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

Harald Hampel, MD, PhD, Mohammad Afshar, MD, PhD, Frédéric Parmentier, PhD, Coralie Williams, MSc, Adrien Etcheto, MSc, Federico Goodsaid, PhD, Emmanuel O Fadiran, PhD, Christopher U Missling, PhD

1Department of Neurology, Sorbonne University, Paris, France; 2Ariana Pharma, Paris, France, 3Regulatory Pathfinders LLC, San Francisco, CA, 4Anavex Life Sciences Corp., New York, NY
Disclosures

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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 activity, 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

Chronically Distressed Cells: Misfolded Proteins

Restoring Homeostasis and Neuroplasticity

stimulating cells to regain functionality & plasticity

Sig-1R / σ1 = Sigma-1 Receptor
IBP = Binding Immunoglobulin Protein

Glembotski et al., Circulation Research. 2007;101:975-984

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
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

- Frontal Cortex
- Brain Stem

Dose of AV2-73:
- 0 mg/kg
- 1 mg/kg
- 10 mg/kg
- 30 mg/kg

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

<table>
<thead>
<tr>
<th>Concentration AV2-73 (ng/mL), Part B</th>
<th>ADCS-ADL Delta from Baseline (Week57)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low (n=9)</td>
<td></td>
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<tr>
<td>Medium (n=7)</td>
<td></td>
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<tr>
<td>High (n=8)</td>
<td></td>
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</tbody>
</table>

p = 0.03

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

<table>
<thead>
<tr>
<th>Focus on genes associated with measures: MMSE &amp; ADCS-ADL</th>
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</thead>
<tbody>
<tr>
<td><strong>BCH</strong></td>
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<tr>
<td><strong>ABCA7</strong></td>
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<tr>
<td><strong>CR1</strong></td>
</tr>
<tr>
<td><strong>MS4A4E</strong></td>
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<tr>
<td><strong>BDNF</strong></td>
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<tr>
<td><strong>COMT</strong></td>
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<tr>
<td><strong>CTNNBL1</strong></td>
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<tr>
<td><strong>FRMD4A</strong></td>
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<tr>
<td><strong>PDE7A</strong></td>
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<tr>
<td><strong>SORL1</strong></td>
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<tr>
<td><strong>ZNF224</strong></td>
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<tr>
<td><strong>APOE</strong></td>
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<tr>
<td><strong>AFF3</strong></td>
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<tr>
<td><strong>AMT</strong></td>
</tr>
<tr>
<td><strong>ARFGEF2</strong></td>
</tr>
<tr>
<td><strong>BCL11A</strong></td>
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<tr>
<td><strong>C12orf65</strong></td>
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</tbody>
</table>

Focus on other relevant genes
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\(^2\).
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.
- 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

**Delta MMSE**  
(Week 57 from Baseline)

- SIGMAR1 Pro2 variant (n=5)  
  (rs1800866)
- SIGMAR1 Gln2 (n=15)

\[ p = 0.048 \]

**Delta ADCS-ADL**  
(Week 57 from Baseline)

- SIGMAR1 Pro2 variant (n=5)  
  (rs1800866)
- SIGMAR1 Gln2 (n=15)

\[ p = 0.023 \]

\*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

\[ p = 0.015 \]

\[ p = p\text{-value of Mann–Whitney U test} \]

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

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

**Primary Endpoints at 48 Weeks**
- ADAS-Cog
- ADCS-ADL
- Safety and tolerability of ANAVEX®2-73

**Key Secondary Endpoints**
- CDR-SB
- Structural and functional MRI
- Biomarkers: Abeta_{40}/Abeta_{42}, T-tau, P-tau, NFL, YKL-40, neurogranin, BACE1

**Pre-specified Endpoints**
- Genetic variants SIGMAR1 (rs1800866), COMT (rs113895332/rs61143203) with influence on treatment effect

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N=450

**Early AD patient population**
- Confirmed amyloid pathophysiology (CSF/amyloid PET)
- Patients aged 60 to 85 years
- MMSE score 20-28
- DNA and RNA sequencing

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Randomization 1:1:1

- ANAVEX®2-73 High dose
- ANAVEX®2-73 Medium dose
- Placebo

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*Restricted to maintain complete blinding*
Acknowledgments

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