Systematic Processing of Full Genomic Analysis of ANAVEX®2-73 Phase 2a Alzheimer's Disease Study Identifies Biomarkers Enabling a Precision Medicine Approach

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Disclosures

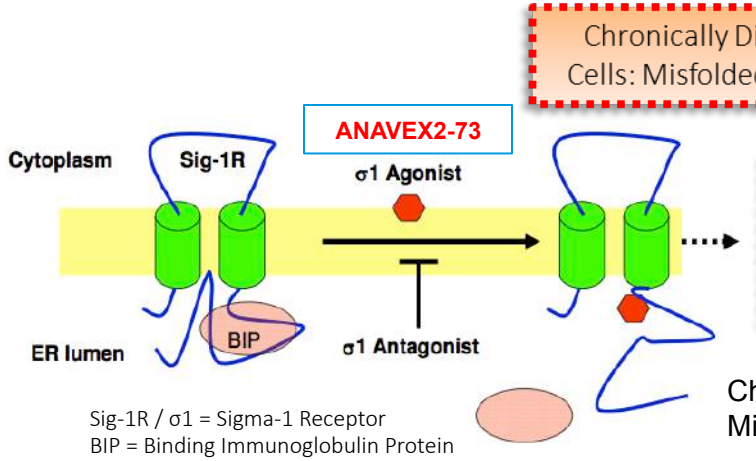
Harald Hampel serves as Senior Associate Editor for the Journal Alzheimer's & Dementia; he is the speaker of the Alzheimer Precision Medicine Initiative (APMI), he received lecture fees from Biogen and Roche, research grants from Pfizer, Avid, and MSD Avenir (paid to the institution), travel funding from Functional Neuromodulation, Axovant, Eli Lilly and company, and Oryzon Genomics, consultancy fees from Axovant, Anavex, Oryzon Genomics, Functional Neuromodulation, and participated in scientific advisory boards of Functional Neuromodulation, Axovant, Eli Lilly and company, Oryzon Genomics, Roche Diagnostics

Introduction ANAVEX®2-73 Phase 2a Alzheimer study

- **ANAVEX®2-73** is a **novel compound** relevant to AD and neurodegenerative, neurological diseases
- **Targeting the Sigma-1 receptor (SIGMAR1)**
- **Selective under pathological conditions while sparing normal physiological actvity, thus limiting adverse side effects[#]**
- **ANAVEX®2-73 is an orally available small molecule** that serves as an intracellular chaperone and functional modulator of **calcium homeostasis** and **synaptic plasticity** through targeting protein-misfolding, oxidative stress, mitochondrial dysfunction, inflammation, cellular stress

[#]Nguyen et al. J Pharmacol Sciences 127 (2015) 17-29

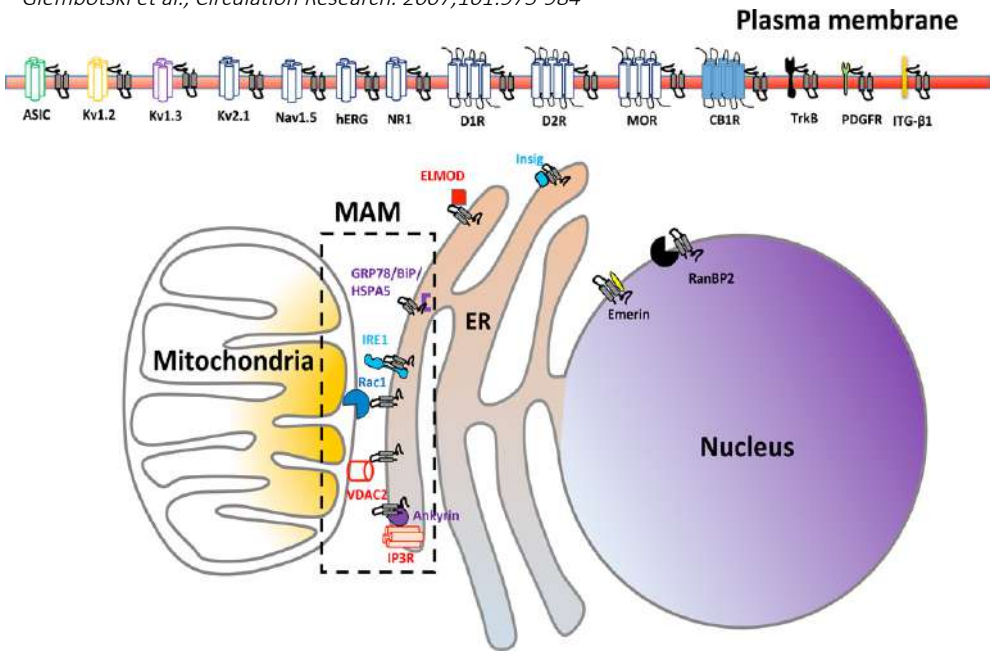
ANAVEX®2-73 activates the Sigma-1 receptor restoring cellular homeostasis



