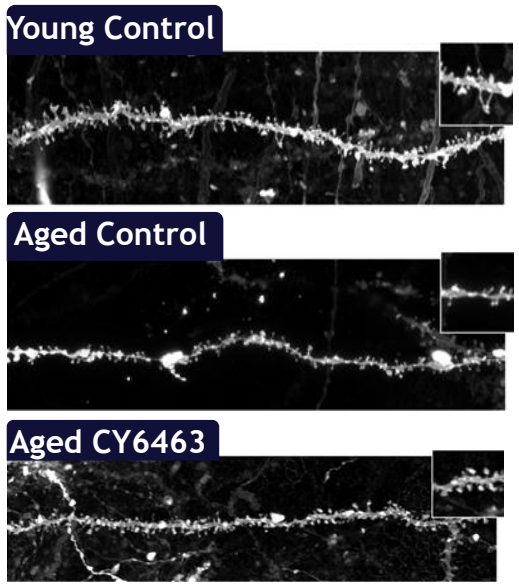
- First-in-class BBB-permeable, positive allosteric modulator of sGC
- Amplifies endogenous NO-sGC-CGMP signaling to address central aspects of disease pathophysiology

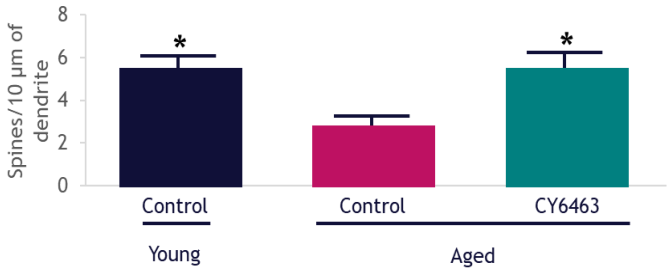
Preclinical data and extensive academic work validate the crucial role of the NO-sGC-cGMP pathway in brain physiology

Important role in learning and memory

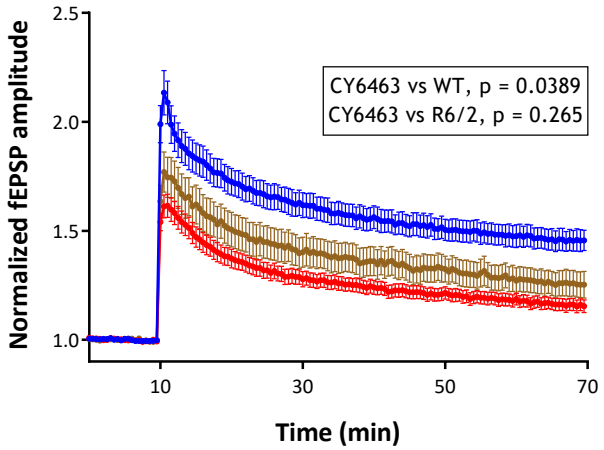
CY6463 improves endpoints relevant to cognition

