

Background

Epigenetics in neuropsychiatry: While not yet a standard tool in clinical psychiatry, epigenetics shows promise in precision medicine, and measuring DNA methylation via epigenetic "aging clocks" can provide valuable insights for the development of novel "anti-aging" therapies

- Emerging studies indicate that accelerated biological aging, identified through DNA methylation analysis, may not only serve as a biomarker for Alzheimer's disease (AD) risk and progression but could also contribute to the pathogenesis of AD.^{1,2}
- Methylation within a specific gene can either enhance or reduce its expression; therefore, mechanisms by which differentially methylated cytosine-guanine base pairs (CpGs) influence pathways, or networks that regulate the progression of AD, are complex
- Because promoter methylation (PM) may inversely correlate with gene expression, analyzing gene-level aggregate PM can provide insight into potential transcriptional impact
- Biological clocks, such as PhenoAge and GrimAge, have recently been found to predict progression from cognitively normal aging to mild cognitive impairment or AD, worsening cognitive abilities, and neuroimaging markers of AD.³⁻⁵

Bezisterim (NE3107), an anti-inflammatory analog of androst-5-ene-3 β ,7 β ,17 β -triol, appears to show good safety and tolerability and is being developed for neurodegenerative diseases, such as AD.^{6,7}

- An exploratory analysis of whole blood DNA methylation from samples collected during a 7-month, randomized, placebo-controlled study in patients with mild-to-moderate probable AD (NCT04669028), found promoter-level methylation changes in key inflammatory pathways that contribute to AD pathology, as well as a reduction in epigenetic age acceleration (EAA) with bezisterim vs placebo⁸
- Analysis of aggregate methylation of CpGs in 9 of 13 clocks, those for which data were available, revealed 45 overlapping targets relating to AD that were differentially methylated by bezisterim vs placebo
- Genes were predominantly involved in inflammation and cognition, including those serving as transcriptional regulators that orchestrate broader gene networks

Relevant Genes and Neurological Assessments

GENES: *DGKZ*, diacylglycerol kinase zeta; *EIF4E*, eukaryotic translation initiation factor 4E; *MTOR*, mechanistic target of rapamycin; *NRBP1*, nuclear receptor-binding protein 1; *PTGER2*, prostaglandin E receptor 2; *RUNX2*, RUNX family transcription factor 2; *SAT1*, spermidine/spermine N1-acetyltransferase 1; *SOAT1*, sterol O-acyltransferase 1; *STAT3*, signal transducer and activator of transcription 3.

NEUROLOGICAL ASSESSMENTS: ADCOMS, Alzheimer's Disease Composite Score; ADL, Alzheimer's Disease Cooperative Study-Activities of Daily Living; CGIC, Alzheimer's Disease Cooperative Study-Clinical Global Impression of Change; Cog12, Alzheimer's Disease Assessment Scale-Cognitive Subscale; GST, Global Statistical Test; MMSE, Mini-Mental State Exam.

Patient Population and Study Objectives⁶

Patients aged 65 to 85 years diagnosed with mild-to-moderate probable AD were included in the original double-blind study⁶

- Mild-to-moderate AD defined as:
 - Clinical Dementia Rating (CDR) Standard Global Score of 1 to 2, inclusive
 - MMSE score of ≥ 14 and ≤ 24 at both screening and baseline
 - No prior evidence of vascular abnormalities or tissue damage via historical MRI or CT brain scans
- Randomized 1:1 to placebo or bezisterim 20 mg twice a day for 30 weeks

Samples from this study were further explored to determine if effects of bezisterim on DNA methylation of inflammatory programs might have a clinically relevant impact on age acceleration, cognition, and diseases of aging (Figure 1)

- 13 biological clocks associated with bezisterim EAA reduction (Figure 2) were further explored to identify correlations to clinical neurological measures