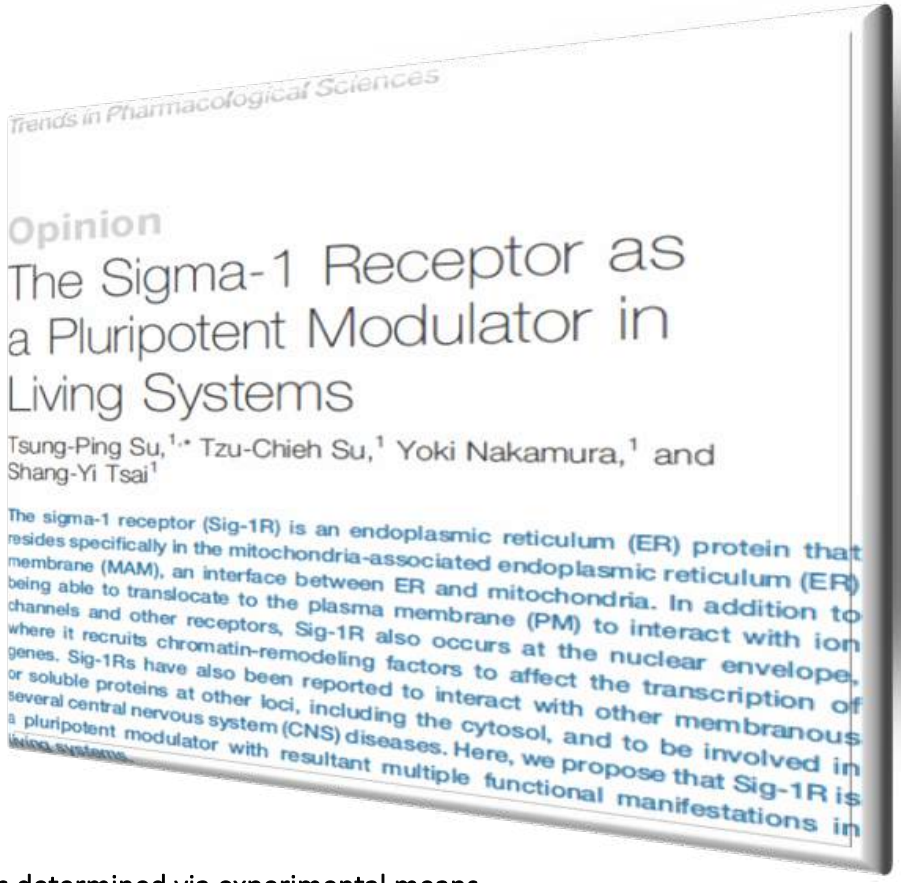
Restoring Homeostasis and Neuroplasticity

stimulating cells to regain functionality & plasticity

Source: Schematic approximation adapted from Miki et al, Dec 9. doi: 10.1111/neup.12080 Neuropathology 2013
Glembotski et al., Circulation Research. 2007;101:975-984



Su et al., Trends Pharmacol Sci. 2016


Trends in Pharmacological Sciences

Opinion

The Sigma-1 Receptor as a Pluripotent Modulator in Living Systems

Tsung-Ping Su,[1,*] Tzu-Chieh Su,[1] Yoki Nakamura,[1] and Shang-Yi Tsai[1]

The sigma-1 receptor (Sig-1R) is an endoplasmic reticulum (ER) protein that resides specifically in the mitochondria-associated endoplasmic reticulum (ER) membrane (MAM), an interface between ER and mitochondria. In addition to being able to translocate to the plasma membrane (PM) to interact with ion channels and other receptors, Sig-1R also occurs at the nuclear envelope, where it recruits chromatin-remodeling factors to affect the transcription of genes. Sig-1Rs have also been reported to interact with other membranous or soluble proteins at other loci, including the cytosol, and to be involved in several central nervous system (CNS) diseases. Here, we propose that Sig-1R is a pluripotent modulator with resultant multiple functional manifestations in

Two-trans-membrane SIGMAR1 is an ER protein that resides in the mitochondrial assoc. ER membrane (MAM)
Translocates to the cytosol/plasma membrane and interacts with numerous receptors, ion channels and proteins as determined via experimental means

Evidence that activation of SIGMAR1 impacts relevant pathophysiological pathways

- **Sigma-1 receptor ligands** have been shown to modulate multiple aspects of neurodegenerative processes, affecting both neurons and glia
- i.e. resulting in *reduction* of beta amyloid, hyperphosphorylated tau, oxidative stress, neuroinflammation - leading to synaptic dysfunction and neuronal loss

Journal of Pharmacological Sciences 127 (2015) 17–29

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Critical review

Role of sigma-1 receptors in neurodegenerative diseases

Linda Nguyen [a, b, c], Brandon P. Lucke-Wold [d], Shona A. Mookerjee [e], John Z. Cavendish [d], Matthew J. Robson [f], Anna L. Scandinaro [a, b, c], Rae R. Matsumoto [a, b, c, e, *]

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Neuroprotective effects of sigma-1 receptor agonists against beta-amyloid-induced toxicity

