Epigenetic modulation of age acceleration and Alzheimer's disease genes associated with bezisterim anti-inflammatory treatment

Christopher Reading[1*], Jiayan Yan[1], Clarence Ahlem[1], Penelope Markham[1], Stephen O'Quinn[1], Joseph M. Palumbo[1], Varun B. Dwaraka[2*]
[1]BioVie Inc., Carson City, NV, USA , [2]TruDiagnostic, Inc., Lexington, KY, USA

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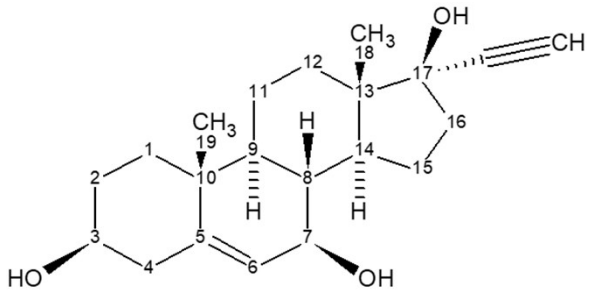
Bezisterim, aging and Alzheimer's

Bezisterim



- 17α-ethynyl-androst-5-ene-3β,7β,17β-triol
- Anti-inflammatory insulin sensitizer
- Binds ERK1/2, LRP1, RPS6KA3, and SIRT2
- Inhibits inflammatory NFκB activation, cytokine and chemokine transcription, with an attractive safety profile to date

Age is the top risk for Late Onset Alzheimer's Disease (AD)

In a published 30-week randomized, placebo-controlled study in 33 mild/moderate individuals with AD, bezisterim[1]:

- Decreased epigenetic age acceleration (EAA) in five clocks
- Improved neurologic endpoints in correlation with the SkinBloodAge clock
- Decreased glucose, cholesterol, MCP-1, and increased HOMA2 %B and %S

%B=beta-cell functioning; %S=insulin sensitivity; ERK=extracellular-regulated kinase; HOMA2=Homeostasis Model Assessment 2; MCP-1=Monocyte Chemoattractant Protein-1; RS6K=p90 ribosomal S6 kinase

1. Reading CY, Schmunk L, Martin-Herranz D, et al. An exploratory analysis of bezisterim treatment associated with decreased biological age acceleration, and improved clinical measure and biomarker changes in mild-to-moderate probable Alzheimer's disease. *Front Neurosci, 19*, 1516746. doi:10.3389/fnins.2025.1516746

AD subjects 30-week placebo-controlled clinical trial

Clinical Measures

Whole blood DNA

Bezisterim 895,323 CpG β values

Placebo 895,323 CpG β values

31 clinical assessment values for both bezisterim and placebo

Age acceleration

PBO 13/13 Clocks

P < 0.10

BEZI 13/13 Clocks

Age deceleration

CpG predicted Clock Gene matches

Differential Average Promoter Methylation (DPM) between bezisterim and PBO

11,322 genes (107,721 promoter CpGs)

T test for significance (P<0.05)

4,282 Genes

72 correlations for bezisterim
13 correlations for placebo
(FDR alpha <0.05)

Sum of promoter methylation 448 genes

FDR set to alpha 0.05

2,154 Genes

45 Clock Genes

Top 5 HOXA4, TYW3, B3GALT4, CRYZ, CHPY

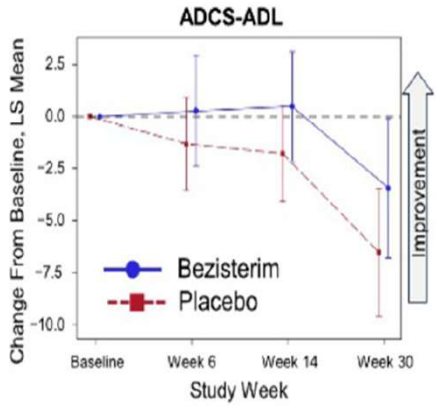
AD/Aging/Inflamm

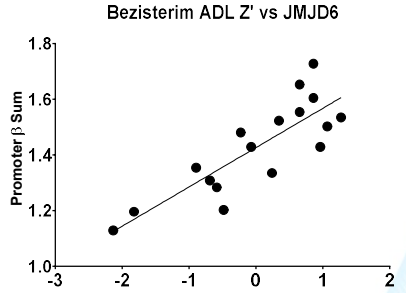
448 Genes (45 Clock)

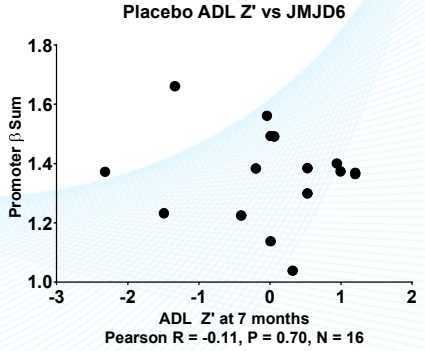
↑DPM ≥5% Top 5: IKBKG, TGIF HDAC11 TRIM27, ZHX2

↓ DPM ≤ -5% Top 5: CDK16, DUSP22,UBE2C PLSCR2, NME3

13 Different Epigenetic Age Acceleration (EAA) "clocks" based on CpGs







Key findings of current work to be discussed

Integrated analysis of molecular mechanisms

- Bezisterim reduced EAA in an additional 13 clocks
- Bezisterim altered average promoter CpG methylation of 448 genes compared to placebo
  - Increases in CpG promoter methylation of key AD-associated genes are likely associated with decreased gene expression; decreases are likely associated with increased gene expression

Changes in promoter methylation were consistent with improvements across the 13 aging clocks, AD measures, and metabolic inflammation measures

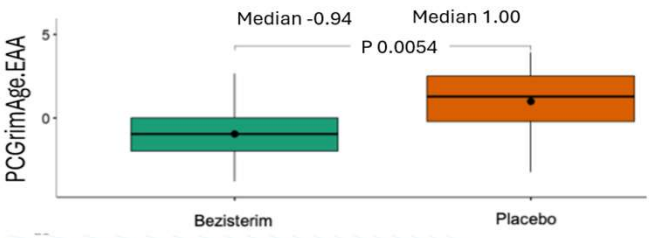
In addition, bezisterim subject promoter methylation beta sums of each of 72 genes correlated with clinical measure changes

