Bezisterim Effects on Biological Age, Alzheimer's Epigenetics, and Neurologic Assessments

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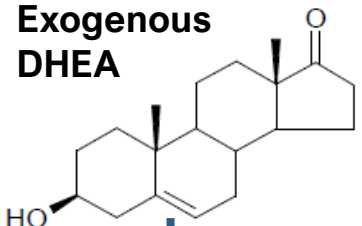
ARDD Copenhagen, 2024

In 2000, we asked

“Why does DHEA show

- **anti-inflammatory**
- **insulin-sensitizing**
- **neuroprotective**
- **anti-aging effects**

in mice, and not in humans?”

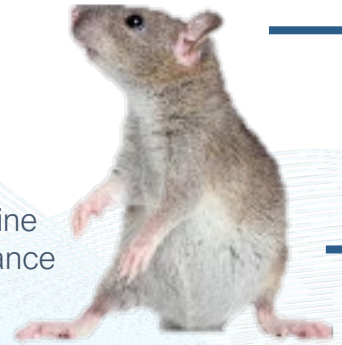


MICE — **INFLAMMATION** — **MEN**

√ **ACTIVE**

X **INACTIVE**

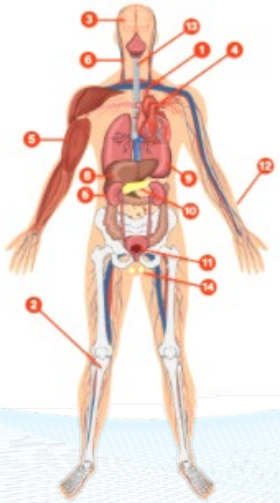
- NFκβ
- cytokines
- chemokines
- IRS1/2 p-Serine
- insulin resistance



- amyloid b
- cognitive decline
- Alzheimer’s
- neuron death

- hypertriglyceridemia
- adipogenesis
- obesity
- diabetes
- longevity

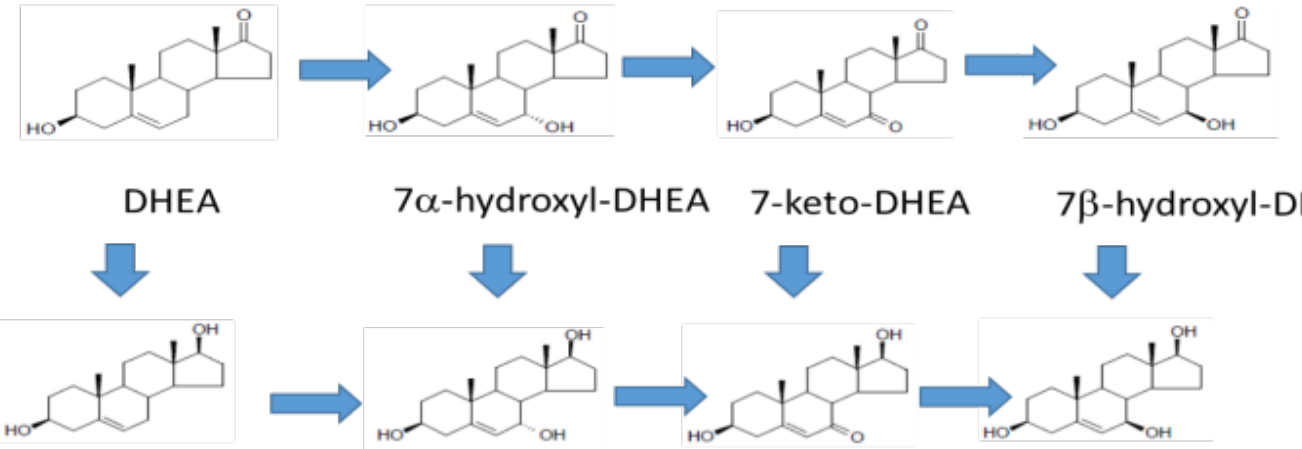
Harvard Health Publishing
HARVARD MEDICAL SCHOOL

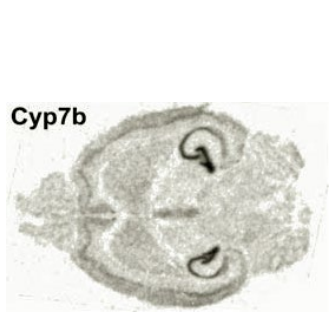


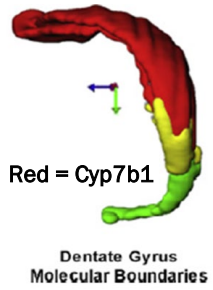
- insulin resistance
- hyperglycemia
- obesity
- dyslipidemia
- osteoporosis
- cognitive decline
- Alzheimer’s
- decreased quality of life