Agostino Marrazzo,[1] Filippo Caraci,[1] Elisa Trovato Salinaro,[1] Tsung-Ping Su,[3] Agata Copani[1,2,CA] and Giuseppe Ronsisvalle[1]

Neuropsychopharmacology (2013) 38, 1706–1723

www.neuropsychopharmacology.org

Blockade of Tau Hyperphosphorylation and $A\beta_{1-42}$ Generation by the Aminotetrahydrofuran Derivative ANAVEX2-73, a Mixed Muscarinic and σ_1 Receptor Agonist, in a Nontransgenic Mouse Model of Alzheimer's Disease

Valentine Lahmy[1,2,3,4], Johann Meunier[4], Susanna Malmström[4], Gaelle Naert[1,2,3], Laurent Givalois[1,2,3], Seung Hyun Kim[5], Vanessa Villard[4], Alexandre Vamvakides[6] and Tangui Maurice*[1,2,3]

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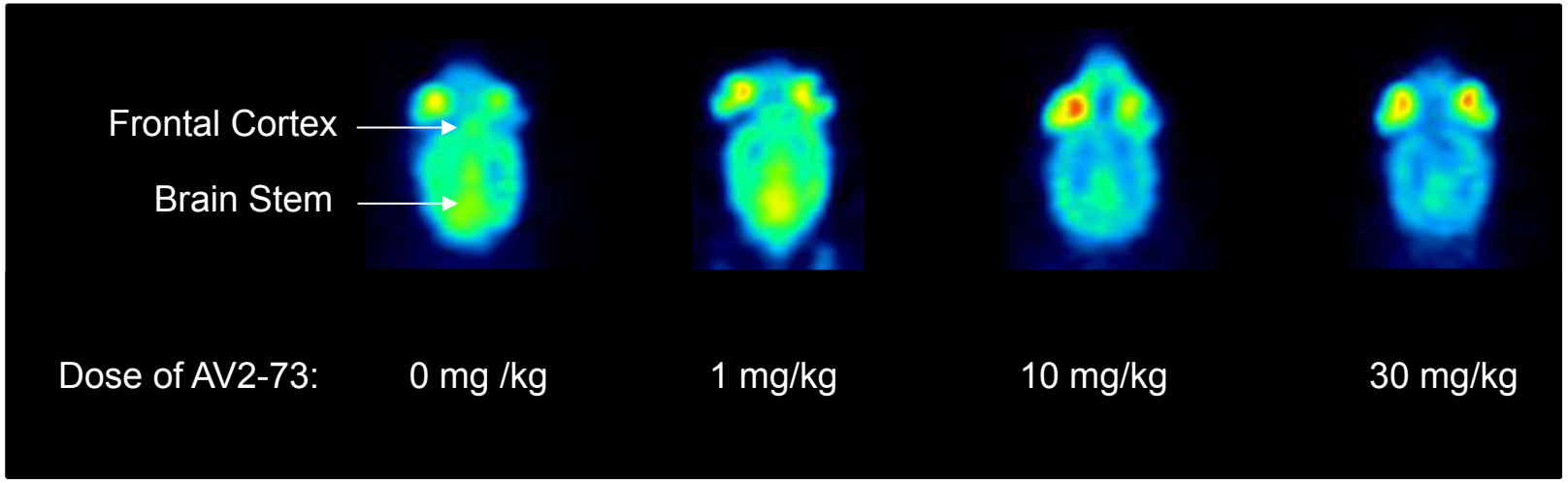
ANAVEX®2-73 phase 2a Alzheimer study

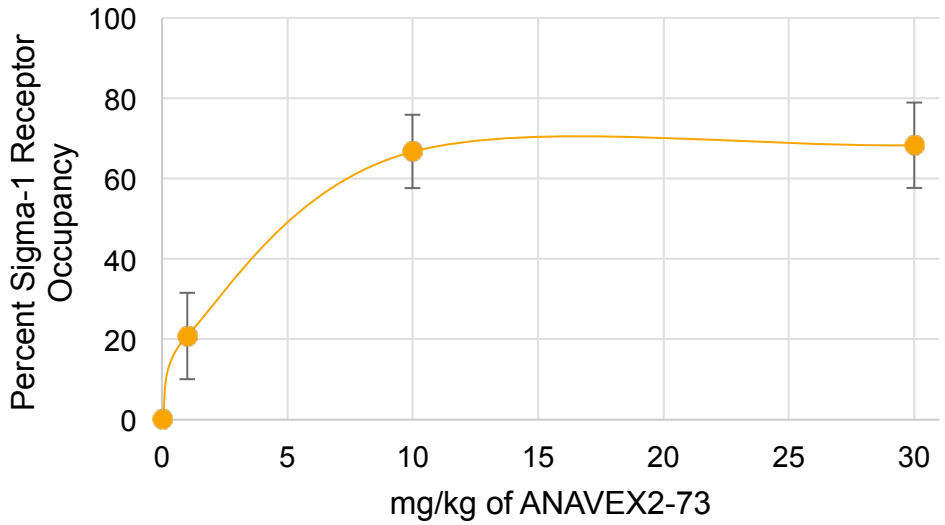
- 57-week proof-of-concept randomized open-label phase 2a study of ANAVEX®2-73 in 32 mild-moderate AD dementia patients (MMSE 16-28)[#]
- Results showed favorable safety profile
- Dose-dependent target engagement
- Favorable dose-response using cognitive and functional endpoints