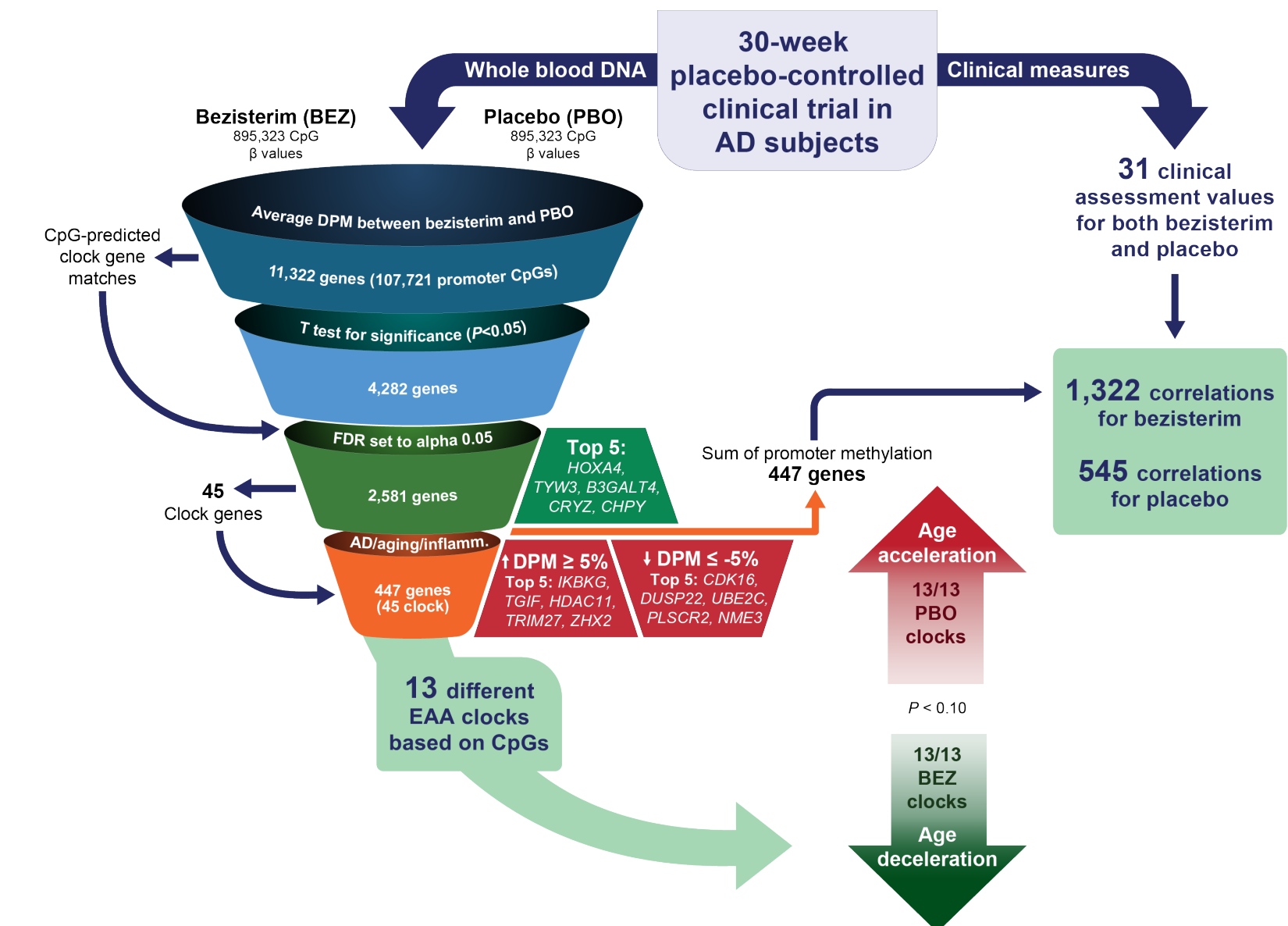
REFERENCES

1. Nakatsu D, et al. *Stem Cell Reports*. 2023;18(5):688-705. doi:10.1016/j.stemcr.2023.01.004. Erratum in: *Stem Cell Reports*. 2023;18(5):1246. doi:10.1016/j.stemcr.2023.03.017 2. Bonham L, et al. *Res Sq [Preprint]*. 2024 Oct 28;rs.3.rs-5273529. doi:10.21203/rs.3.rs-5273529/v1. Update in: *NPJ Dement*. 2025;1(1):7. doi:10.1038/s44003-025-00071-3 3. Merchant JP, et al. *Commun Biol*. 2023;6(1):503. doi:10.1038/s44003-023-04791-5 4. Schumuk LJ, et al. *Aging Cell*. 2025;24(4):e14444. doi:10.1111/acel.14444 5. Fruhwirth S, et al. *J Neuroimmunol*. 2024;390:578342. doi:10.1111/jneuroim.2024.578342 6. Reading CL, et al. *Front Neurosci*. 2025;19:1516746. doi:10.3389/fnins.2025.1516746. Erratum in: *Front Neurosci*. 2026;19:1-3. doi:10.3389/fnins.2025.1758523 7. Lu M, et al. *Am J Physiol Endocrinol Metab*. 2010;298:E1036-48. doi:10.1152/ajpendo.00668.2009 8. Reading C, et al. Bezisterim-associated anti-inflammatory epigenetic modulation of age acceleration and Alzheimer's disease genes. medRxiv preprint. <https://doi.org/10.1101/2025.10.20.25338385> 9. Dang Y, et al. *Pharmaceuticals (Basel)*. 