Thousands of publications

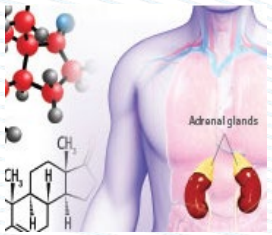
Bezisterim Developed by Understanding the Differential Metabolism of Androst-5-ene-3,17-diol Between Rats, Canines, Monkeys and Humans[1]

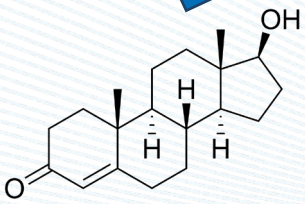




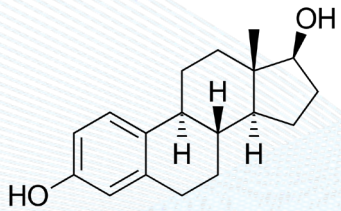


Neurosteroid: Cyp7B defines hippocampal dentate gyrus[3,4]

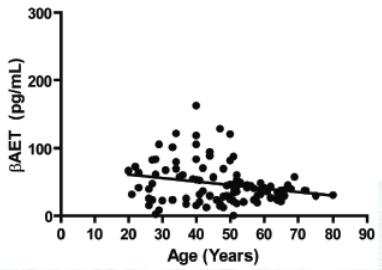




Testosterone

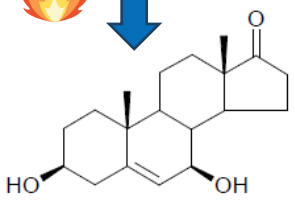


Estradiol



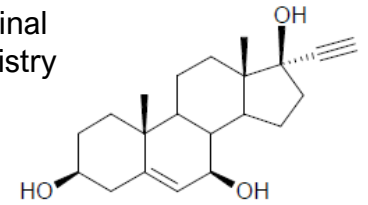
βAET decreases with age[2]

Oxidation



7β-hydroxyl-DHEA
INACTIVE

Medicinal Chemistry



17α-ethynyl-βAET (bezisterim)

Metabolic stability
Oral bioavailability
Blood-brain permeable

1. Ahlem 2011 *Steroids* 76 66; 2. Auci 2011 *Obesity* 19 806; 3. Rose 2001 *J Biol Chem* 276 23937; 4. Thompson 2008 *Neuron* 60 1010

Bezisterim

In vitro, in vivo and clinical findings align with expected pharmacology

- Binds extracellular-signal regulated kinase[5], (ERK), and inhibits inflammatory[6,7], but not homeostatic[8] ERK, NFκB and TNF signaling. It is more potent than βAET for inhibition of NFκB transcription
- These activities have been demonstrated in primary **macrophages**, and in **monocyte/macrophage cell lines**, as well as in vivo animal models
- Decreased inflammatory mediators and insulin resistance in animal models and human subjects with obese impaired glucose tolerance or T2D[9,10]
- Improved motor activity and decreased neurodegeneration in Parkinson's disease models[11], and improved motor activity in a phase 2a PD study[12]
- Has a well-tolerated safety profile to date[13]
- Positive signals of clinical activity in dementia in AD clinical studies

5. Reading 2012 PLoS ONE 7 e32147; 6. Wang 2010 J Pharmacol Exp Ther 333 70; 7. Lu 2010 Am J Physiol Endocrinol Metab 298 E1036; 8. Lambert 2017 Front Neurosci 11 45; 9. Reading 2013 Obesity 21 E343; 10. Reading 2013 Mediators Inflamm 2013 814989; 11. Philippens 2023 Movement Disorders https://bioviepharma.com/publications/ ; 12. Ahlem 2023 ADPD https://bioviepharma.com/publications/ ; 13. Reading 2024 ADPDDS https://bioviepharma.com/publications/

Positive Signals of Bezisterim Clinical Activity in Dementia

Open label 14-week study in MCI and Mild AD

- Improved neurological, neuroimaging, and dementia and inflammation biomarkers in an open-label 14-week study in MCI and mild AD[14]
- Observed 3.4-year decrease from baseline in biological age (Horvath SkinBlood Clock), and altered monocyte methylome[15], but array batch effects confounded the interpretation