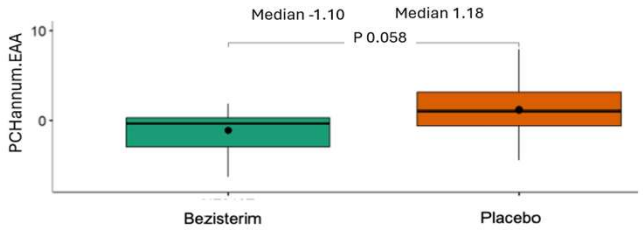
Bezisterim reduced epigenetic age acceleration (1 of 2)

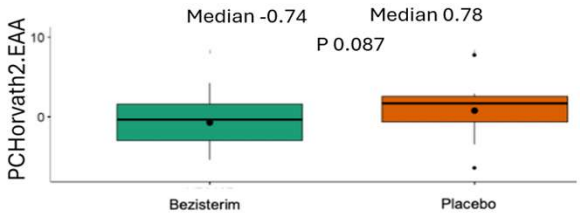
- Decelerated aging in 13 aging clocks after 30 weeks of bezisterim
- Negative residuals of model-predicted biological age and chronological age indicate decelerated aging, whereas positive differences indicate accelerated aging

First 5 of 13 Clocks

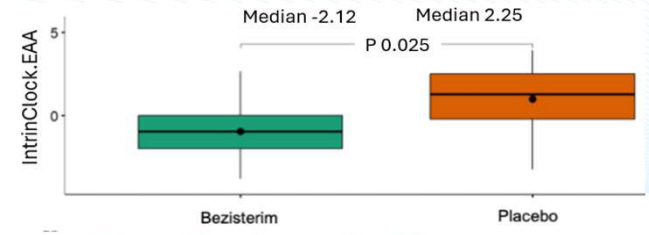
Principal Component First- and Second-Generation EAA Clocks (GrimAge, Hannum, and Horvath2)

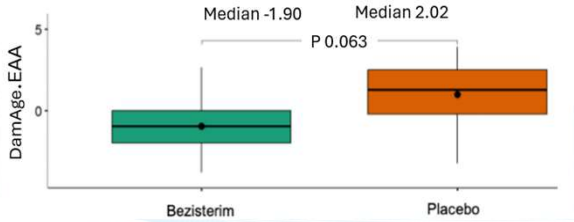






Intrinsic and Damage EAA Clocks (IntrinClock and DamAge)

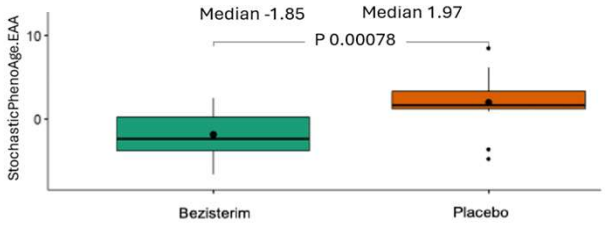


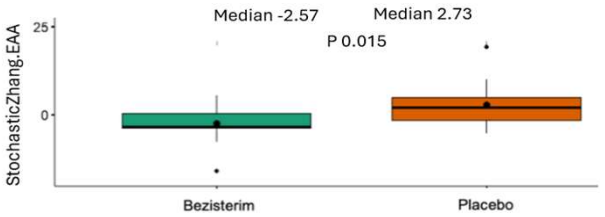


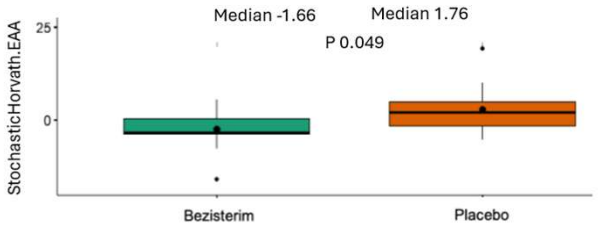
Bezisterim reduced epigenetic age acceleration (2 of 2)

- Remaining 8 of 13 Clocks

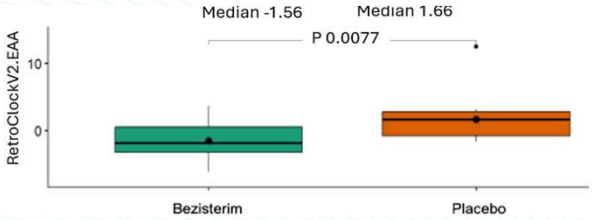
Stochastic EAA Clocks (PhenoAge, Zhang, and Horvath)

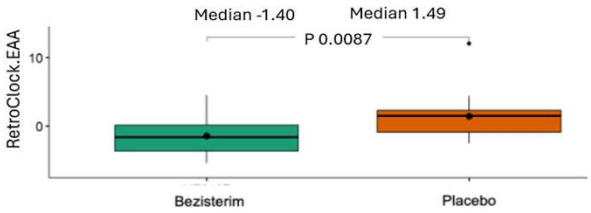




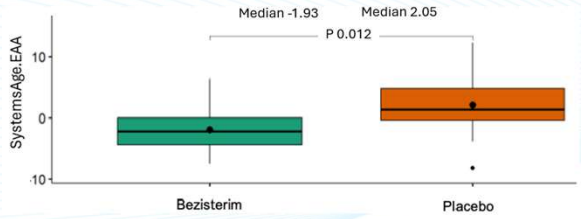


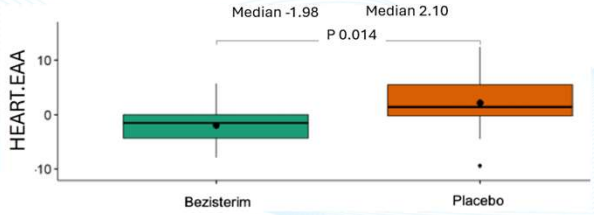
Retroviral Integration EAA Clocks (Retroclock2 and Retroclock)