2022;15(10):1177. doi:10.3390/ph15101177 10. Mérida I, et al. *Biochem J*. 2019;476(8):1205-1219. doi:10.1042/BCJ20180620 11. Yao W, et al. *Mol Psychiatry*. 2022;27(3):1618-1629. doi:10.1038/s41380-021-01377-7. Erratum in: *Mol Psychiatry*. 2022;27(3):1630. doi:10.1038/s41380-021-01417-2 12. Pérez RF, et al. *J Gerontol A Biol Sci Med Sci*. 2022;77(9):1743-1749. doi:10.1093/gerona/giac068 13. Perluigi M, et al. *Free Radic Biol Med*.

Methods⁶

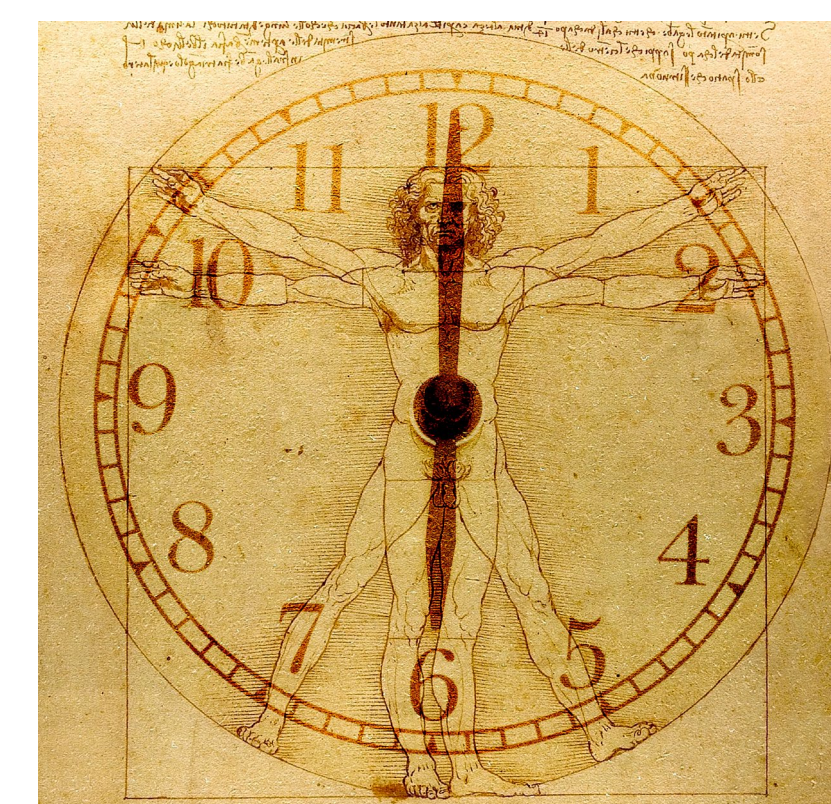
- Here, we expand on prior findings with EAA clocks, linking age-deceleration signals with inflammatory, metabolic, and neurodegenerative pathways
- Blood samples were analyzed using the Illumina EPIC v2 platform with standardized preprocessing and quality control
- A comprehensive panel of clocks was analyzed, and differential promoter methylation (DPM) statistics were calculated in samples from bezisterim-treated (n=17) and placebo-treated (n=16) patients, with DPM targets being contextualized within inflammatory and neurodegenerative knowledge spaces (Figure 1)