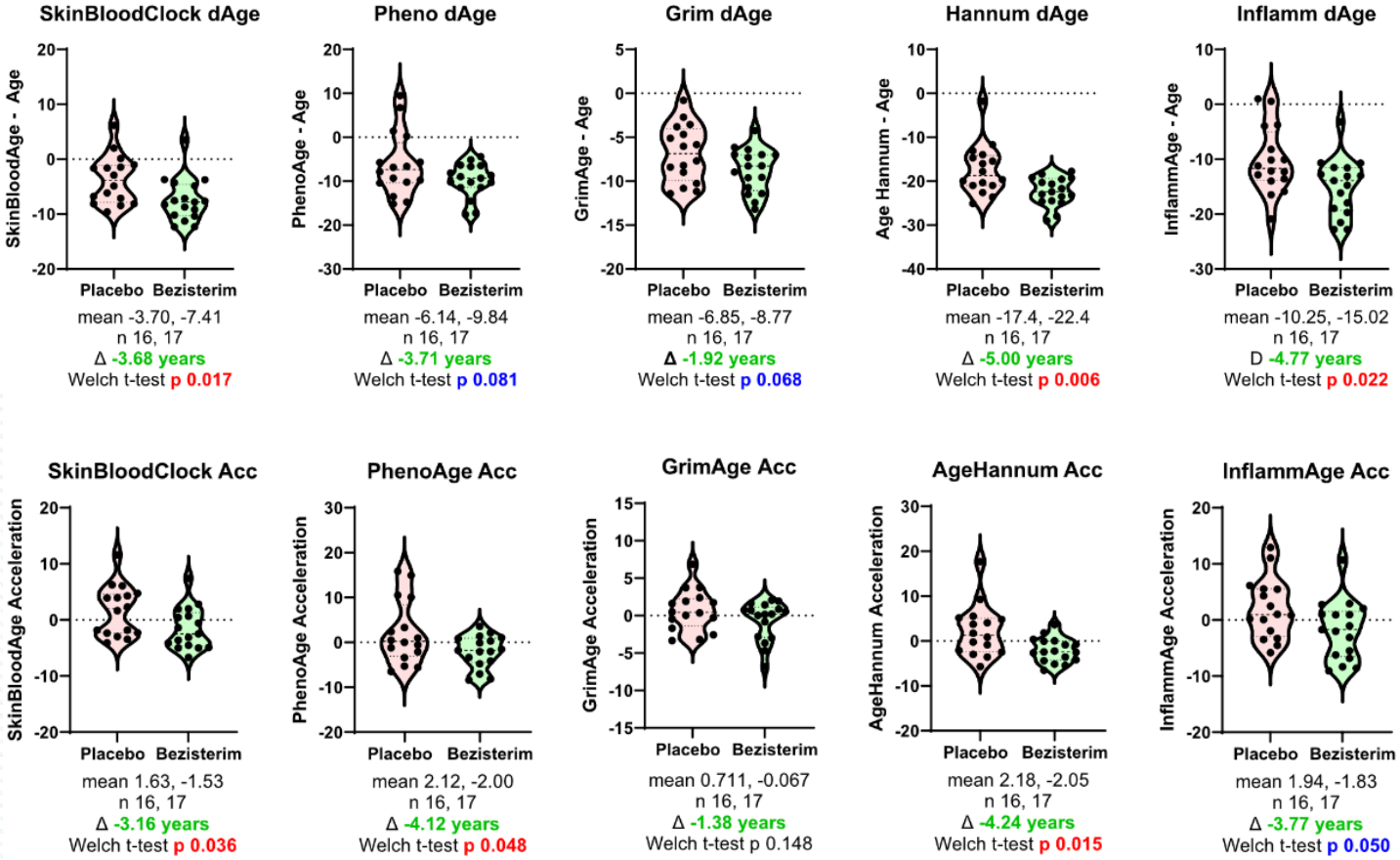
Placebo-controlled 30-week study in Mild/Mod AD[16,17]

- Positive signals found in -
  - Neurological and biomarker measures in 50 per-protocol subjects (26 placebo and 24 bezisterim)
  - DNA methylation data for 33 subjects at completion (16 placebo and 17 bezisterim)

Focus of this presentation

14. Haroon 2024 *Medicine* 103 e39027; 15. Reading 2023 AAIC https://bioviepharma.com/publications/ ;16. Reading 2024 Submitted for publication; 17. Reading 2021 *Neurodegener Dis Manag* 11 289;

Bezisterim Subjects Showed a Lower Biological Age Than Placebo Subjects at 30 Weeks



Difference between biologic and chronological age (dAge) as calculated by different epigenetic aging clocks[18-22]

(SkinBlood Clock difference consistent with previous study[15])