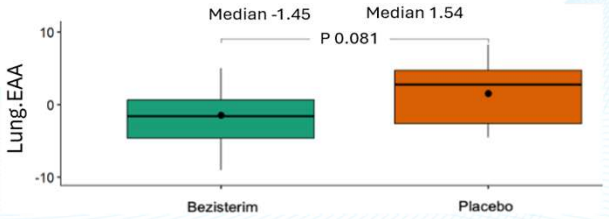




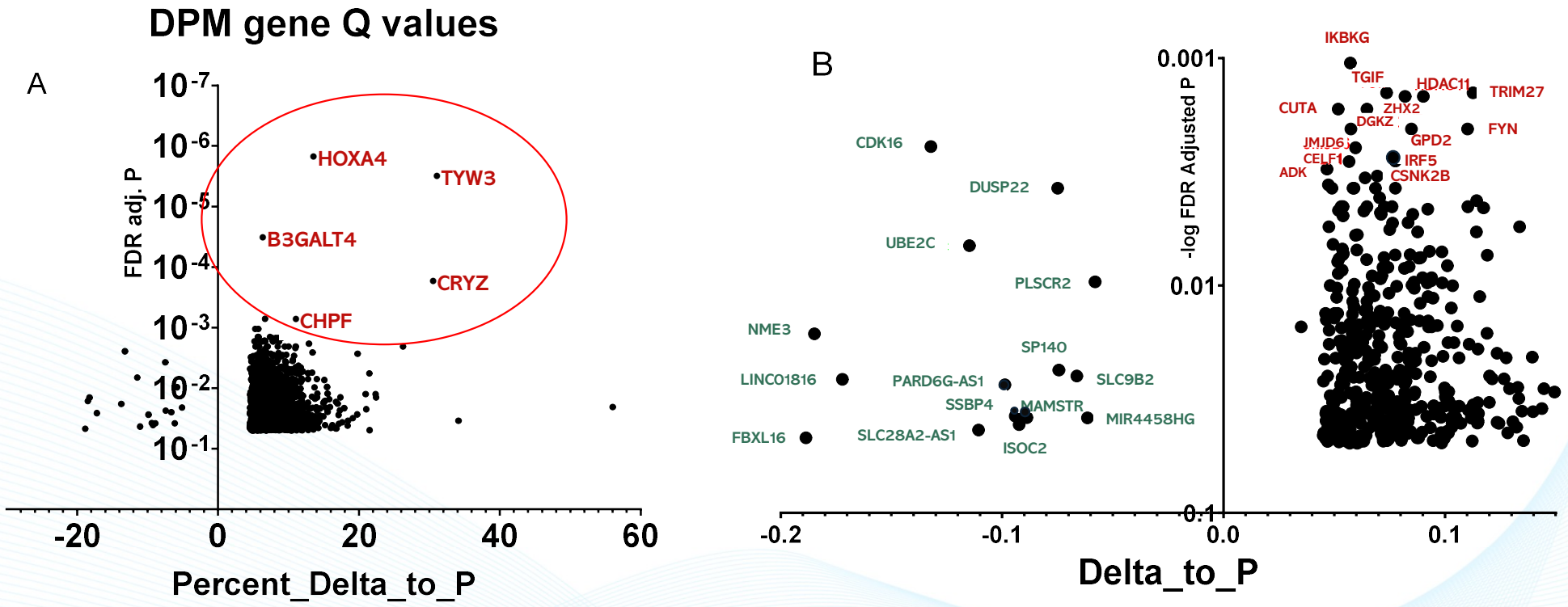
Systems Age EAA Clocks (SystemsAge, Heart, and Lung)







Bezisterim altered average promoter CpG methylation



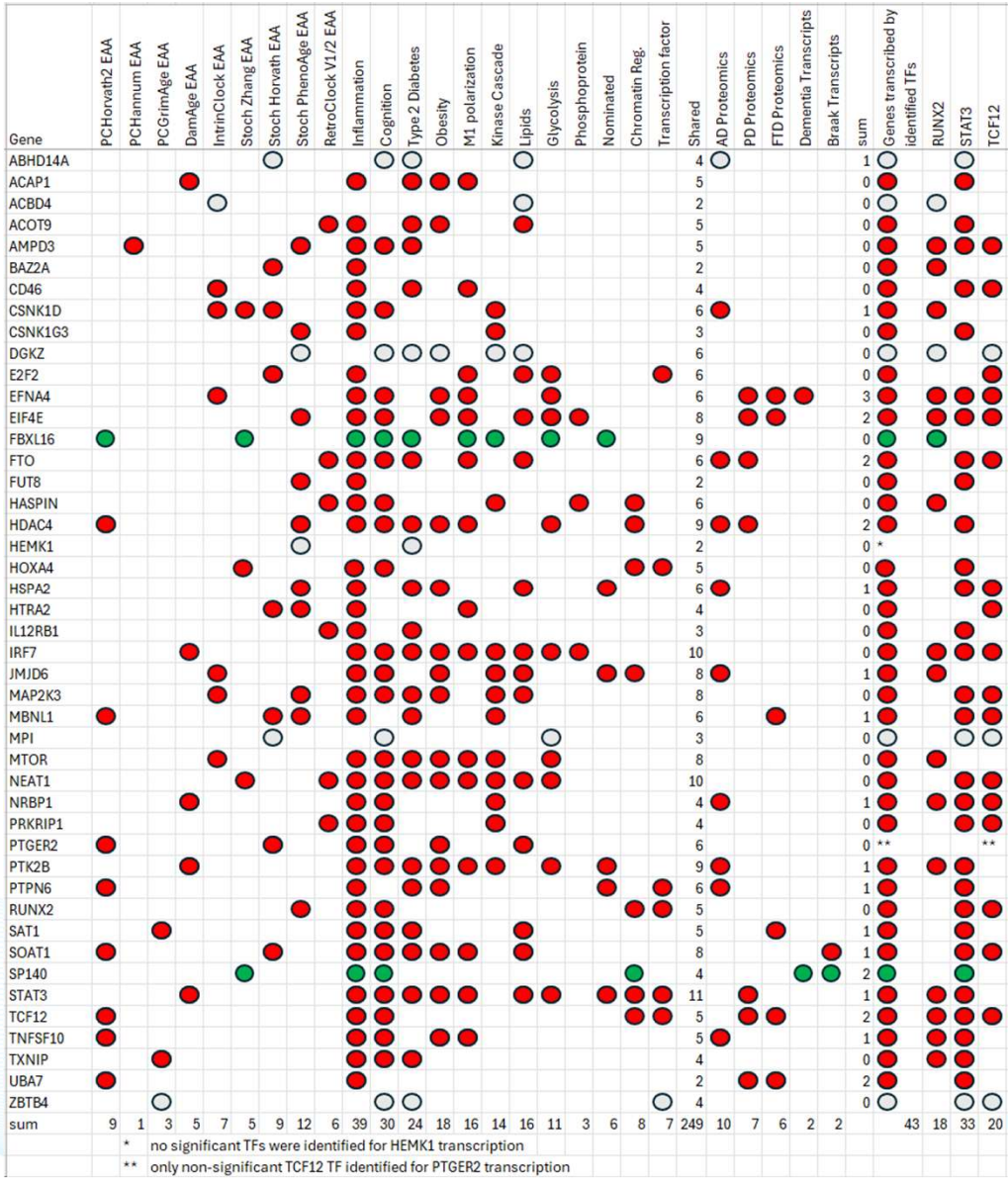
FDR=Benjamini–Hochberg false discovery rate.