[#]ClinicalTrials.gov Identifier: NCT02244541

Confirmation with quantitative PET Scan:
Dose-dependent ANAVEX®2-73 target engagement with the Sigma-1 receptor

2D [^{18}F]FTC-146-PET imaging of ANAVEX®2-73

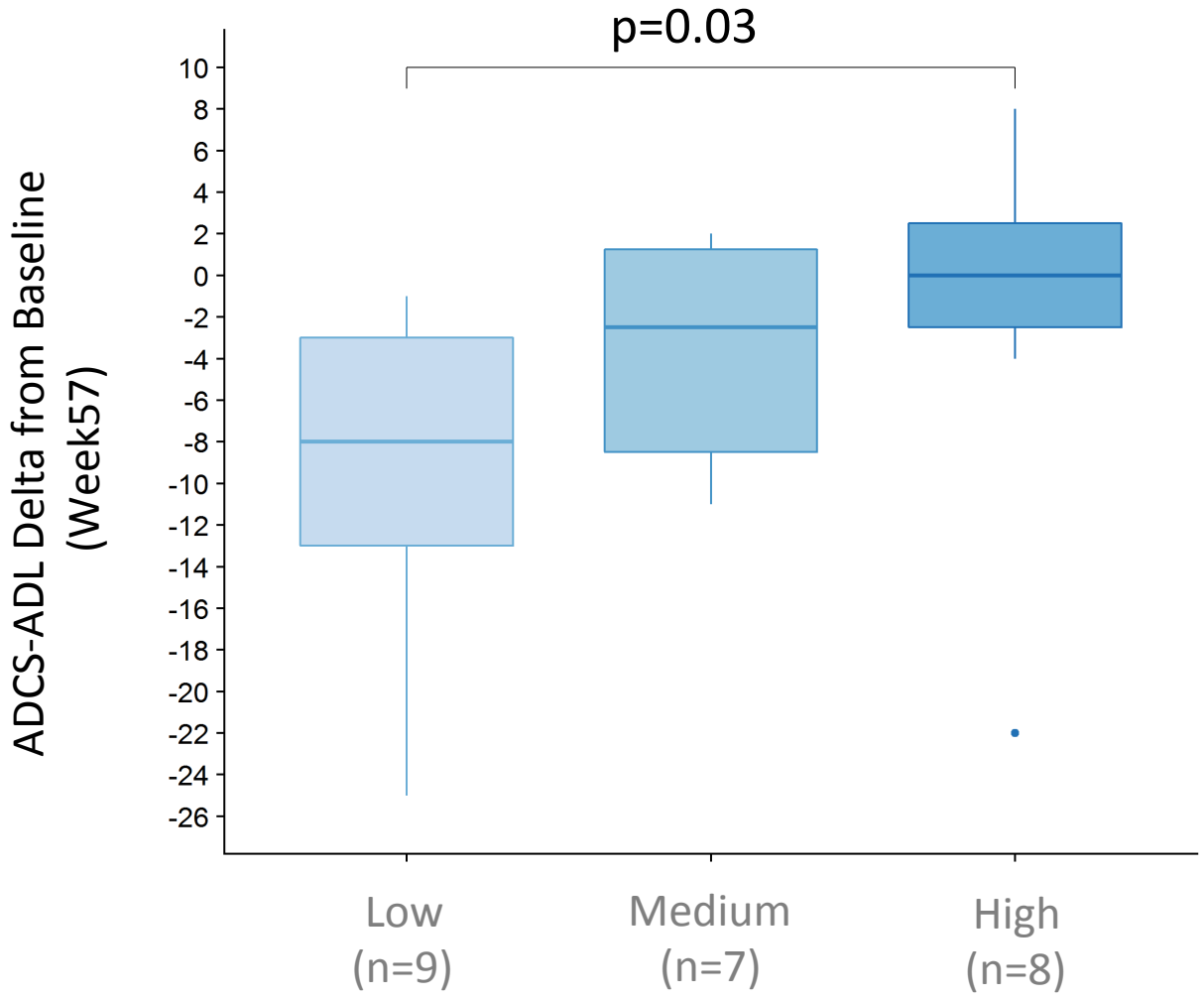




Sigma-1 receptor target occupancy study with quantitative PET analysis of ANAVEX®2-73 (*Presented at AAIC 2018 - P4-262)*

Significant relation between ANAVEX®2-73 concentration and response Alzheimer's Disease Cooperative Study Activities of Daily Living 23-item scale (ADCS-ADL)

Concentration ANAVEX2-73 High => ADCS-ADL Delta High



p = p-value of Mann–Whitney U test

All n=24 patients in study at week 57

ANAVEX®2-73 phase 2a extension Alzheimer study

- +104/+104-week extension study in 21 patients[#]