A consistent trend was observed when using age acceleration (similar to dAge but not confounded by chronological age)

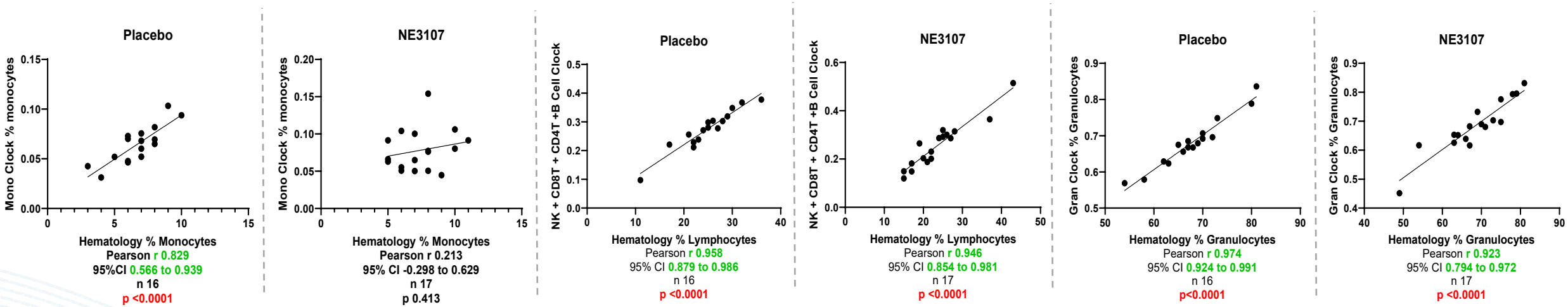
Note consistent placebo acceleration, Bezisterim deceleration

Raw data from Epigenetic Clock Development Foundation; Analyses of diverse epigenetic aging clocks performed by Chronomics/Hurdle Group, including their new InflammAge Clock[22]

18. Horvath 2018 *Aging* 10 1758; 19. Levine 2018 *Aging* 2018 10 573; 20. Lu 2019 *Aging*. 2019 11 303; 21. Hannum 2013 *Mol Cell* 49 359; 22. Schmunk *bioRxiv*. 2023. doi.org/10.1101/2023.12.21.572866

Bezisterim Appeared to Modify Week 30 Monocyte Methylome

Consistent with hypothesis of a transition from pro- to anti-inflammatory



Cell Type	Placebo Pearson r, P	Bezisterim Pearson r, P	Fisher transformation Z test statistic, P
Monocytes	0.829, **<0.0001**	0.213, 0.413	*2.51,* ***0.012***
Lymphocytes	0.958, **<0.0001**	0.946, **<0.0001**	*0.334, 0.738*
Granulocytes	0.974, **<0.0001**	0.923, **<0.0001**	1.44, 0.149

- Hematology and clock data are correlated for lymphocytes and granulocytes
- For pllacebo, but not bezisterim subjects, monocytes are not correlated
- Consistent with the previous open-label study result and hypothesis of a transition from pro-inflammatory (M1) to anti-inflammatory (M2) myeloid cells[15]

15. Reading 2023 AAIC https://bioviepharma.com/publicationss/

Analysis of Promoter Methylation

- Increased promoter methylation has been associated with decreased gene expression, however gene expression was not measured in this study
- Monocytes represent less than 10% of leukocytes; if significant promoter methylation differences were predominantly represented in monocytes[23], the magnitude of changes is much higher in monocytes.
- Significant (FDR $p<0.05$) differences in promoter CpG methylation β values (bezisterim – placebo) identified were almost exclusively increased methylation by bezisterim