Potentially Beneficial DPM Gene Examples

Increased DPM Genes	Promoter CpGs	DPM	P FDR < 0.05	Activity		References
B3GALT4	50	+6%	2.7e-5	Hypomethylated in AD brain, microglial neuroinflammation, pro-inflammatory kinase cascades, lipid dysregulation, neurodegeneration and cognition		B3GALT4, 2025; Hayashi et al., 2004; Madrid et al., 2018; Matsubara et al., 2017; Oikawa et al., 2015; Sha et al., 2022; X. Wang et al., 2021; Yanagisawa, 2015
FYN	15	+11%	0.0021	Kinase cascades, neurotoxicity and synaptic impairments caused by Aβ, tau tyrosine phosphorylation, cognitive decline, microglial neuroinflammation, inflammasome, insulin resistance, and T2D		Meur & Karati, 2025; Guglietti, Sivasankar, Mustafa, Corrigan, & Collins-Praino, 2021; Panicker et al., 2019; Lee et al., 2013; Bastie et al., 2007
JMJD6	18	+6%	0.0024	**Clock biomarker and epigenetic regulator identified as a key driver in AD**, Cognitive decline, driver of AD network, Abeta, pTau, inflammation,TRAF6, NFkB, obesity, lipid metabolism; increased in AD plasma; nominated aging and AD target, forms 5-OH-lysine on histones and inhibits methylation		Merchant et al., 2023; Tikhanovich et al., 2015; Hu et al., 2015; J. Zhou et al., 2022; Ali et al., 2025; J. Agora, n.d.; Unoki et al., 2013
Decreased DPM Genes	**Promoter CpGs**	**DPM**	**P FDR < 0.05**	**Activity**		**References**
DUSP22	8	-7%	0.004	Anti-inflammatory phosphatase, hypermethylated in AD, expression decreases inflammatory kinase cascades and dysregulated phosphoproteins, decreases pTau, DNAm correlates with Braak stages, cognitive decline, PTSD and schizophrenia, expression is negatively correlated with glucose, obesity, cholesterol, M1 polarization,		An et al., 2021; Boks et al., 2018; Ge et al., 2022; Howie, Rijal, & Ressler, 2019; Patysheva, Prostakishina, Budnitskaya, Bragina, & Kzhyshkowska, 2023; Sanchez-Mut et al., 2014; Sanchez-Mut & Graff, 2015
MAMSTR	7	-9%	0.038	Anti-inflammatory transcriptional coactivator of MEF2C that restrains microglial inflammatory response and is lost in brain ageing in an IFN-I-dependent manner; highly significant hypermethylated region in AD associated with Braak stages, microglial neuroinflammation, and cognitive decline/		L. Zhang et al., 2020; Deczkowska et al., 2017

Bezisterim Potentially Beneficial DPM Epigenetic Age Acceleration Clock Genes Shared with Aging, AD, and Metabolic Disease

- 45 potentially beneficial DPM EAA clock genes were also associated with diseases of aging
- 87% of these genes are associated with **inflammation**
- 67% impact **cognition**
- **Red** circles are genes with **increased** DPM associated with inflammation
- **Green** circles are genes with **decreased** DPM associated with inflammation
- **Gray** circles are genes with **increased** DPM <u>not</u> associated with inflammation
- All of these with available transcription factor data can be transcribed by one of three transcription factors which are also DPM genes



Potentially beneficial changes to 448 aging and disease risk gene promoters

- Based on these data, we investigated additional DPM genes for reported associations with inflammatory, neurologic, and metabolic disease
- In addition to the 45 clock genes we identified an additional 403 of the 2154 DPM genes that shared traits similar to the clock genes
- For AD, we identified 106 DPM genes that showed potentially beneficial DPM to improve reported plasma levels in AD. In addition, we identified 62 DPM genes with potential benefit for plasma levels in Parkinson's disease
- We identified DPM genes with potential improvements in published AD microglial transcript levels for 30 shared Dementia and 20 shared Braak pathology score genes

Are bezisterim changes associated with clinical benefit? (slide 1 of 2)

The sum of promoter methylation for each potentially beneficial DPM gene was investigated for correlation to previously reported clinical measures:

- Chronologic age
- Neurologic assessments: *ADCOMS, ADL, CDR-SB, CGIC, GST, NPI*
- Inflammatory measures: MCP1, TNF, RANTES, Monocytes, CRP, C1q
- Metabolic parameters: waist-to-hip ratio, weight, systolic blood pressure, fasting glucose, insulin, triglycerides, cholesterol, fructosamine, HOMA2 %B and %S
- AD biomarkers: pTau217, GFAP, NfL, Aβ 42/40 ratio

Aβ=amyloid –beta; CRP=C-reactive protein; GFAP= Glial Fibrillary Acidic Protein; NfL=Neurofilament Light chain; RANTES=regulated on activation, normal T-cell expressed and secreted (CCL5); TNF=tumor necrosis factor.

Are bezisterim changes associated with clinical benefit? (slide 2 of 2)

For bezisterim subjects, there were 72 significant (FDR < 0.05) correlations of clinical measures with differential sums of promoter methylation beta values

- 48 genes with increased DPM sum
- One gene with decreased DPM sum

For placebo, there were 13 significant correlations with 11 genes

There were more correlations with bezisterim than placebo (*P* = 5.5e-12, Fisher Exact Test)

Bezisterim and placebo potentially beneficial clinical measure and gene correlations

Potentially beneficial clinical measure correlations

	Bezisterim Correlations		Placebo Correlations	
Measures	Sum	Beneficial	Sum	Beneficial
ADCOMS	7	7	1	0
ADL	25	25	1	1
C1q	1	1		
CDR SB	10	10		
Cog 12	3	2	1	0
CGIC	1	1		
GST	10	10	1	0
MMSE	1	0		
Chol	6	6		
Glucose	1	0		
Trig	3	3		
WHR	1	1		
Weight	3	3		
BPs			6	0
GFAP			3	0
TOTAL	72	69	13	1