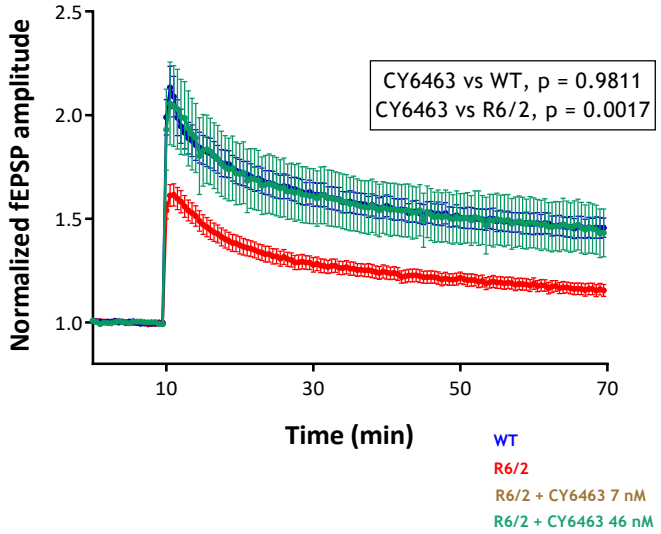
Morphological plasticity



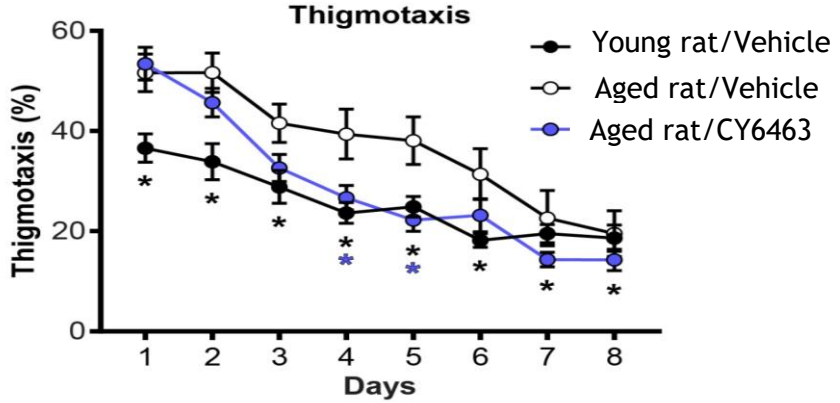


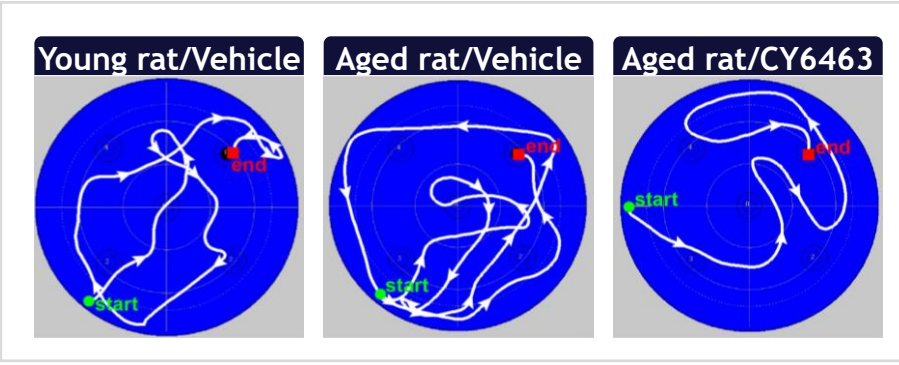
Ex-vivo LTP





In-vivo learning and memory

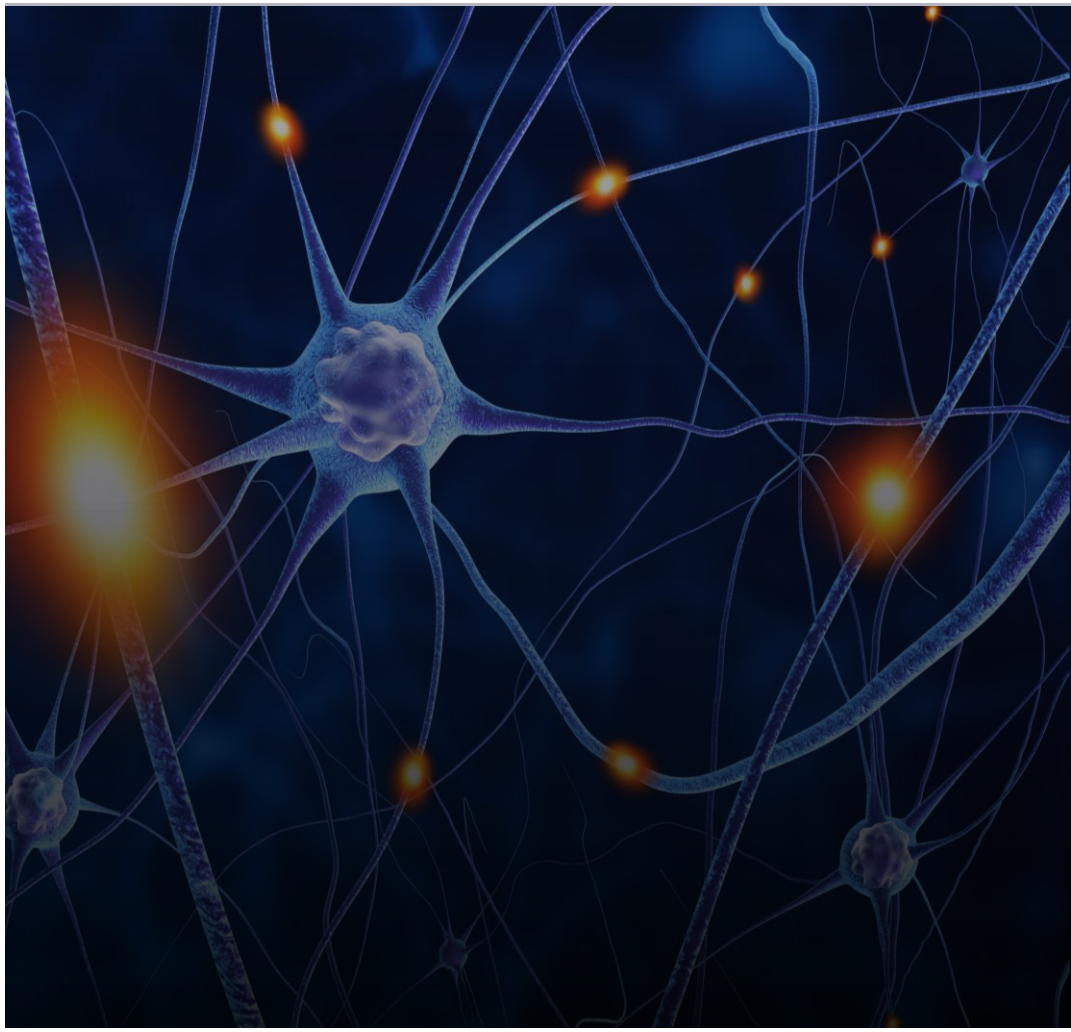




*p<0.05 vs. the aged control group

CY6463 amplifies a fundamental CNS signaling pathway

- NO-sGC-cGMP pathway plays a critical role in brain function
- sGC stimulation with CY6463 amplifies NO-sGC-cGMP signaling
- Morphological, *ex vivo* and *in vivo* data demonstrate important role of sGC in synaptic plasticity, learning and memory, and 6463's ability to restore deficits in these endpoints



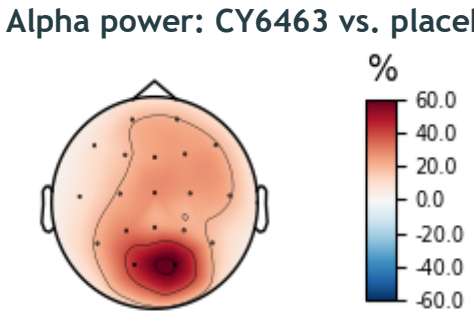
CLINICAL DEVELOPMENT STRATEGY

Chris Winrow, PhD
Head of Translational Medicine

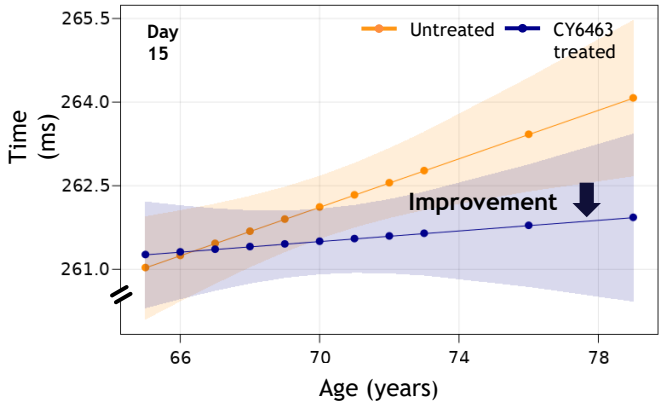
CY6463 showed rapid improvement in biomarkers of cognition

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

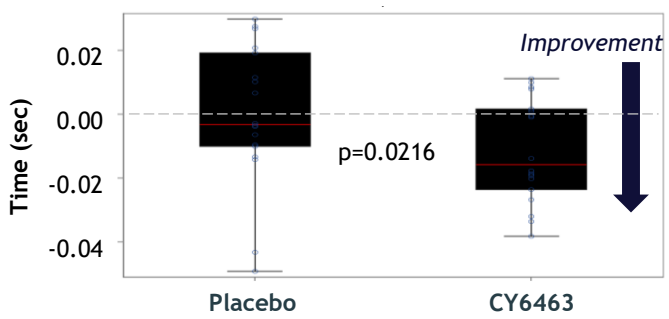
Increased alpha and gamma power



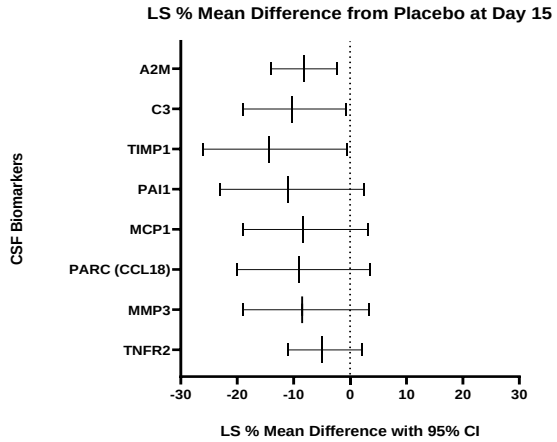
Improved N200 latency



Faster saccadic eye movement reaction time



Reduced neuroinflammatory biomarkers



CY6463 data point to potential in cognition

Preclinical CNS pharmacology

- Neuronal function
- Neuro-inflammation
- Bioenergetics
- Vascular function

+

Clinical CNS pharmacology*

- Increased posterior alpha and gamma power
- Improved N200 latency
- Faster saccadic eye movement (SEM) and reaction time
- Reduced neuroinflammatory biomarkers in CSF