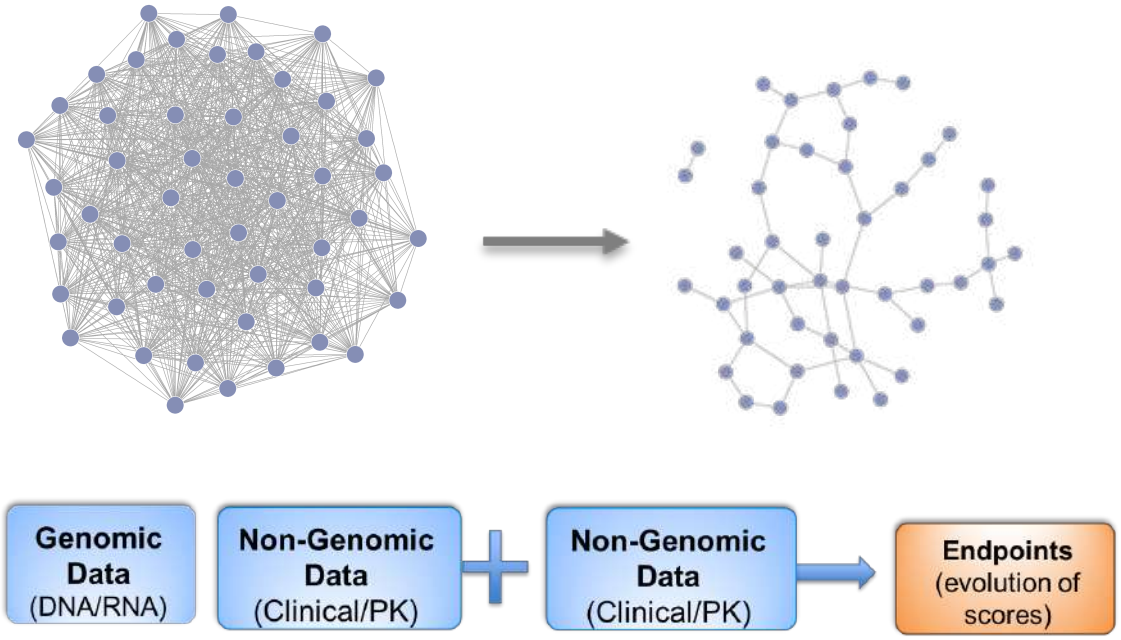
- With full NGS exome (DNA) and transcriptome (RNA) sequencing

[#]*ClinicalTrials.gov Identifier: NCT02756858*

RNA/DNA sequences/study data analyzed using neuroinformatics platform KEM®

Systematic unbiased generation of all possible causal associations in a multi-parametric dataset

- >20 million relations extracted and characterized from study data
- Identification & ranking of biomarkers relating to outcome derived from a small n of samples, avoiding overfitting



Advanced Machine Learning - Artificial Intelligence Platform Supporting Clinical Trial Design

KEM® using Formal Concept Analysis (FCA)

Comprehensively analyzes complex datasets by measuring all logical relations within a dataset, exploring all combinations of parameters and endpoints

Identifies most relevant and powerful causal relations, revealing hidden relationships

Successfully utilized in oncology and other disease areas

Focus on 102 Genes

BCHE	LARGE1	PLD3	IL18	KMT2D	MAPT
ABCA7	MEF2C	PICALM	IFNG	PSEN1	SLC14A1
CR1	NFIX	NME8	RORB	IL10	ERCC8
MS4A4E	PDE4D	MS4A6E	RORA	IL17A	SLC24A4
BDNF	SHANK3	MS4A4A	PER3	GRIN2B	APP
COMT	ST3GAL3	INPP5D	PER2	MTUS1	RTN1
CTNNBL1	SUOX	HLA-DRB4	PER1	KCNH1	RIN3
FRMD4A	TCF4	HLA-DRB1	NR1D1	PSEN2	DPYD
PDE7A	THRB	FERMT2	NPAS2	FMR1	TOMM40
SORL1	UBA7	EPHA1	CRY2	SRRM4	BCA7
ZNF224	DAG1	DTNBP1	CRY1	KANSL1	PCP4
APOE	SNCA	DSG2	CLOCK	PTK2B	FOXP1
AFF3	GBA	CLU	ARNT	UBQLN1	CLN3
AMT	CNTN1	CELF1	PPARG	VSNL1	TREM2
ARFGEF2	SIGMAR1	CD33	GCKR	GMPPB	BIN1
BCL11A	RAB10	CD2AP	ADAMTS9	REST	MDH1
C12orf65	ZCWPW1	CASS4	CHN1	LRRK2	SNAP25

Focus on genes associated with measures: MMSE & ADCS-ADL

Focus on other relevant genes

Analyses provided patient selection *Gene Variant Markers*

DNA variant analysis shows patient **selection gene (variant) biomarkers** related to SIGMAR1 gene

The presence of specific **variants results in worse outcomes**, and that of others in **improved outcomes** for MMSE[1] and ADCS-ADL[2]