Fisher Exact P = 4.3e-11

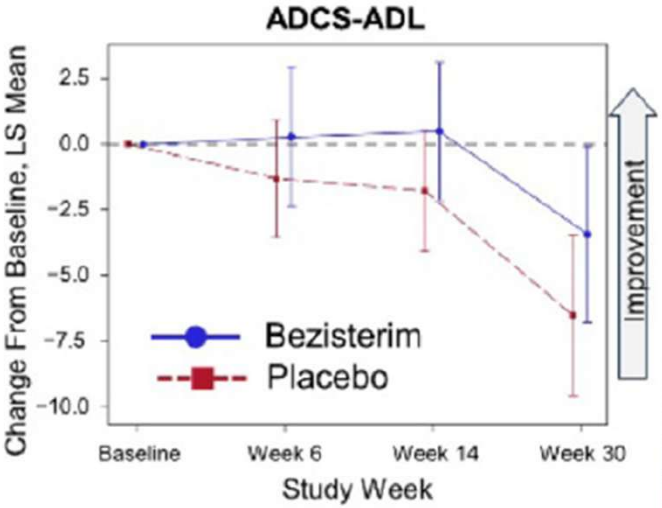
Potentially beneficial gene correlations

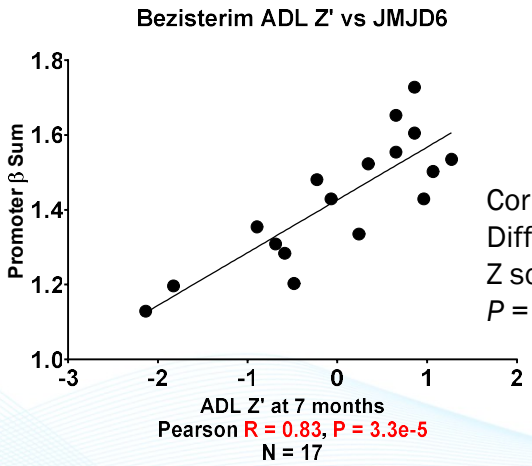
	Bezisterim Correlations		Placebo Correlations	
Gene	Sum	Beneficial	Sum	Beneficial
CDK2AP1	3	3		
CSNK1D			1	0
CTSL			1	0
DGZK	4	4		
ETVI			1	0
GPD2			1	0
IL6R	5	5		
IRF5			1	0
JDP2	4	4		
KLF7	5	5		
LEPR			1	1
LIME1	4	1		
MTHR			1	0
NEU1	3	3		
NINJ2			3	0
NRBP1	4	4		
P2RX1	4	4	1	0
SMARCD2			1	0
SAT2	4	4		
TGIF1	3	3		
TUBA4A			1	0
TOTAL	43	40	13	1

Fisher Exact P = 9.9e-9

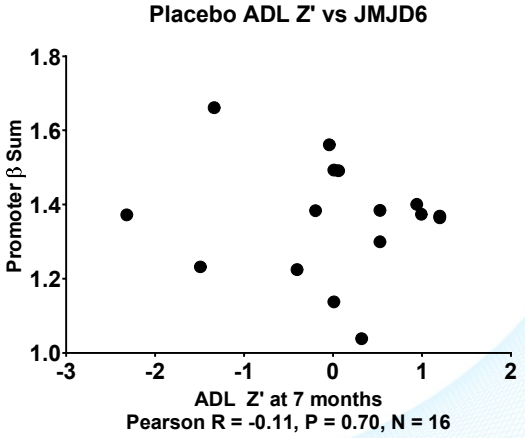
Activities of Daily Living (ADCS-ADL) with JMJD6 was the most significant correlation with DPM genes

- For bezisterim, JMJD6, an epigenetic regulator and driver of AD, was correlated with ADL (R = 0.83, *P* = 3.3e-5, FDR P 0.0010)
  - This was significantly different from placebo (z score *P* = 0.0007)

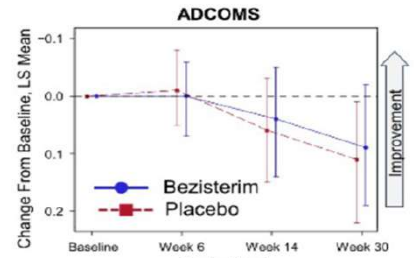


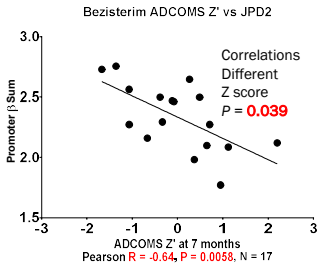


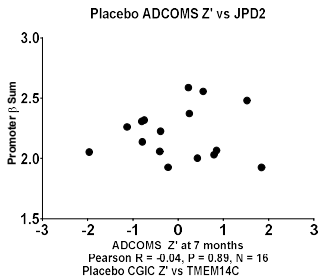
Correlations Different Z score *P* = 0.0007

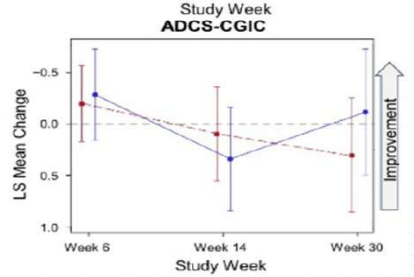


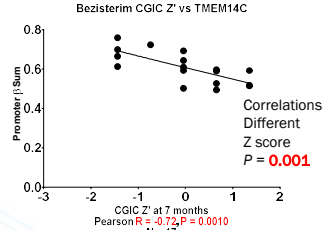
Additional examples of bezisterim potentially beneficial DMP gene correlations with clinical measures