=

Potential to improve cognitive function

**In a 15-day study in 24 healthy elderly subjects*

Cognitive impairment is a debilitating facet of many CNS diseases

Neurodegenerative

~2M	ADv *ongoing*
~35M	Alzheimer's Disease
~13M	Lewy Body Dementia
~5M	Parkinson's Dementia

Neuropsychiatric

~21M	CIAS *ongoing*
~150M	Major Depressive Disorder
~27M	Bipolar Disorder
~10M	Autism

Mitochondrial

Orphan	MELAS *ongoing*
Orphan	Leigh Syndrome
Orphan	Kearns-Sayre Syndrome

Event-related

~21M (US)	Traumatic brain injury
~12M	Stroke
~5M (US)	Cancer/chemotherapy-induced cognitive impairment

References on file

Represents approximate prevalence of patients with cognitive impairment associated with other CNS diseases, worldwide in millions, except where noted as US prevalence.

Biomarker-guided development strategy in well-defined populations with cognitive impairment

ADv **Neurodegenerative**

CIAS **Neuropsychiatric**

Improving Cognition

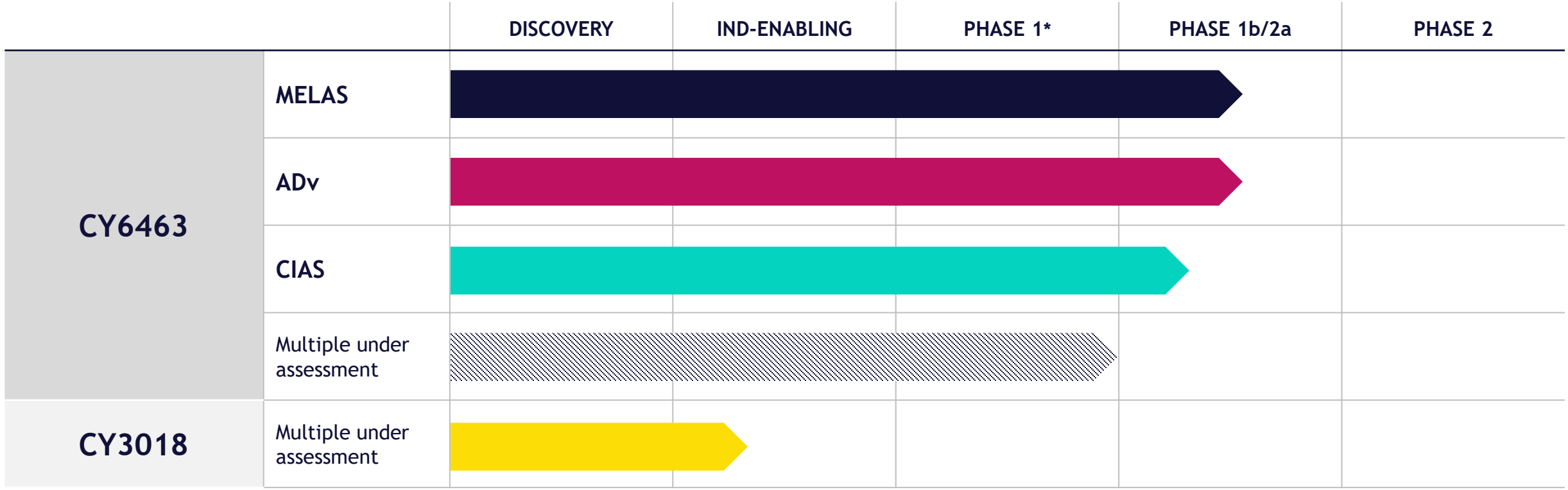
- Parallel studies in distinct populations
- Efficient, signal-seeking studies inform larger and longer studies
- Disease-relevant biomarkers accelerate and guide development
- Translation of insights across programs increases odds of success

Mitochondrial Disease MELAS

Significant additional opportunities

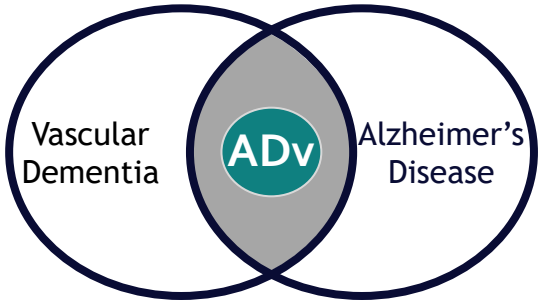
ADv | *Alzheimer's Disease with vascular pathology (ADv)*
CIAS | *Cognitive Impairment Associated with Schizophrenia*
MELAS | *Mitochondrial Encephalomyopathy, Lactic Acidosis, and Stroke-like episodes*

Advancing parallel, signal-seeking, exploratory studies in priority patient populations



**Two phase 1 studies were completed in healthy young and old (>65 years of age) volunteers confirming targeted CNS exposure and activity*

ADv Ph2a study expected to initiate 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
Patient targeting	• Confirmed AD pathology (PET or CSF) • 2+ cardiovascular risk factors • Mild-moderate subcortical small-vessel disease on MRI • Mini mental state exam score (20-26)

