Study of the Mechanism of Action of *Blarcamesine* (ANAVEX®2-73): Whole Blood Transcriptomics Analysis (RNAseq) Identifies Treatment Impact on Compensatory Pathways by Restoring Key Neurodegenerative Pathways Functionality, including Alzheimer's and Parkinson's Disease Pathways

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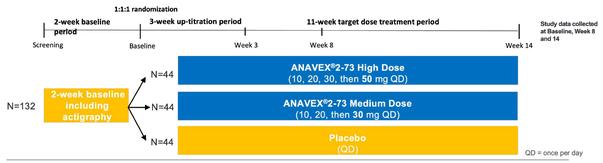
Overview

- ANAVEX®2-73 (*blarcamesine*) is a Sigma-1 receptor (SIGMAR1) agonist that mechanistically focuses on a new target relevant to Alzheimer's disease, Parkinson's disease and other neurological diseases
- SIGMAR1 activation is a compensatory mechanism to chronic CNS diseases
- The direct occupancy of ANAVEX®2-73 at SIGMAR1 has been established previously using quantitative PET scan
- Full genomic analysis of ANAVEX®2-73-PDD-001 Phase 2 study in patients with Parkinson's Disease Dementia (PDD) assessed biomarkers of response exploring potential for a Precision Medicine approach
- The overall goal of this study is to specify the ANAVEX®2-73 response pathways by identifying the most differentially expressed genes and characterizing the pathways involved

ANAVEX®2-73 PoC Phase 2 PDD Study Design

A Phase 2 trial to Assess the Safety, Tolerability and Efficacy of ANAVEX®2-73 (*blarcamesine*) Oral Capsules in the Treatment of Parkinson's Disease Dementia



- **PDD Patient Population**
  - Diagnosis of probable Parkinson's disease dementia
  - Diagnosis of idiopathic Parkinson's disease
  - Patients aged ≥ 50 years
  - MoCA score 13-23
- **Key Primary and Secondary Endpoints**
  - Safety and tolerability
  - CDR Cognitive Domain of Attention
  - Sleep function
  - MDS-UPDRS
  - Actigraphy (24-hour monitoring)
  - Entire DNA and RNA sequencing
- **Pre-specified Endpoints**
  - Genetic variants SIGMAR1 (rs1800866),
  - COMT(rs113895332/rs611432 03) with influence on treatment effect

ANAVEX®2-73-PDD-001 is a Proof of Concept (PoC) Phase 2, multicenter, randomized, double-blind, placebo-controlled, parallel-group, 3-arm, 14-week study

Data description

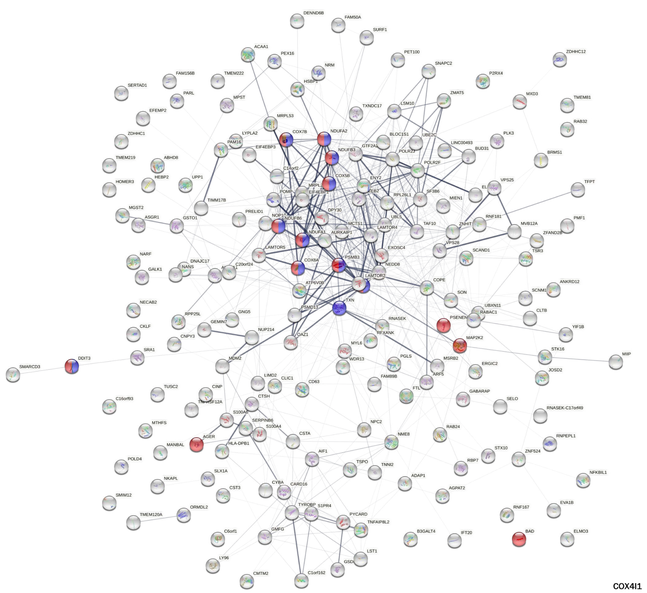
- Whole blood transcriptomics analysis (RNAseq) of patients with Parkinson's disease dementia was performed for the ANAVEX®2-73-PDD-001 study at two timepoints: Baseline and Week 14 (end of trial)
- All available RNA samples with RIN≥4[1] (130 samples) were analyzed
- The expression of 14,150 genes at these two timepoints were analyzed from both placebo and ANAVEX®2-73 treated patients