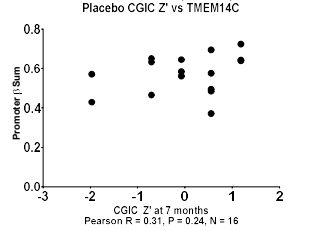


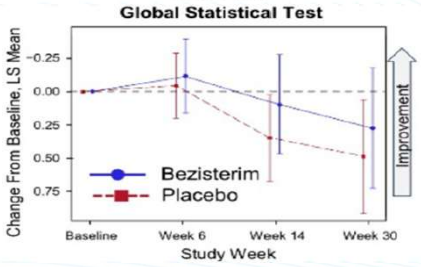


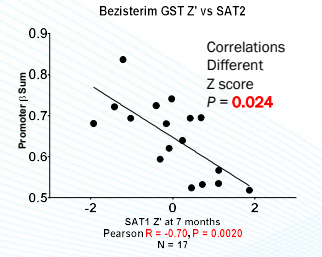


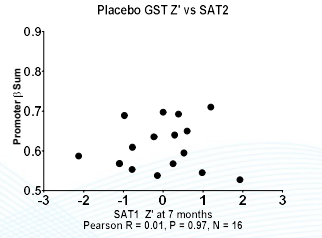


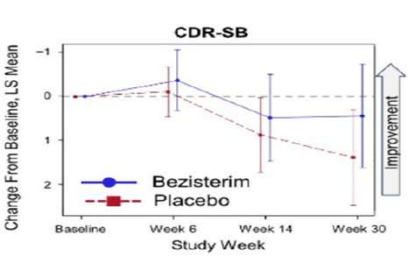


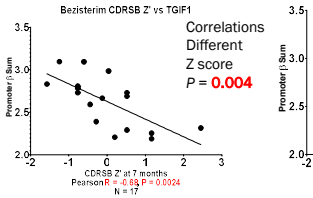


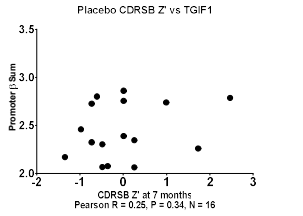


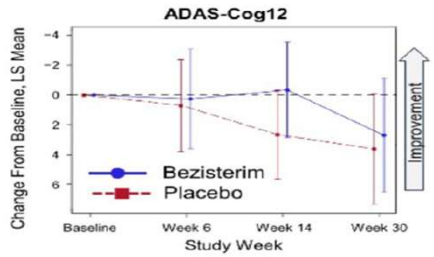


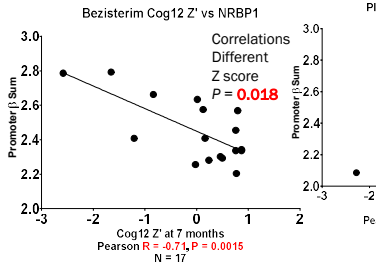


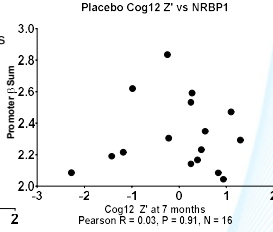


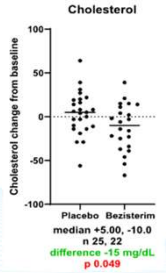


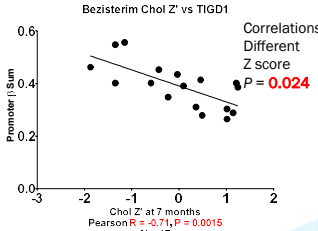


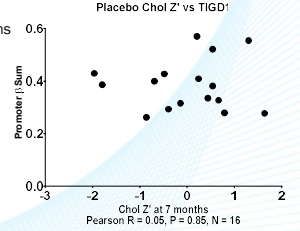












ADCOMS: Alzheimer's disease composite score; CGIC: Clinician global impression of change;
GST: composite of ADAS-Cog12 (Cognition 12 score) and CDR SB (Clinical Dementia rating sum of boxes).

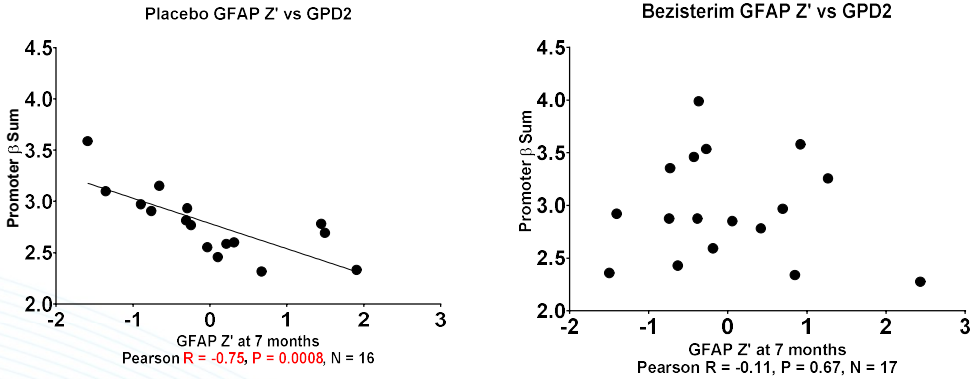
Placebo promoter methylation correlations with clinical measures

- For placebo, there were 13 significant correlations with 11 genes
  - Systolic blood pressure was correlated with 6 genes (CSNK1D, CTSL, ETV1, MTHFR, SMARCD2 and TUBA4A)
  - GFAP RISK was correlated with 3 genes (GPD2, IRF1, and P2RX1); it was uncorrelated in bezisterim subjects
  - The gene NINJ2 was correlated with decline in 3 clinical measures (ADCOMS, Cog12, and GST)

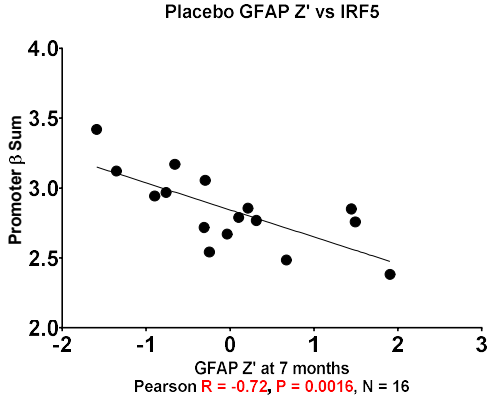
ADCOMS=Alzheimer's Disease COMposite Score; Cog12=Alzheimer's Disease Assessment Scale-Cognitive Subscale; GST=Glutathione S-transferase.

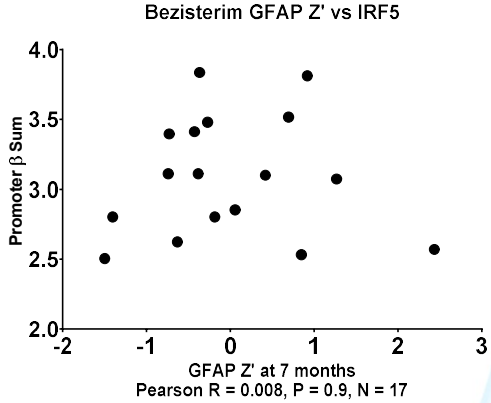
Examples of placebo potential risk DMP gene correlations with clinical measures (1 of 2)