Figure 1: Graphical Representation of Study Design and Outcomes



Results: Reduced EAA With Bezisterim⁸

Figure 2: Thirteen biological clocks with bezisterim-associated EAA reduction



- PCGrimAge
- PCHannum
- PCHorvath2
- IntrinClock
- DamAge
- Stochastic PhenoAge
- Stochastic Zhang
- Stochastic Horvath
- RetroClockV2
- RetroClock
- SystemsAge
- Heart
- Lung

Bezisterim treatment significantly reduced EAA vs placebo across 13 independent biological clocks

Results: Correlation Between Potentially Beneficial DPM Changes and Improvement in Clinical Outcomes⁸

From 13 biological clocks (Figure 2), we identified 9 clock-associated genes in which significantly increased promoter methylation (PM) correlated with directional clinical neurocognitive assessments (Table 1).

- For example, higher bezisterim-associated PM of *PTGER2* was correlated with higher MMSE and lower CGIC (Figure 3)
- Seven of these are AD hub genes (Figure 4).

Table 1: Bezisterim Alters PM of 9 EAA Clock Genes Correlated With AD Neurological Assessments and Indicative of Cognitive Decline

Gene	DPM, FDR P	EAA Clock(s)	Correlated Assessment(s)	Pearson R, P	AD Hub Gene	Bezisterim-Associated AD Improvement
<i>SAT1</i>	+7%, 0.017	PCGrimAge.EAA	MMSE	+0.53, 0.028	Yes	Significantly correlated to higher MMSE score ^{9,a}
<i>DGKZ</i>	+6%, 0.002	StocPhenoAge.EAA	Cog12	-0.53, 0.027	No	Correlated with lower Cog12 and GST scores ^{10,b}
			GST	-0.51, 0.037		
<i>NRBP1</i>	+6%, 0.047	StocPhenoAge.EAA	ADL	+0.57, 0.038	Yes	Correlated with lower Cog12 and GST scores ^{11,c}
			Cog12	-0.65, 0.0051		
			GST	-0.51, 0.035		
<i>EIF4E</i>	+6%, 0.049	StocPhenoAge.EAA	ADL	+0.51, 0.038	Yes	Correlated with higher ADL score ^{12,d}
<i>RUNX2</i>	+11%, 0.048	StocPhenoAge.EAA	CGIC	-0.56, 0.020	Yes	Correlated with lower CGIC score ^{1,e}
<i>MTOR</i>	+6%, 0.0011	IntrinClock.EAA	Cog12	-0.50, 0.043	Yes	Correlated with lower Cog12 score ^{13,f}
			MMSE	+0.63, 0.0064		
<i>PTGER2</i>	+9%, 0.011	Horvath2.EAA	ADCOMS	-0.53, 0.029		
		StocHorvath.EAA	CGIC	-0.58, 0.014	Yes	Correlated with higher MMSE and lower ADCOMS, CGIC, Cog12, and GST scores ^{14,g}
			Cog12	-0.60, 0.012		
			GST	-0.58, 0.17		
<i>SOAT1</i>	+9%, 0.00035	Horvath2.EAA	CGIC	-0.50, 0.043	No	Correlated with lower CGIC, Cog12, and GST scores ^{15,h}
		StocHorvath.EAA	Cog12	-0.49, 0.048		
<i>STAT3</i>	+9%, 0.00080	DamAge.EAA	ADL	+0.49, 0.048	Yes	Correlated with higher ADL score ^{16,i}