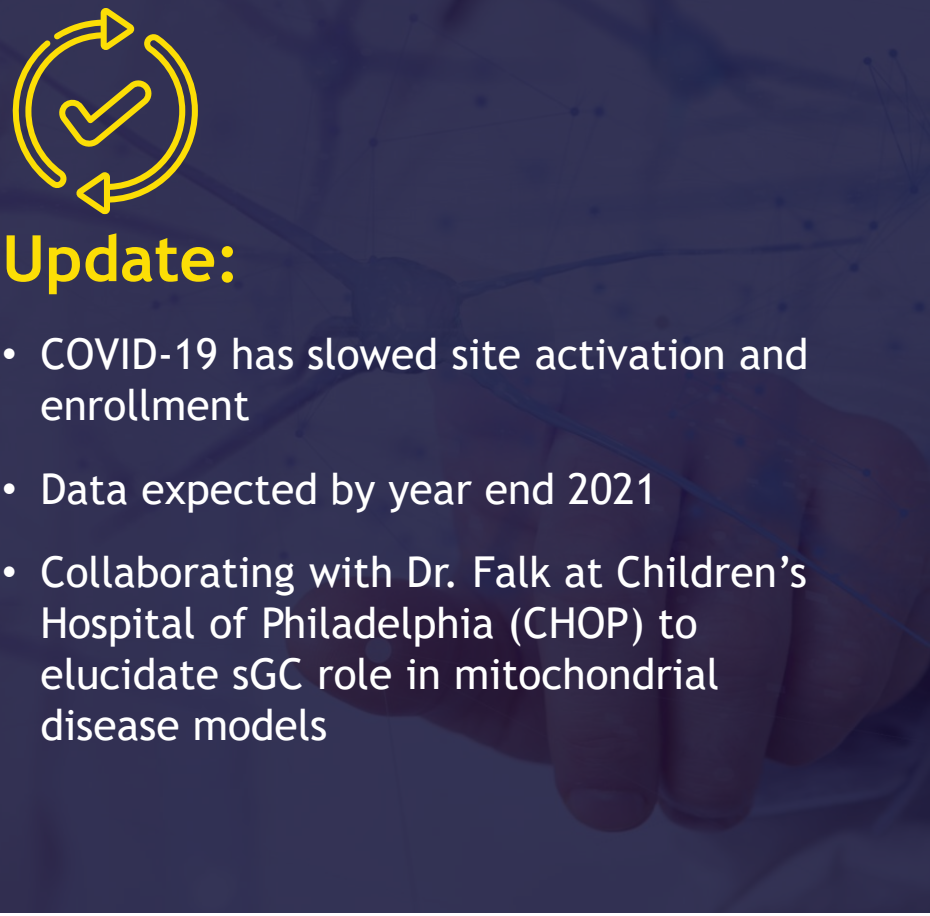
With the Alzheimer's Association's Part the Cloud-Bill Gates Partnership

Update:

- IND cleared; protocol incorporates input from leading KOLs
- Study start-up activities initiated
- First patient enrollment expected mid-2021 barring any COVID-19-related delays
- Collaborating with Dr. Budson at Boston University on a study to examine the relationship between ERP/EEG and cognitive measures in dementias

MELAS Ph2a study: data now expected by year end 2021

Objectives	Exploratory, signal-seeking study to evaluate safety, tolerability, and pharmacodynamic effects (MRI, biomarkers)
Study design	• 29 days, open label • Once-daily CY6463 • Up to 20 adult participants (targeting 12 completers)
Patient targeting	• Genetically confirmed mitochondrial disease with neurological features of MELAS • Elevated plasma lactate (disease biomarker)
Sites	**Centers of excellence for mitochondrial medicine:** CHOP, MGH, Children's National Hospital, Columbia University, Johns Hopkins University



Update:

- COVID-19 has slowed site activation and enrollment
- Data expected by year end 2021
- Collaborating with Dr. Falk at Children's Hospital of Philadelphia (CHOP) to elucidate sGC role in mitochondrial disease models

sGC PATHWAY: ROLE IN COGNITIVE IMPAIRMENT ASSOCIATED WITH SCHIZOPHRENIA (CIAS)

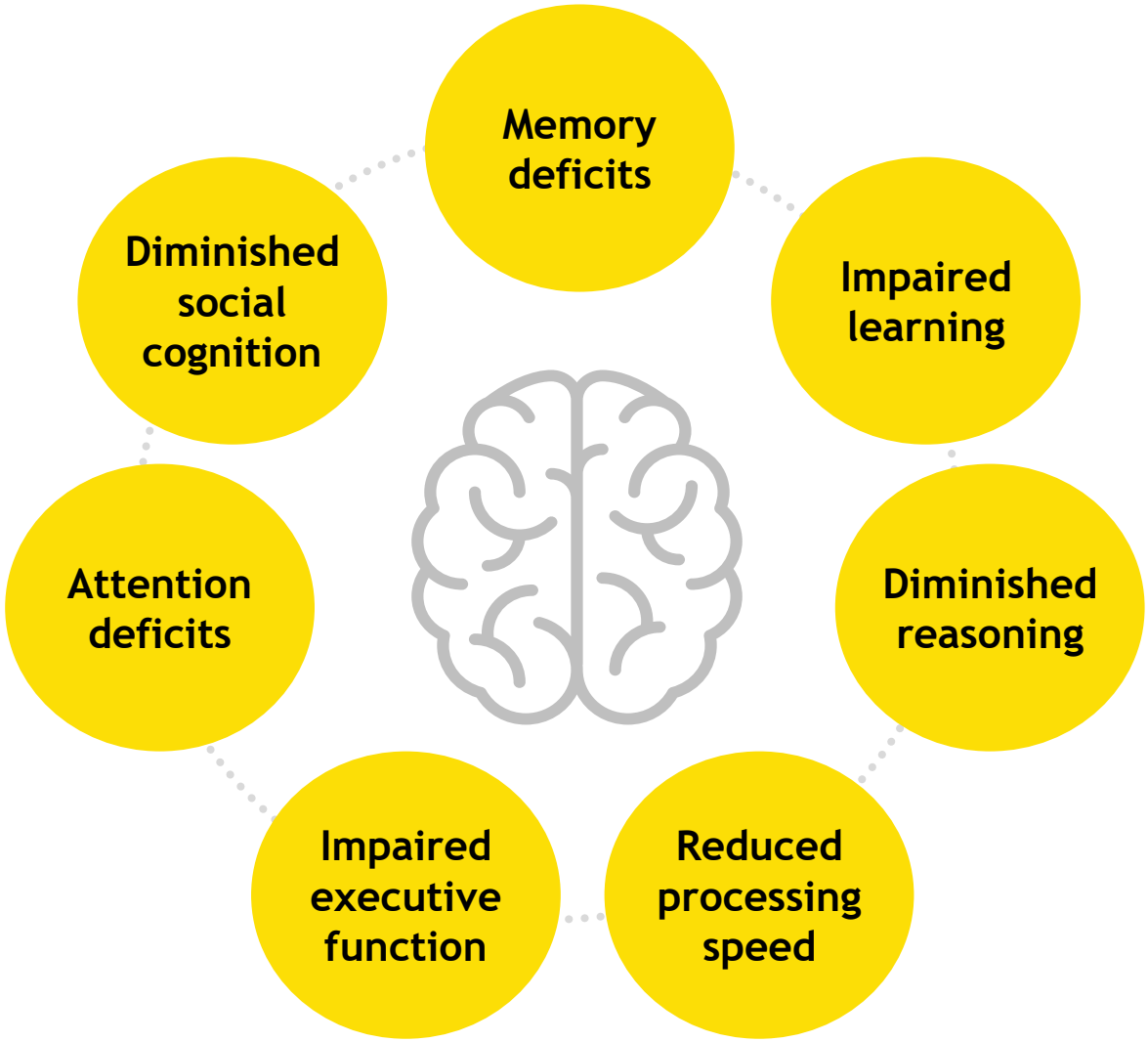
Andreas Reif, MD
Department of Psychiatry, Psychosomatic Medicine & Psychotherapy
University Hospital Frankfurt