[1] *Mini Mental State Examination (MMSE)*
[2] *Alzheimer's Disease Co-operative Study – Activities of Daily Living Inventory (ADCS-ADL)*

Gene variant patient selection biomarkers confirmed through *RNA expression*

RNA expression analyses can confirm the information derived from DNA (gene) variants as patient selection biomarkers

RNA expression analyses show **concordance with results for the SIGMAR1 variant markers**

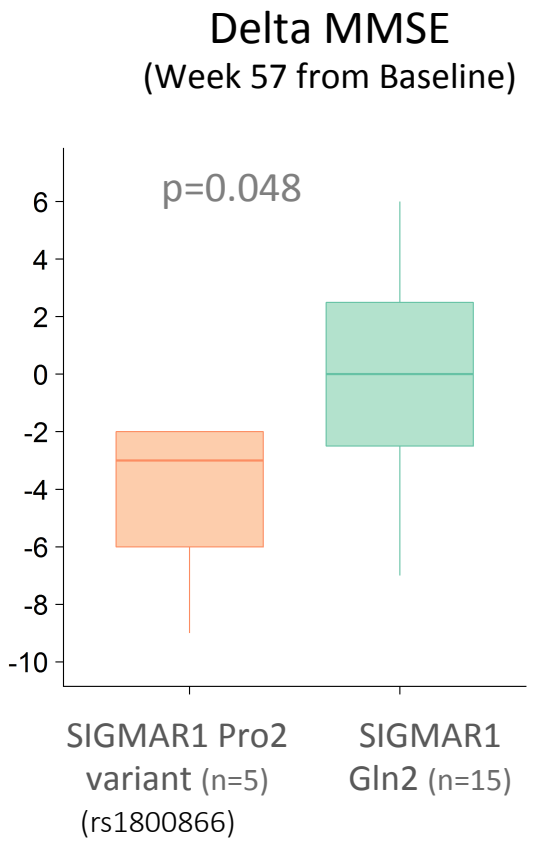
ANAVEX®2-73 Alzheimer Phase 2a extension study

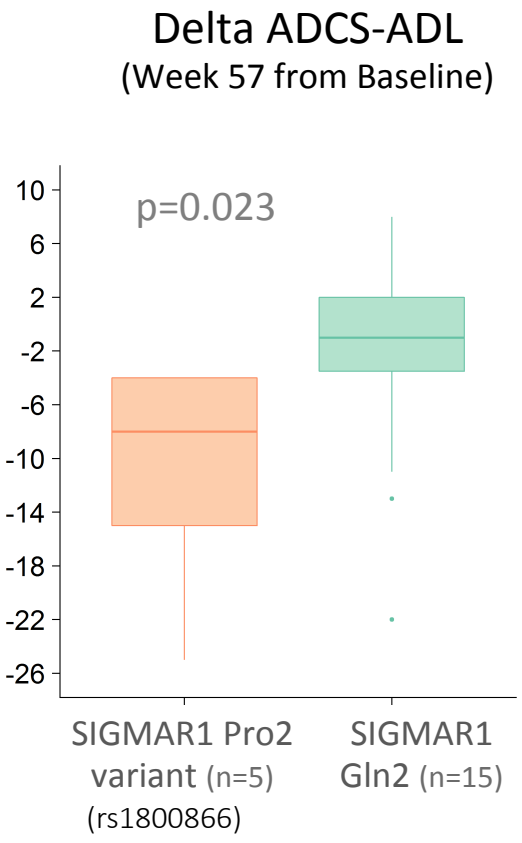
- AD patients with **wild-type SIGMAR1 gene** were found to have **improved benefit** from ANAVEX®2-73

- AD patients with a **variant of the SIGMAR1 gene (rs1800866)**, were found to have a **limited benefit** from ANAVEX®2-73

- Majority of AD population, about **80% has no variant SIGMAR1 gene**, hence the majority of patients is expected to benefit from ANAVEX®2-73