Abbreviations: FDR, false discovery rate; P, p-value; PKCo, protein kinase C, alpha

FOOTNOTES

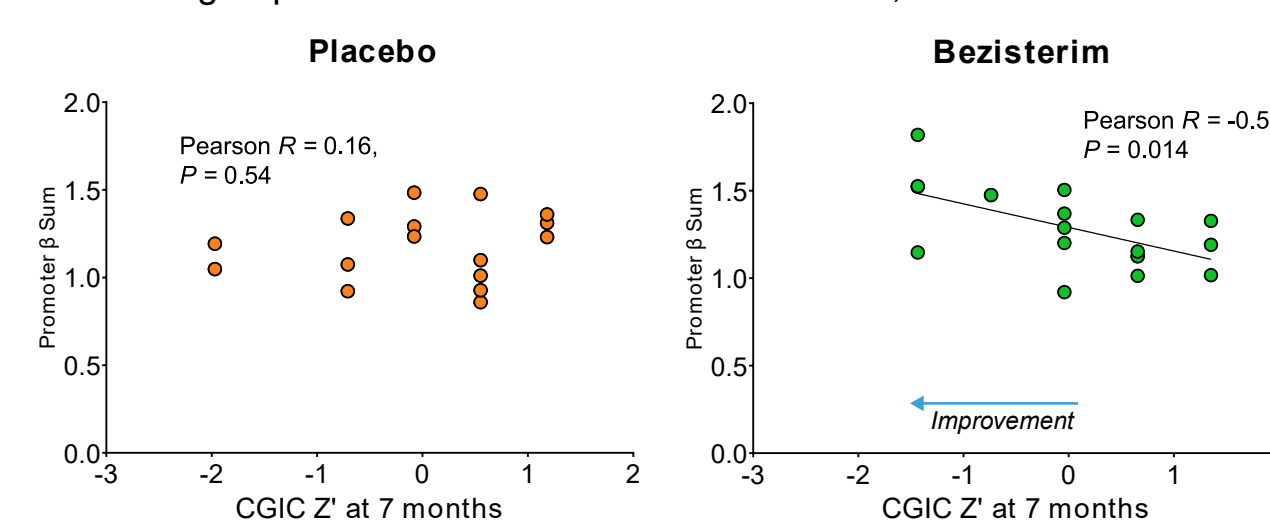
- SAT1* is associated with cognitive impairment in AD models.⁹
- DGKZ* may enhance PKCo activity and synaptic degeneration in AD.¹⁰
- NRBP1* expression is associated with recognition function disorder.^{10,11}
- EIF4E* methylation patterns have been associated with dementia.¹²
- Higher *RUNX2* expression inhibits neurogenesis in AD senescence.¹
- Higher *MTOR* expression was associated with age-dependent cognitive decline.¹³
- Inhibition of *PTGER2* prevents cognitive impairment.¹⁴
- Knockdown of *SOAT1* using siRNA or treatment with the *SOAT1*-inhibitor K604 improved cognitive function in an AD mouse model.¹⁵
- Higher *STAT3* PM is associated with decreased cognitive function; *STAT3* deletion in astrocytes strongly ameliorated spatial learning and memory decline in AD-model mice. Importantly, these protective effects on network dysfunction and cognition were recapitulated in AD-model mice systemically treated with a preclinical *STAT3*-inhibitor drug.^{16,17}