Schizophrenia is a complex, chronic, and disabling disorder

Cognitive impairment is a core facet of schizophrenia

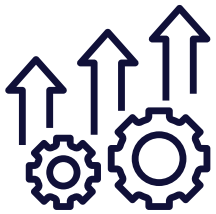
Nearly all people with schizophrenia (98%) have cognitive deficits

21M worldwide, 2.7M in US with schizophrenia and growing



Cognitive impairment underlies functional disability in schizophrenia and is not addressed by current treatments

Deficits in perception and cognition in schizophrenia are associated with poor social and vocational functioning



Over three quarters of the estimated US annual cost of schizophrenia ($156B in 2013) is attributable to indirect costs including costs due to unemployment and productivity loss

Improving cognition

Functional recovery

Increasing ability to participate fully in the community and live independently

Longstanding investigation of NO-sGC-cGMP pathway in schizophrenia

Molecular Psychiatry (2006) 11, 286–300
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www.nature.com/mp

ORIGINAL ARTICLE

A neuronal nitric oxide synthase (NOS-I) haplotype associated with schizophrenia modifies prefrontal cortex function

A Reif[1], S Herterich[2], A Strobel
U Walter[2], A Schmitt[1], AJ Fallg

[1]Department of Psychiatry and Psych
Psychophysiology and Functional Ima
Laboratory, Department of Clinical Bio
[3]Department of Differential and Perso
Germany; [4]Institute of Medical Biomet
of Internal Medicine II, Technical Univ

and Behavior

Official publication of the International Behavioural and Neural Genetics Society

Genes, Brain and Behavior (2015) 14: 46–63 doi: 10.1111/gbb.12193

Review

Neuronal nitric oxide synthase (*NOS1*) and its adaptor, *NOS1AP*, as a genetic risk factors for psychiatric disorders

F. Freudenberg[†], A. Alttoa[‡,§] and A. Reif[†,*]

Keywords: ADHD, biomarker, bipolar disorder, impulsivity,

European Neuropsychopharmacology (2016) 26, 741-755

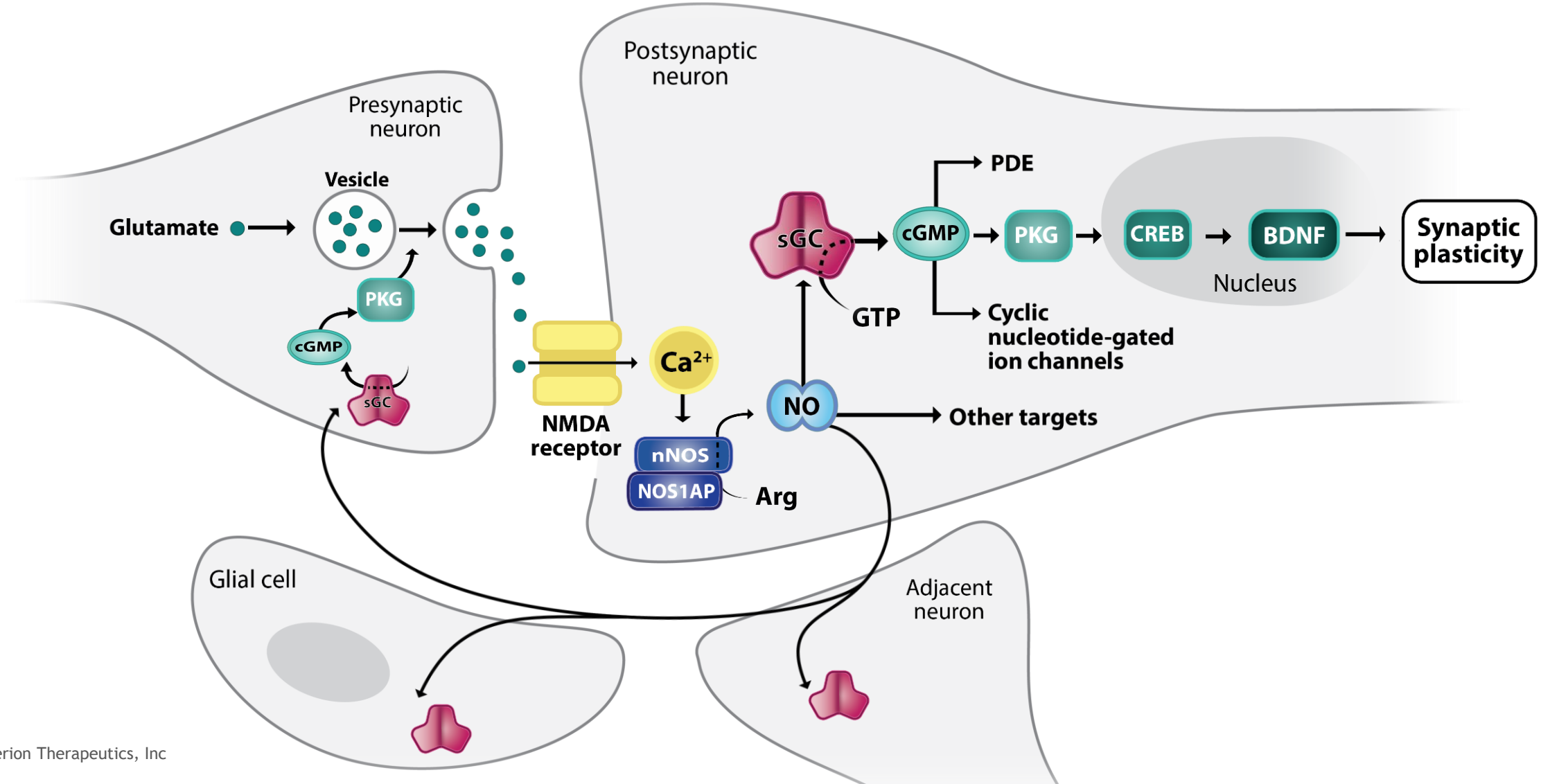
ELSEVIER

www.elsevier.com/locate/euroneuro

Interaction of NOS1AP with the NOS-I PDZ domain: Implications for schizophrenia-related alterations in dendritic morphology

Esin Candemir[a,b,c], Leonie Kollert[b], Lena Weißflog[a,b], Maria Geis[b], Antje Müller[b], Antonia M Post[a,b], Aet O'Leary[b,d], Jaanus Harro[d], Andreas Reif[a,b], Florian Freudenberg[a,b,*]

sGC plays a key role in NMDA receptor signaling



CIAS linked to reduced NO-sGC-cGMP signaling and associated altered dendritic spine morphology