Correlations Different Z score *P* = 0.024

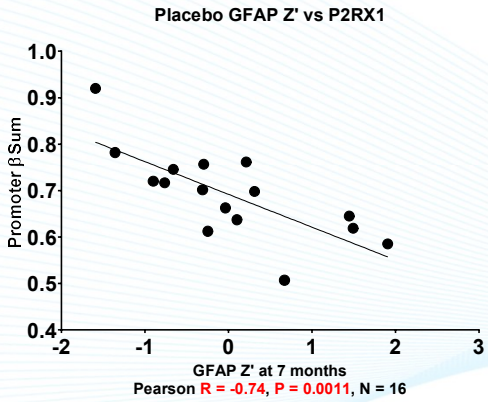


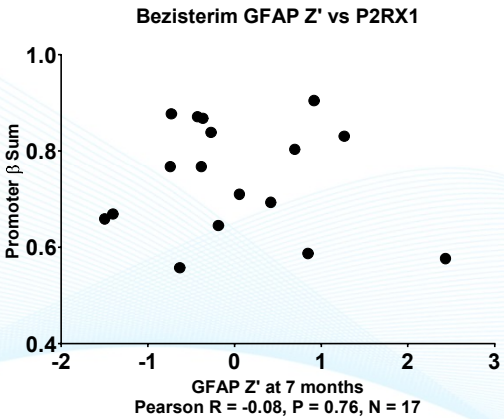
Correlations Different Z score *P* = 0.017





Correlations Different Z score *P* = 0.024

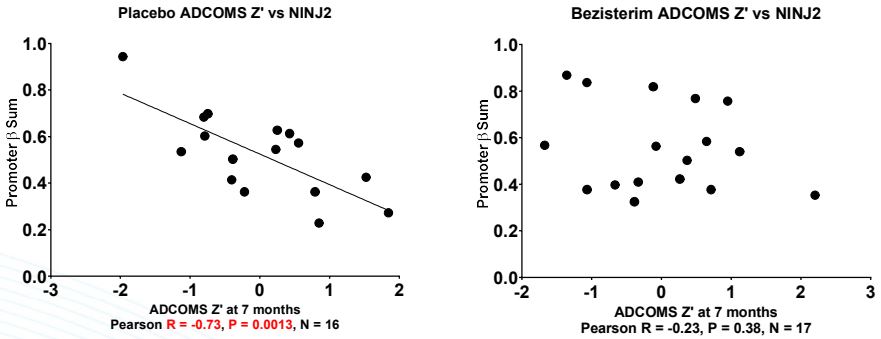




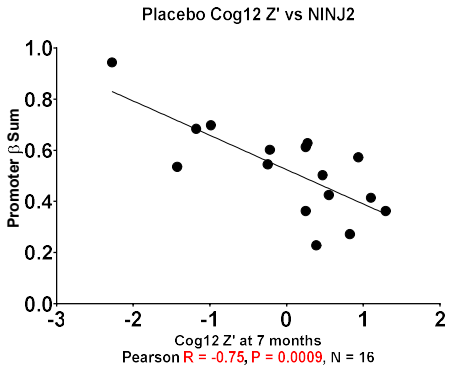
Decreased DPM ∝ increased GFAP

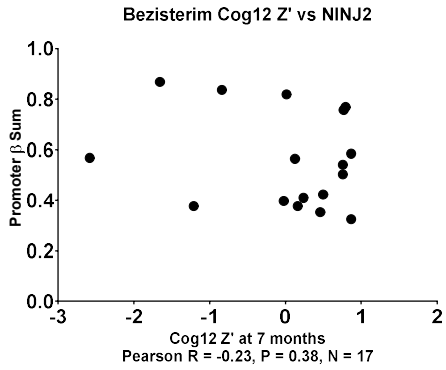
Examples of placebo potential risk DMP gene correlations with clinical measures (2 of 2)

Correlations Trend Different Z score *P* = 0.055

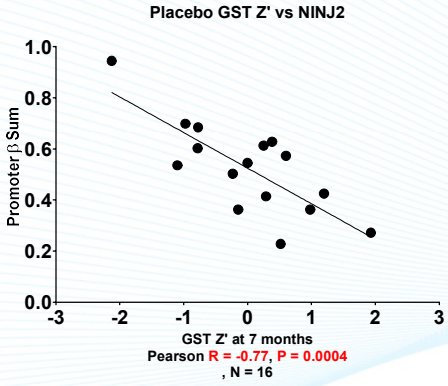


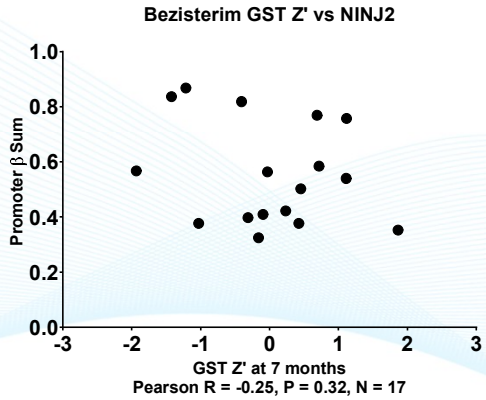
Correlations Trend Different Z score *P* = 0.055





Correlations Different Z score *P* = 0.047



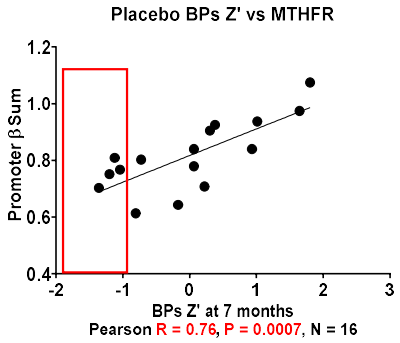


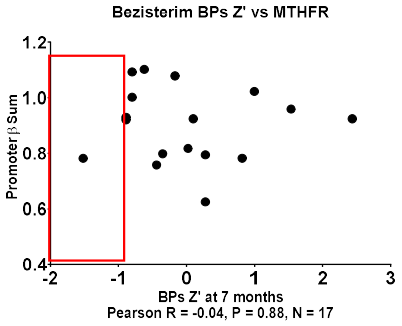
Decreased DMP ∝ Cognitive Decline

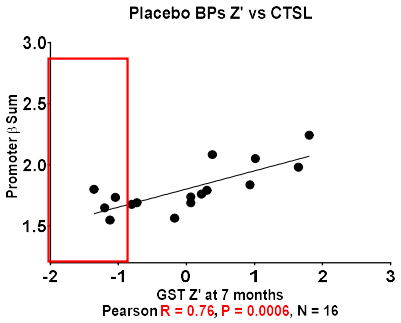
Correlations with systolic blood pressure suggest detection of underlying disease pathology

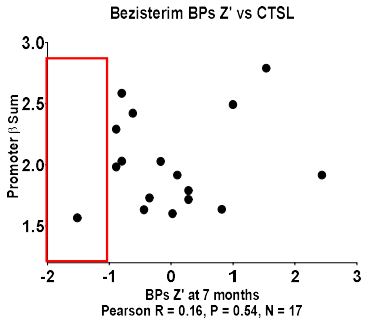
- Previously reported imaging data from an open-label study of 23 subjects with mild cognitive impairment and mild AD[1]
  - Bezisterim was associated with clinician-rated improvements in relative cerebral blood flow (rCBF) using arterial spin label imaging and blood-oxygen-dependent functional connectivity within the brain. rCBF is strongly correlated with FDG-PET brain imaging and metabolic coupling in AD.
- In the current study, there were six placebo correlations with systolic blood pressure.
- The following correlation graphs identify a potential for decreased rCBF in placebo subjects which may be related to brain hypometabolism. This relationship was uncorrelated with bezisterim subjects.