Figure 3: Sample Association of PM With Neurological Assessments

Differentially increased PM of *PTGER2* with bezisterim is correlated with improved MMSE and CGIC scores

CGIC vs *PTGER2*

Between-groups difference in correlations: $Z' = 2.14, P = 0.035$



MMSE vs *PTGER2*

Between-groups difference in correlations: $Z' = -1.79, P = 0.073$

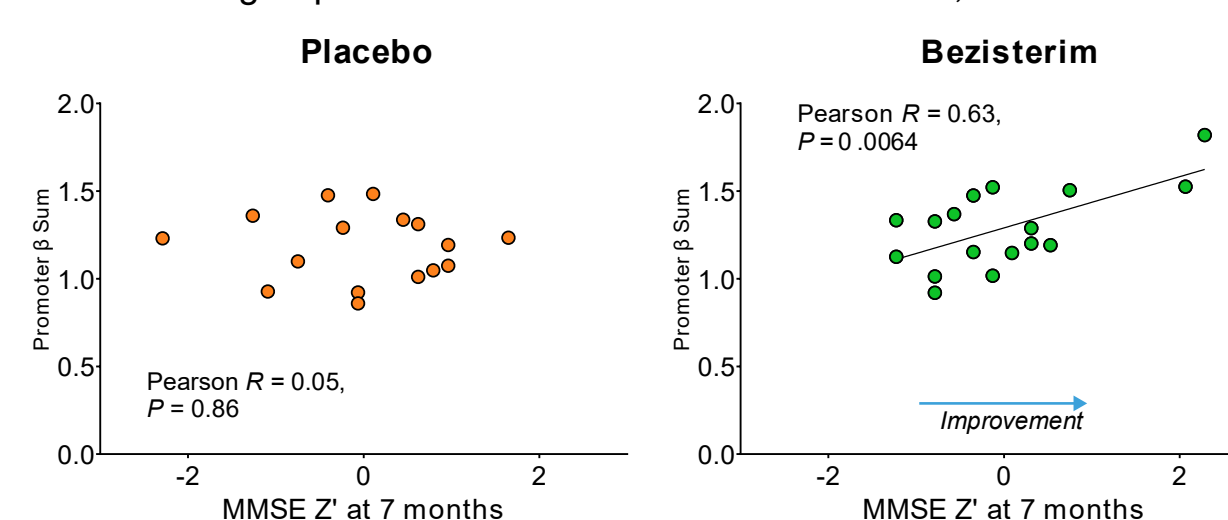
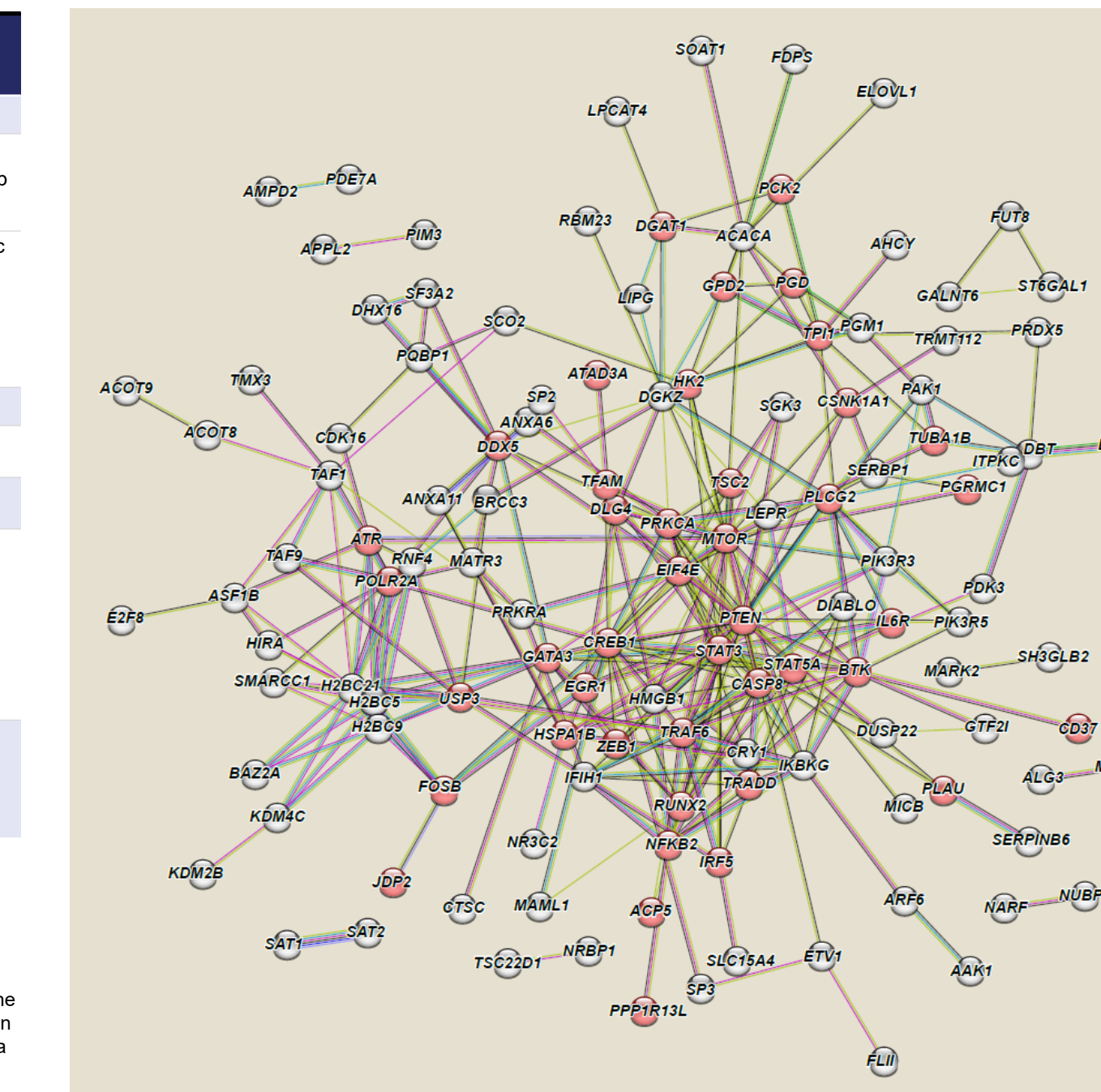