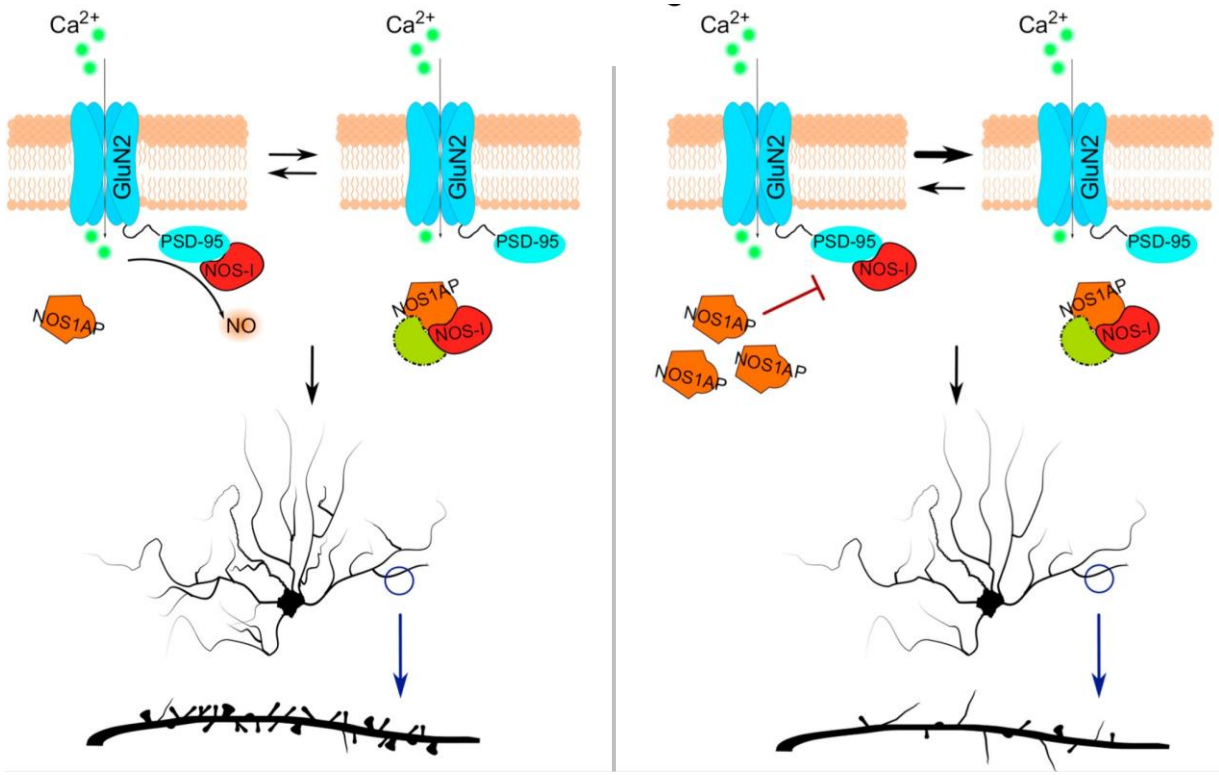
Overexpression of NOS1AP leads to sequestering of NOS-I from NMDA receptor

Reduced NO-sGC-cGMP signaling → altered dendritic spine number and morphology

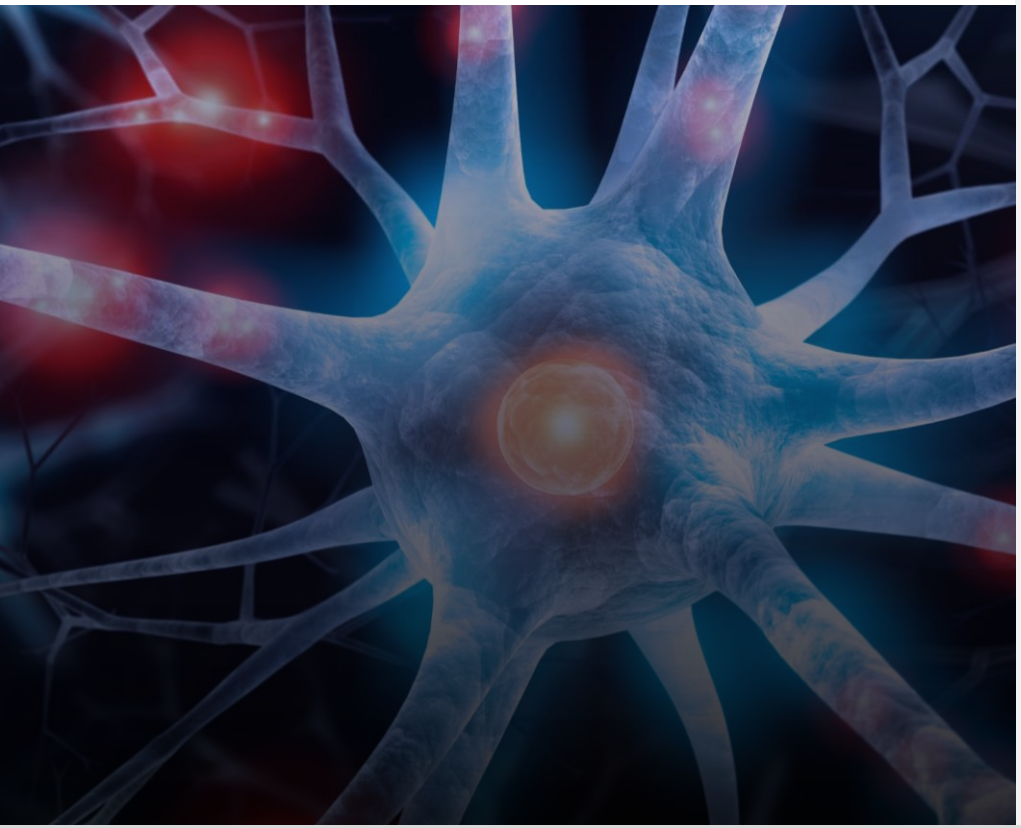
Reduced synaptic plasticity → disturbance of prefrontal cortex and hippocampal function, detectable by EEG

Cognitive impairment

Theoretical model for NOS1AP interactions



NO-sGC-cGMP dysfunction is implicated in pathophysiology of CIAS



Multiple lines of evidence implicate reduced NO-sGC-cGMP signaling in schizophrenia and specifically in cognitive deficits in schizophrenia

Hypoglutamatergic state, which is linked to low NO, is known part of schizophrenia pathophysiology

Compromised prefrontal and hippocampal NO signaling is implicated in cognitive deficits in schizophrenia