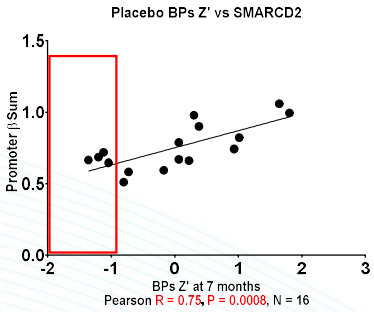
1. Haroon J, Jordan K, Mahdavi K, et al. A phase 2, open-label study of anti-inflammatory NE3107 in patients with dementias. *Medicine (Baltimore)*. 2024;103(30):e39027. doi: 10.1097/MD.0000000000039027.

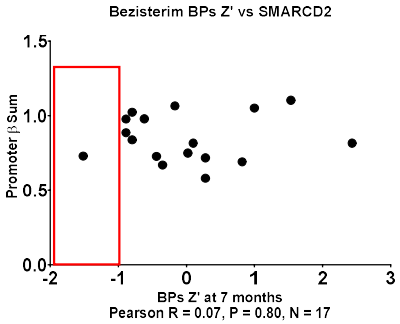


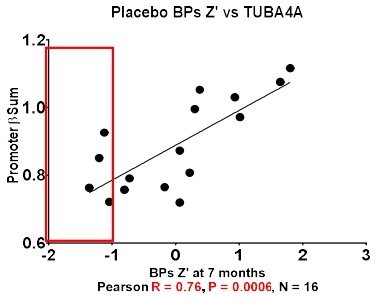


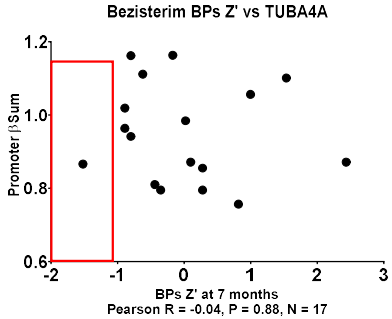


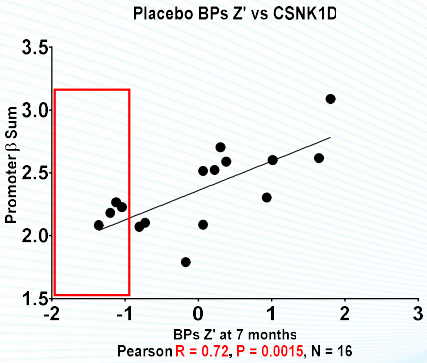


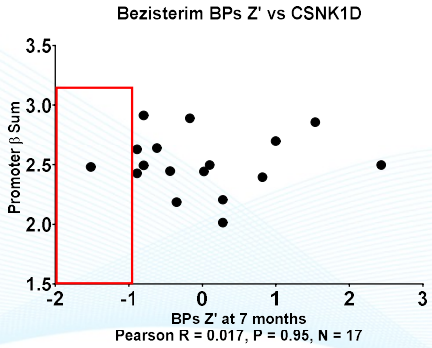


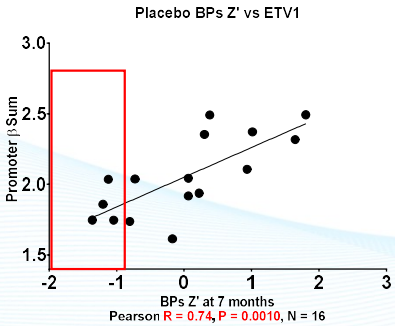


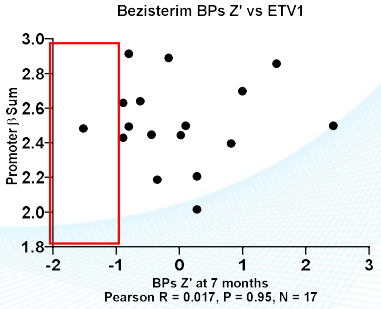












Potentially improved rCBF with bezisterim

Summary and conclusions (1 of 2)

- Clinical benefit in bezisterim subjects was associated with epigenetic modulation of age and disease-related genes
- Bezisterim modulated epigenetic effects in a group of 448 genes associated with inflammatory responses and diseases of aging
- Promoter-wide methylation analysis may be more powerful than individual differences in CpGs when exploring conditions or treatments for healthspan or diseases of aging
- Bezisterim modulated promoter methylation of 69 genes that are covariates of AD clinical measures in a potentially beneficial manner
- JMJD6 (the top gene correlated with ADL change) is an exciting example of the importance of promoter methylation differences in relationship to clinical measures
- In placebo subjects, correlations of 3 genes with increased GFAP, NINJ2 with cognitive decline, and 6 genes with potential decreases in rCBF might lead to epigenetic biomarkers for bezisterim activity, and for brain hypometabolism.

Summary and conclusions (2 of 2)

- Bezisterim decreased epigenetic age acceleration in a total of 18 biological aging clocks that have been shown to be associated with aging in general
  - In particular, bezisterim decreased EAA in 3 stochastic clocks, which are based on random methylation changes in aging, rather than clocks such as the DamAge and GrimAge clocks that are associated with effects of diseases of aging
- Based on EAA clocks, and evidence of correlations between potential gene expression differences and clinical responses for neurological and metabolic measures, it is tempting to speculate that bezisterim may increase healthspan in general
- The specifics of biomarkers that can be derived from this study await replication in a larger AD population
  - We are currently collecting DNA methylation data for bezisterim trials in PD (NCT06757010) and in Long COVID (NCT06847191), with the possibility of understanding the generalizability of these findings in other neurodegeneration settings
  - A manuscript with the full data will be on MedRxiv preprint server next week.