Figure 4: AD Network Hub Genes



Bezisterim potentially beneficial DPM genes with PM correlations to improved neurological assessments (ADCOMS, ADL, CGIC, Cog12, GST, and MMSE)

- Genes identified as known AD network hub genes are identified in red

Summary of Findings⁸

We detected treatment-related DPM in 2581 genes from a study in mild-to-moderate AD (preprint server reference) and further explored 447 of these genes that showed potentially beneficial DPM for EAA clock-associated covariates.

- Modifications of AD hub gene networks responsible for driving AD were identified
- Bezisterim was associated with significant DPM of 179 AD hub genes involved in AD progression

Conclusions

- In this small sample of patients with AD, we identified potentially beneficial epigenetic PM changes that correlate with improvements in neurological clinical assessments after treatment with bezisterim, an anti-inflammatory analog of a natural biologically active sterol
- These findings support the clinical utility of targeting epigenetic mediators of neuroinflammation in diseases of aging with novel anti-inflammatory agents like bezisterim

DISCLOSURES

JMP, CLR, JY, CA, and PM are employees of BioVie Inc. SO'Q is a consultant for BioVie Inc. VBD is a consultant for BioVie, Inc., and an employee of TruDiagnostic, Inc.

ACKNOWLEDGEMENTS

Funded by BioVie Inc. p-value group provided editorial support.

CONTACT INFORMATION

Joseph M. Palumbo: jpalumbo@bioviepharma.com
Christopher L. Reading: creading@bioviepharma.com