SIGMAR1 Gene Variant associated with decreased response

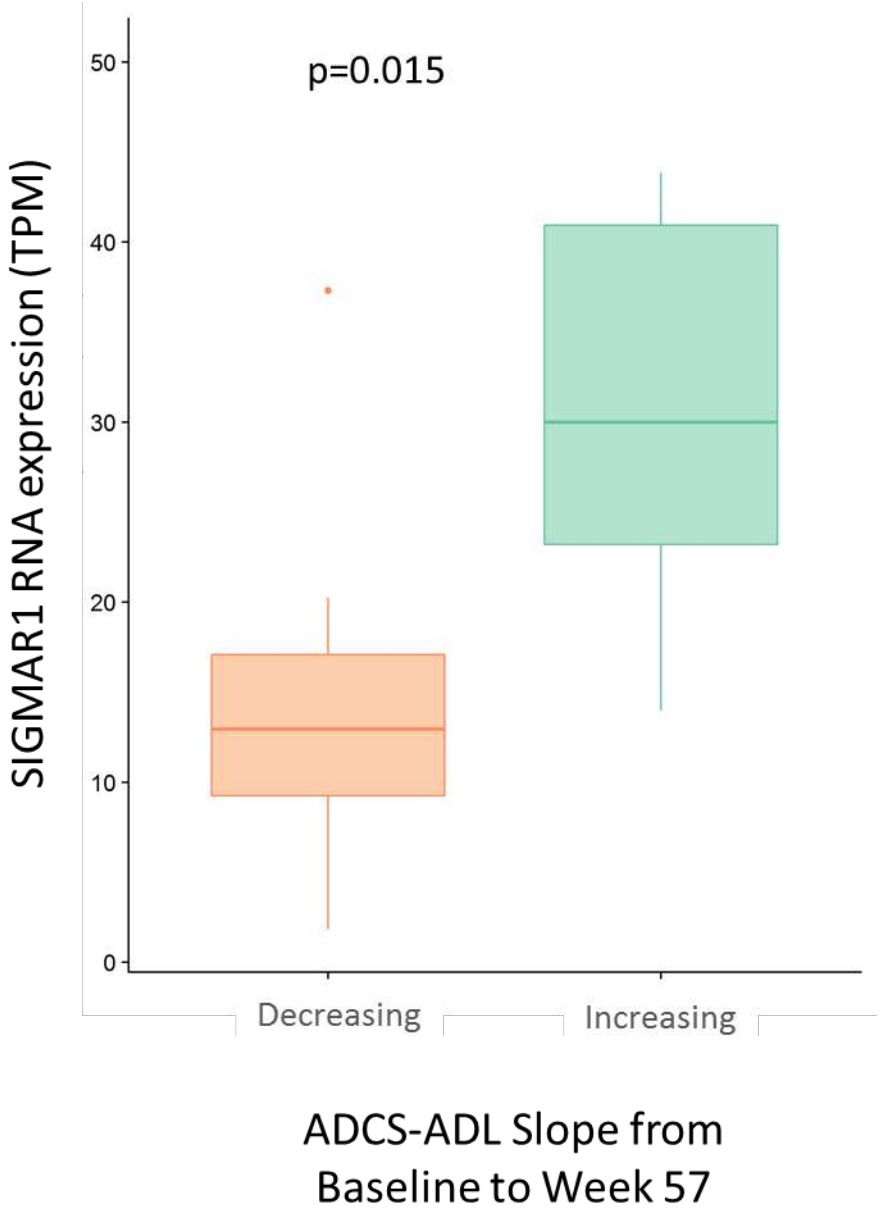




p = p-value of Mann–Whitney U test

All n=20 patients in study at week 57 with available genomic data

Confirmation: Significant correlation of ADCS-ADL response and SIGMAR1 RNA expression levels

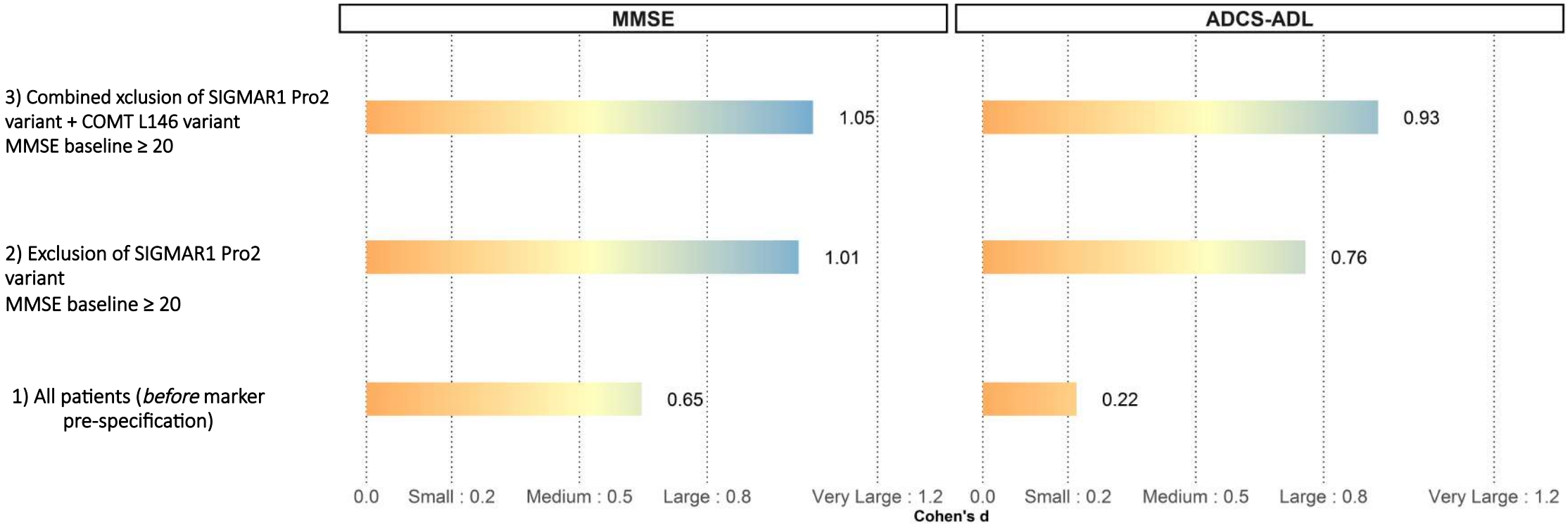


p = p-value of Mann–Whitney U test

All n=20 patients in study at week 57 with available genomic data

Gene markers improve effect size (Cohen's d) with ANAVEX®2-73

Improvement of scores in week 57 from baseline



Higher Cohen's d implies less patients are needed to show a significant difference between placebo arm and ANAVEX®2-73 arm in the Phase2b/3 study