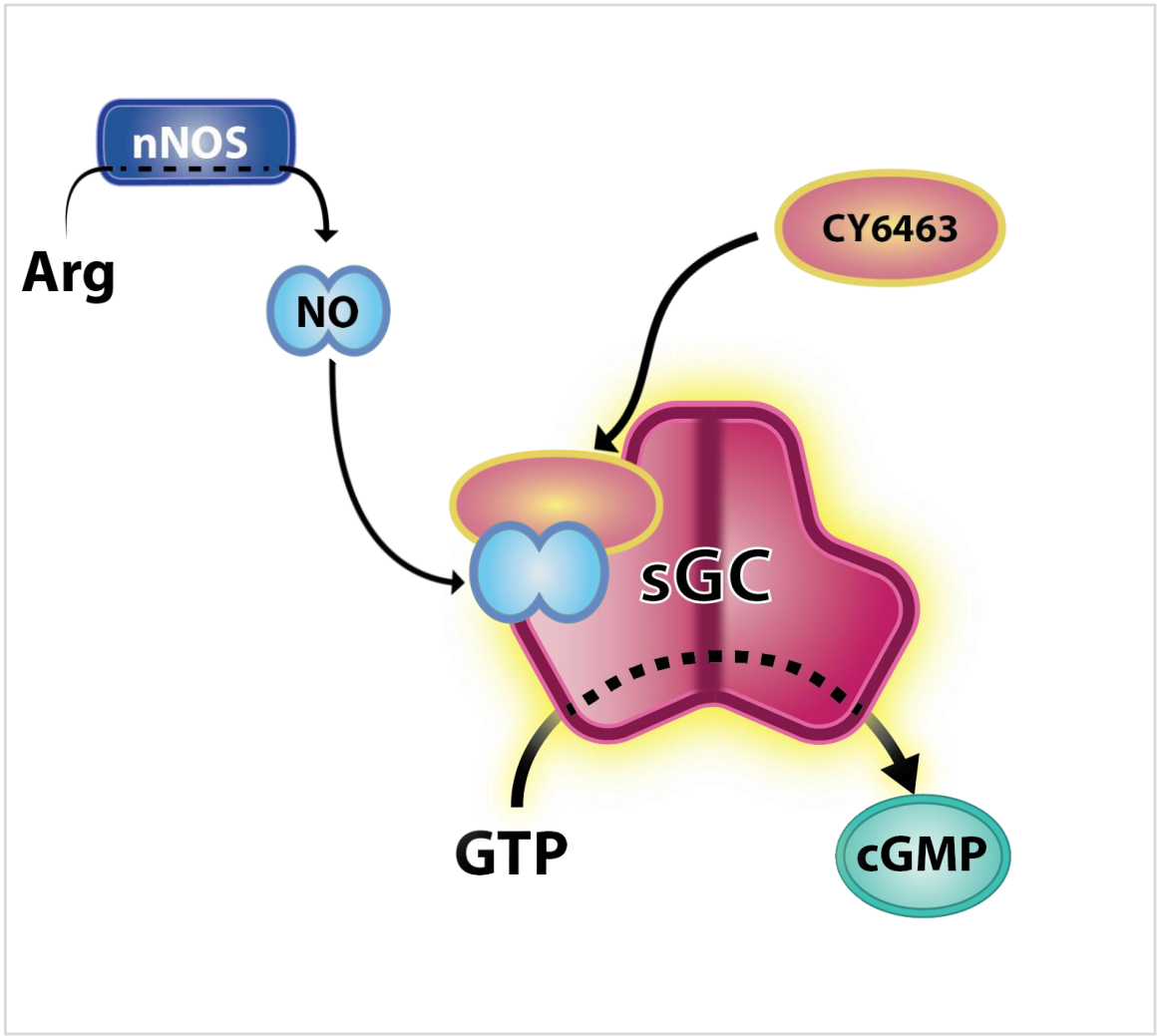
Modulation of the NO-sGC-cGMP pathway is a promising therapeutic approach to the treatment of CIAS

CY6463 PHASE 1B STUDY IN CIAS

Jennifer Chickering, PhD
Senior Director of Clinical Strategy

CY6463 brings novel CNS mechanism to treatment of CIAS

- Reduced NO-sGC-cGMP signaling linked to cognitive impairment in schizophrenia
- sGC stimulation by CY6463 is attractive approach for the treatment of CIAS
  - specific to NO-sGC-cGMP signaling
  - proven druggable target
  - targets all critical brain regions and cell types
  - amplifies endogenous signaling
- Demonstrated impact on multiple biomarkers associated with cognition



Efficient first study in CIAS to inform dose, patient, and biomarker selection for subsequent development

- Confirm CNS target engagement and explore early signal detection
- Evaluate multiple dose levels

CY6463 Ph1b signal-seeking study in CIAS

Controlled

- In-clinic, randomized, placebo-controlled, double-blinded
- Psychiatrically stable adults with schizophrenia, on stable antipsychotic regimen
- QD dosing for 14 days in ~50 participants across sequential cohorts
- Comprehensive safety and pharmacokinetic assessment

Cohort 1

Cohort 2

Cohort 3

Cohort 4

Exploratory

- Broad battery of EEG-based tests to capture spectral and ERP measures
- Computerized battery of cognitive tests
- Flexible, data-responsive cohort dose selection to maximize learning

CIAS biomarker-guided development strategy

Today	Tomorrow	Future
Exploratory Phase 1b Safety + near-term impact on disease-relevant biomarkers	**Larger, longer studies focused on biomarker-identified populations**	**Standard of care adjunctive therapy** Improve cognitive impairment and functional outcomes

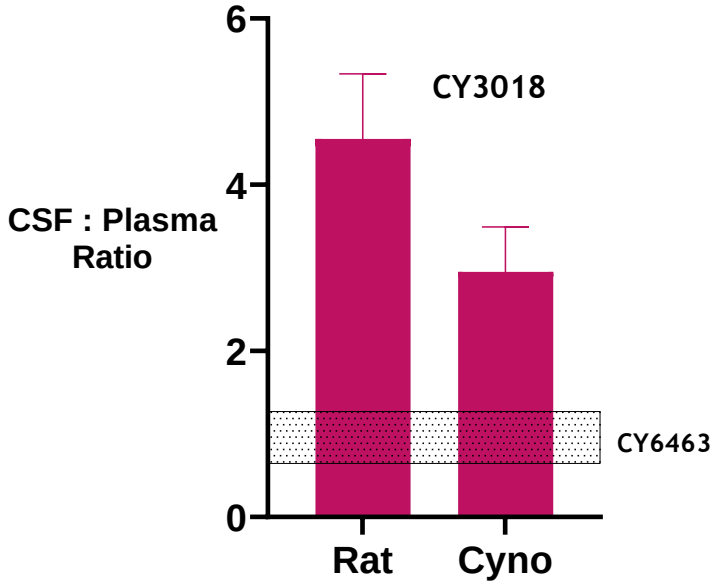
- Debilitating and untreated facet of schizophrenia
- Large and growing unmet need
- High cost to families and society