[1] RIN = RNA integrity number

In the two clusters of genes impacted by ANAVEX®2-73, over 70% of the genes are known to have biological interactions. Pathway enrichment analysis revealed that multiple neurodegenerative pathways, including Alzheimer's disease and Parkinson's disease were significantly (adjusted p-value < 0.005) enriched for these genes

Cluster 1 (213 genes)

79% of the genes (169) have biological interactions

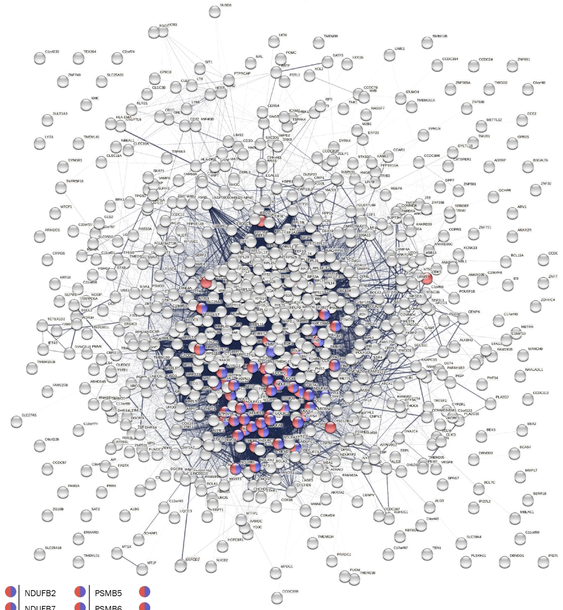


COX5B		PSMA4	
COX7B		PSMB3	
COX8A		PSMD13	
DDIT3		AGER	
NDUFA1		MAP2K2	
NDUFA2		PSENEN	
NDUFB3		TXN	
NDUFB6			

Pathway	adjusted p-value
Alzheimer's disease	0.004
Parkinson's disease	0.004
Other Neurodegenerative diseases	0.004

Cluster 2 (962 genes)

70% of the genes (680) have biological interactions



COX4I1		NDUFB2	PSMB5
COX6A1		NDUFB7	PSMB6
COX6B1		**NDUFB8**	PSMB7
COX6C		NDUFB9	PSMD8
COX7A2		NDUFC1	SLC25A6
COX7C		NDUFC2	UQCR10
CYC1		NDUFS3	UQCR11
NDUFA11		NDUFS4	UQCRH
NDUFA12		**NDUFS5**	UQCRQ
NDUFA13		NDUFS6	CDK5
NDUFA3		NDUFS7	GAPDH
NDUFA4		NDUFS8	HRAS
NDUFA7		PSMA2	HSD17B10
NDUFA8		PSMA6	PARK7
NDUFB1		PSMA7	RPS27A
NDUFB10		PSMB1	TXN2
NDUFB11		PSMB4	

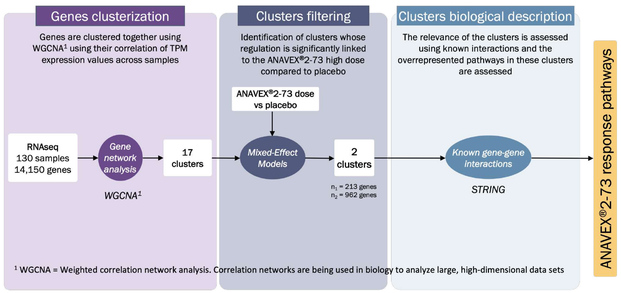
Pathway	adjusted p-value
Alzheimer's disease	<0.001
Parkinson's disease	<0.001
Other Neurodegenerative diseases	<0.001

In the gene networks, genes in KEGG 2022 pathways database are highlighted:

Alzheimer's disease: red nodes
Parkinson's disease: blue nodes

p-value: Fisher's test; adjustment: Benjamini-Hochberg

Multi-disciplinary method to identify ANAVEX®2-73 genes response pathways



Genes clusterization

- Genes were clustered together using WGCNA[1], starting from their correlation of TPM[2] expression values across samples: WGCNA created 17 clusters (size: 13 to 11,190 genes)
- The cluster's eigengene[3] was used as summary of expression level of the cluster

Clusters filtering

- For each cluster, the eigengene is used as target variable of a linear mixed effect model with 3 covariates: Dose, patient, and timepoint
- Clusters without significant (Dunnett's test) contrasts between treated and placebo across all timepoints are filtered out
- The ratio between cluster size and number of pathways was calculated and the top 2 clusters were eventually retained