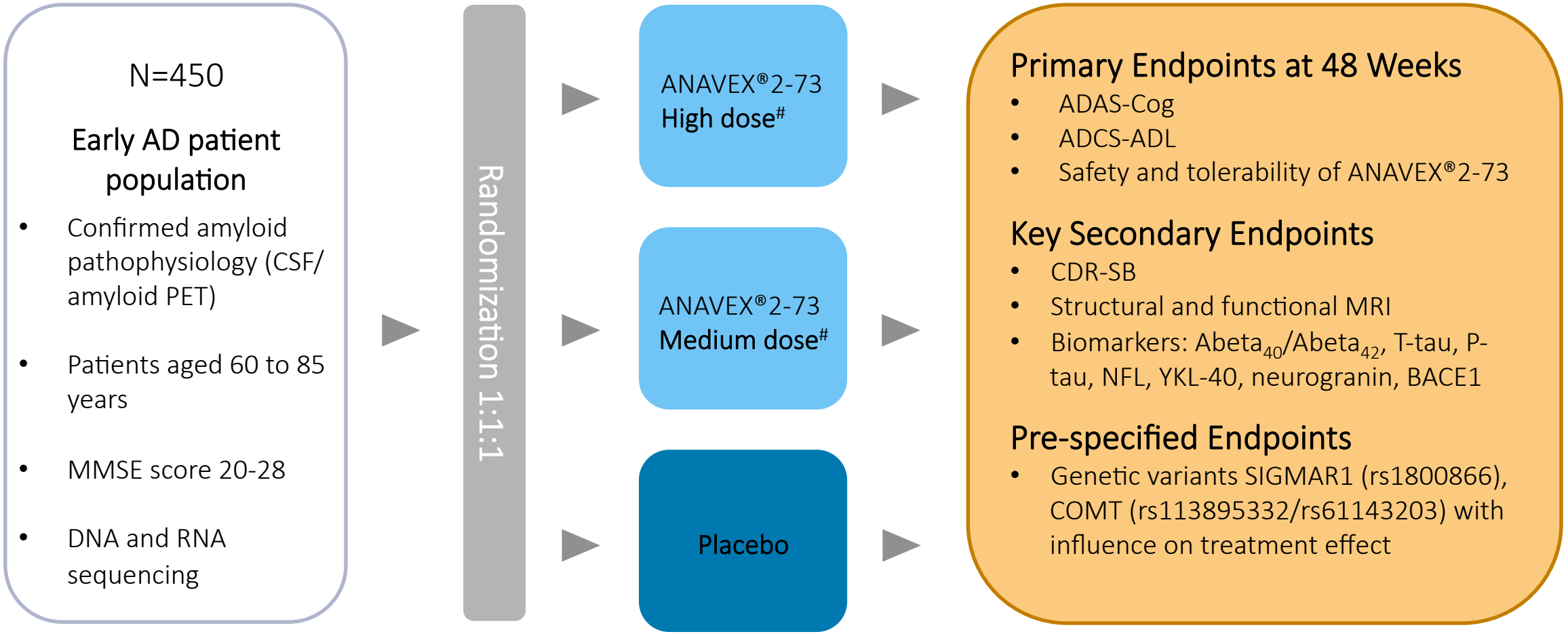
Summary ANAVEX®2-73 study

- **Confirmed safety & dose-dependent target engagement** with SIGMAR1 using **PET**
- **Exome sequencing showed response-linked gene variants using formal concept analyses (FCA)**
- **Identified SIGMAR1/COMT variants** shown to be **linked to limited clinical response**
- Targeted therapy **benefit** on **WT variants** is expected for about **80%** of patient population
- **Confirmation through high SIGMAR1 RNA expression** levels **linked to clinical response**
- **Results consistent** across cognition (**MMSE**) and activities of daily living (**ADCS-ADL**)
- **Identified actionable genetic variants support enrichment with genetic biomarkers in the clinical development of** ANAVEX®2-73

Targeted therapy studies approved to initiate:

- ✓ Alzheimer's Disease - Phase 2b/3
- ✓ Parkinson's Disease Dementia - Phase 2

ANAVEX®2-73 phase 2b/3 early Alzheimer's Disease study design



 # Restricted to maintain complete blinding

Acknowledgments

Developing biomarker-guided targeted therapies for neurodegenerative diseases

Thanks to:

- Principal Investigators & clinical sites' study staff
- Data safety review committee
- Anavex SAB
- Most of all, grateful acknowledgement of the contribution of the participating AD patients and their caregivers

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