NEXT GENERATION sGC STIMULATOR PROGRAM

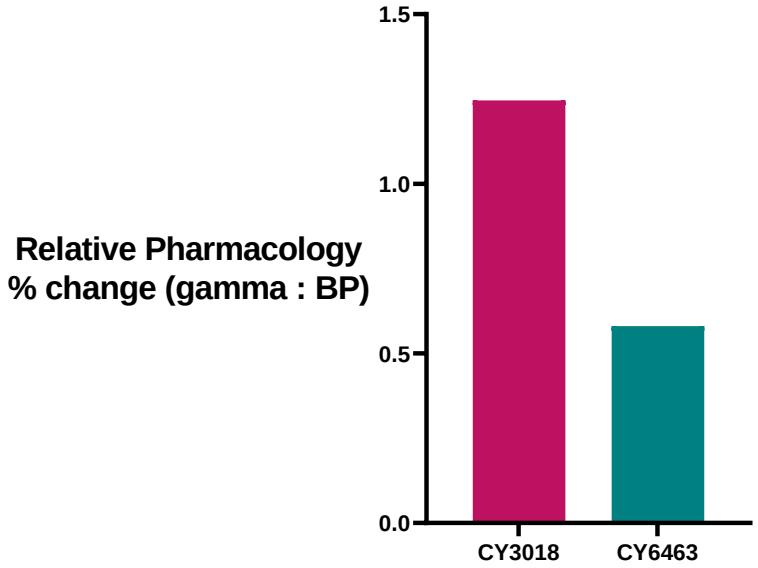
Andy Busch, PhD
Chief Scientific Officer

Next generation sGC stimulator CY3018: selectively targeting the CNS

Greater relative CNS exposure



Greater relative CNS pharmacology



- Greater CSF:plasma ratio for CY3018 translating into greater relative CNS pharmacology
- CY3018 is progressing through IND-enabling development
- Ongoing pharmacology studies to validate amenable CNS indications

Data displayed as mean+ SEM, Relative pharmacology ratio: 1-hour post-dose with vehicle-subtraction

SUMMARY

Peter Hecht, PhD
Chief Executive Officer

On a mission to develop treatments that restore cognitive function

Tapping into a fundamental CNS signaling pathway with CY6463, a first-in-class, CNS-penetrant sGC stimulator

Executing biomarker-guided development strategy in well-defined populations with cognitive impairment

Tackling the enormous burden and breadth of cognitive impairment through an innovative portfolio of indications and molecules


cyclerion
Q&A

Relevant reference publications and notes (1 of 3)

CY6463 improves endpoints relevant to cognition

LTP: hippocampal slices from R6/2 symptomatic mice; Spine: 16-month-old (aged) healthy mice treated for 4 months with CY6463; MWM: ~21 month old rats treated daily with CY6463 throughout the learning phase

Schizophrenia is a complex, chronic, and disabling disorder

Keefe RS, Eesley CE, Poe MP. Defining a cognitive function decrement in schizophrenia. Biol Psychiatry. 2005 Mar 15;57(6):688-91. doi: 10.1016/j.biopsych.2005.01.003. PMID: 15780858.

Charlson FJ, Ferrari AJ, Santomauro DF, et al. Global Epidemiology and Burden of Schizophrenia: Findings From the Global Burden of Disease Study 2016. Schizophr Bull. 2018;44(6):1195-1203. doi:10.1093/schbul/sby058 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6192504/

https://www.treatmentadvocacycenter.org/fixing-the-system/features-and-news/3828-research-weekly-2016-prevalence-of-treated-and-untreated-severe-mental-illness-by-state#:~:text=Schizophrenia%20affected%202.7%20million%20US,a%20prevalence%20rate%20of%202.2%25.

Nuechterlein KH, Green MF, Kern RS, Baade LE, Barch DM, Cohen JD, Essock S, Fenton WS, Frese FJ 3rd, Gold JM, Goldberg T, Heaton RK, Keefe RS, Kraemer H, Mesholam-Gately R, Seidman LJ, Stover E, Weinberger DR, Young AS, Zalcman S, Marder SR. The MATRICS Consensus Cognitive Battery, part 1: test selection, reliability, and validity. Am J Psychiatry. 2008 Feb;165(2):203-13. doi: 10.1176/appi.ajp.2007.07010042. Epub 2008 Jan 2. PMID: 18172019.

Relevant reference publications and notes (2 of 3)

Cognitive impairment underlies functional disability in schizophrenia and is not addressed by current treatments

Bowie CR, Harvey PD. Cognitive deficits and functional outcome in schizophrenia. Neuropsychiatr Dis Treat. 2006;2(4):531-536. doi:10.2147/nedt.2006.2.4.531

Cloutier M, Aigbogun MS, Guerin A, Nitulescu R, Ramanakumar AV, Kamat SA, DeLucia M, Duffy R, Legacy SN, Henderson C, Francois C, Wu E. The Economic Burden of Schizophrenia in the United States in 2013. J Clin Psychiatry. 2016 Jun;77(6):764-71. doi: 10.4088/JCP.15m10278. PMID: 27135986..

Keefe RS, Eesley CE, Poe MP. Defining a cognitive function decrement in schizophrenia. Biol Psychiatry. 2005 Mar 15;57(6):688-91. doi: 10.1016/j.biopsych.2005.01.003. PMID: 15780858.

Longstanding investigation of NO-sGC-cGMP pathway in schizophrenia

Reif A, Herterich S, Strobel A, Ehlis AC, Saur D, Jacob CP, Wienker T, Töpner T, Fritzen S, Walter U, Schmitt A, Fallgatter AJ, Lesch KP. A neuronal nitric oxide synthase (NOS-I) haplotype associated with schizophrenia modifies prefrontal cortex function. Mol Psychiatry. 2006 Mar;11(3):286-300. doi: 10.1038/sj.mp.4001779. PMID: 16389274.

Freudenberg F, Alttoa A, Reif A. Neuronal nitric oxide synthase (NOS1) and its adaptor, NOS1AP, as a genetic risk factors for psychiatric disorders. Genes Brain Behav. 2015 Jan;14(1):46-63. doi: 10.1111/gbb.12193. PMID: 25612209.

Candemir E, Kollert L, Weißflog L, Geis M, Müller A, Post AM, O'Leary A, Harro J, Reif A, Freudenberg F. Interaction of NOS1AP with the NOS-I PDZ domain: Implications for schizophrenia-related alterations in dendritic morphology. Eur Neuropsychopharmacol. 2016

Relevant reference publications and notes (3 of 3)

CIAS linked to reduced NO-sGC-cGMP signaling and associated altered dendritic spine morphology

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Figure 5. Theoretical model for NOS1AP interactions. (a) NOS1AP interactions predicted by previous studies (Carrel et al., 2009, Courtney et al., 2014, Fang et al., 2000, Guan et al., 2008, Jaffrey et al., 2002, Kamiya et al., 2006, Li et al., 2013, Nagasaka et al., 2010, Richier et al., 2010). (b) NOS1AP competes with PSD-95 to interact with NOS-I PDZ domain and disrupts the NOS-I/PSD-95/NMDA receptor complex. The stability of these interactions is important for dendritic development regulation. (c) NOS1AP overexpression leads to abnormal dendritic development by interrupting NOS-I interaction with post-synaptic density and possibly requiring interactions with downstream signaling proteins.