Clusters biological description

- For each cluster, functional interactions between the genes of the cluster were assessed using the STRING database (Edge score >0.150; no text mining edges)
- Genes that have no interaction with any other genes of the cluster were discarded, which led to a reduced cluster
- Pathway enrichment analysis was performed on these reduced clusters. False Discovery Rate (FDR)[4] with adjustment for multiple testing (Benjamini-Hochberg procedure) was used to characterize pathway overrepresentation

[1] WGCNA = Weighted correlation network analysis. Correlation networks are being used in biology to analyze large, high-dimensional data sets
[2] TPM = Transcript per million. TPM value represents a relative expression level that should be comparable between samples
[3] Eigengene = One of a set of a genes matrix that tabulates the mRNA or gene expression of the genes across the samples
[4] FDR = False Discovery Rate: adjustment for multiple testing with the Benjamini-Hochberg procedure

Expression levels of dysregulated neurodegenerative genes were restored by the therapeutic effect of ANAVEX®2-73

- Both identified clusters are up-regulated for treated patients compared to placebo: Cluster 2 eigengene expression was significantly increased for patients treated with ANAVEX®2-73 high oral dose compared to placebo (p = 0.021)
- These genes are known to be down-regulated in the pathology of both Alzheimer's disease[1,2] and Parkinson's disease[3,4]

[1] Liang WS, Reiman EM, Valla J, et al. Alzheimer's disease is associated with reduced expression of energy metabolism genes in posterior cingulate neurons. Proc Natl Acad Sci U S A. 2008;105(11):4441-4446. doi:10.1073/pnas.0709259105

[2] Lunnon K, Keohane A, Pidsley R, et al. Mitochondrial genes are altered in blood early in Alzheimer's disease. Neurobiol Aging. 2017;53:36-47. doi:10.1016/j.neurobiolaging.2016.12.029

[3] Hauser MA, Li YJ, Xu H, et al. Expression profiling of substantia nigra in Parkinson disease, progressive supranuclear palsy, and frontotemporal dementia with parkinsonism. Arch Neurol. 2005;62(6):917-921. doi:10.1001/archneur.62.6.917

[4] Hendrickx DM, Glaab E. Comparative transcriptome analysis of Parkinson's disease and Hutchinson-Gilford progeria syndrome reveals shared susceptible cellular network processes. BMC Med Genomics. 2020;13(1):114. Published 2020 Aug 18. doi:10.1186/s12920-020-00761-6

Key results

- Randomized, placebo-controlled clinical trial in 132 patients with Parkinson's disease dementia (PDD) included prespecified biomarkers of response as well as Whole Exome Sequencing DNA data and full RNA exome expression data collection. This study demonstrated dose-dependent, statistically significant improvement of dementia assessment, Quality of Episodic Memory with ANAVEX®2-73 (p=0.003) as well as significant improvement of Parkinson's assessment, MDS-UPDRS Total score (p=0.034) for patients treated with ANAVEX®2-73 high oral dose once daily during 14-week trial
- ANAVEX®2-73 transcriptomics analysis (RNAseq), identified a gene network that is differentially expressed in Parkinson's disease dementia (PDD) patients treated with ANAVEX®2-73 compared to placebo after 14 weeks of treatment
- Biological relevance of this gene network was assessed through pathway analysis and confirmed the impact of ANAVEX®2-73 treatment on pathways involved in neurodegenerative diseases, especially Alzheimer's disease and Parkinson's disease
- **While genes are down-regulated in Alzheimer's disease and Parkinson's disease, ANAVEX®2-73 singularly impacted expression levels of these genes in multiple pathways by countering the pathological down-regulation of genes in both Alzheimer's (p<0.005) and Parkinson's disease (p<0.005) and other degenerative diseases (p<0.005) and these may represent additional potential biomarkers of disease pathology and response**

Conclusion

- These findings will facilitate contextualization of upcoming readout of ANAVEX®2-73 Phase 2b/3 Alzheimer's disease clinical trial
- Findings further support pivotal studies in Parkinson's disease and Parkinson's disease dementia
- These findings also enable the translational identification of additional potential therapeutic indications for ANAVEX®2-73

References

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- *Reyes et al, Sci Rep. 2021*
- *Hauser et al, Arch Neurol, 2005*
- *Hendricks et al, BMC Med Genomics, 2020*
- *WGCNA: Langfelder & Horvath, BMC Bioinformatics, 2008*
- *STRING: Szklarczyk et al, NAR, 2021*
- *KEGG: Kanehisa & Goto, NAR, 2000*