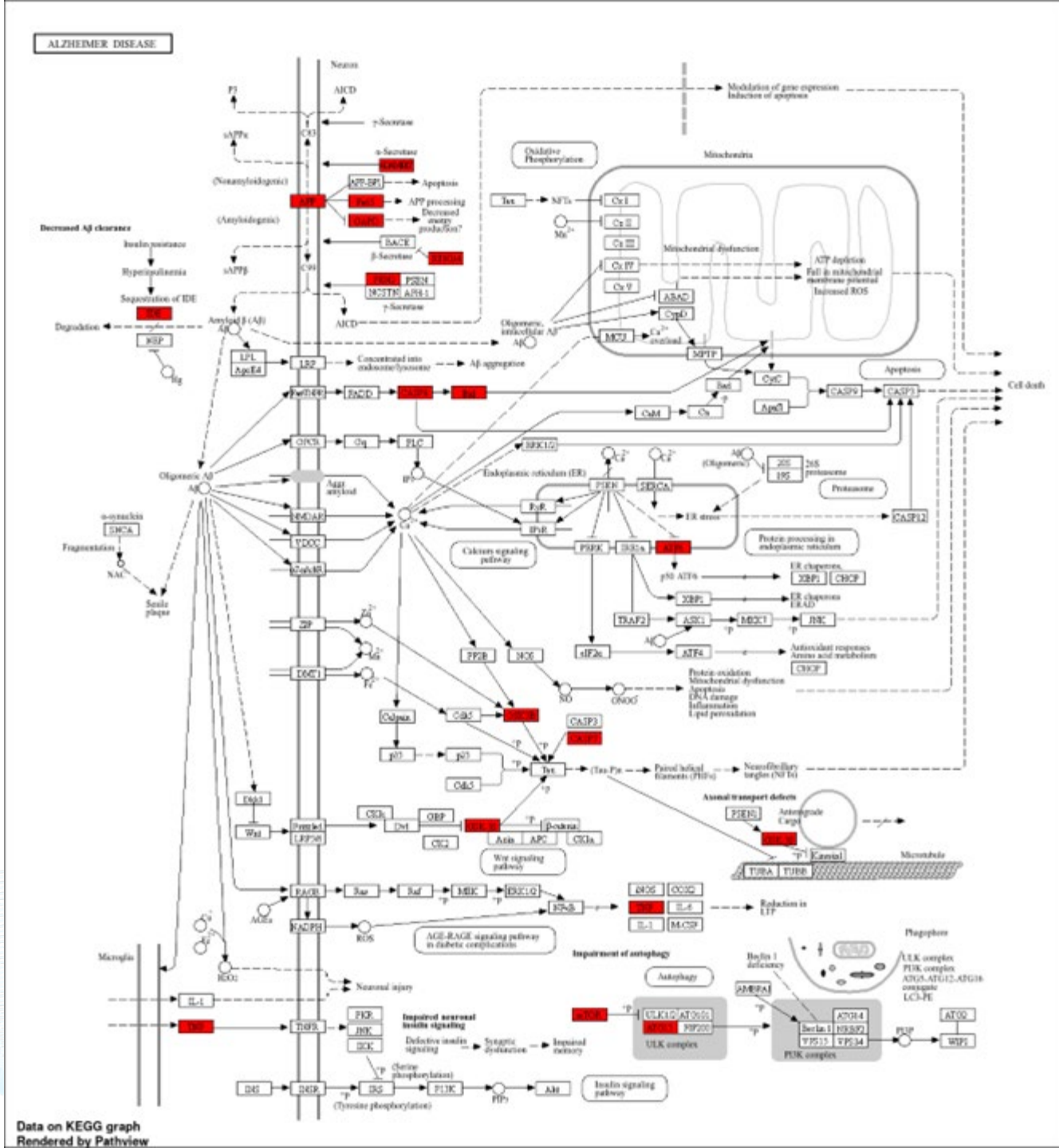
23. Stankey 2024 Nature June 5

Bezisterim Increases in Promoter Methylation of Genes Associated with Disease Progression, Inflammation and Metabolic Regulation

- Compared to placebo, **bezisterim** subjects had significantly (FDR $p<0.05$) **increased methylation** in promoter CpGs of 4934 genes
- The top 50 genes (FDR $p< 0.005$) genes identified **associations with disease progression** for the following:
  - Cognitive decline, AD, PD and other CNS diseases
  - Inflammatory response, neurodegeneration, cell death
  - Obesity, insulin resistance, and T2D
  - Neurodegeneration biomarkers
  - Age acceleration
- Compared to placebo, **bezisterim** subjects had significantly (FDR $p<0.05$) **decreased methylation** in promoter CpGs in 21 genes
- We identified these 6 of the 21 genes were associated with the following **improved homeostasis:**
  - Autophagy, mitochondrial energy
  - Positive regulation of transcription
  - Repression of inflammatory gene expression, decreased ROS
  - Promotion of beta cell regeneration

Alzheimer's Disease Pathway Enrichment Suggests Positive Impact by Bezisterim

- Genes with promoter **methylation significantly increased (p<0.05) in bezisterim compared to placebo AD subjects** were examined for enhancement of the KEGG Alzheimer's disease pathway
- There was a 1.6-fold enrichment (127 of 384 pathway genes)
- FDR p=6.2e^{-6}
  - The red boxes represent proteins produced by genes with bezisterim-increased methylation (ie, decreased expression)
- There was no enrichment for genes with significantly decreased promoter methylation in bezisterim compared to placebo AD subjects

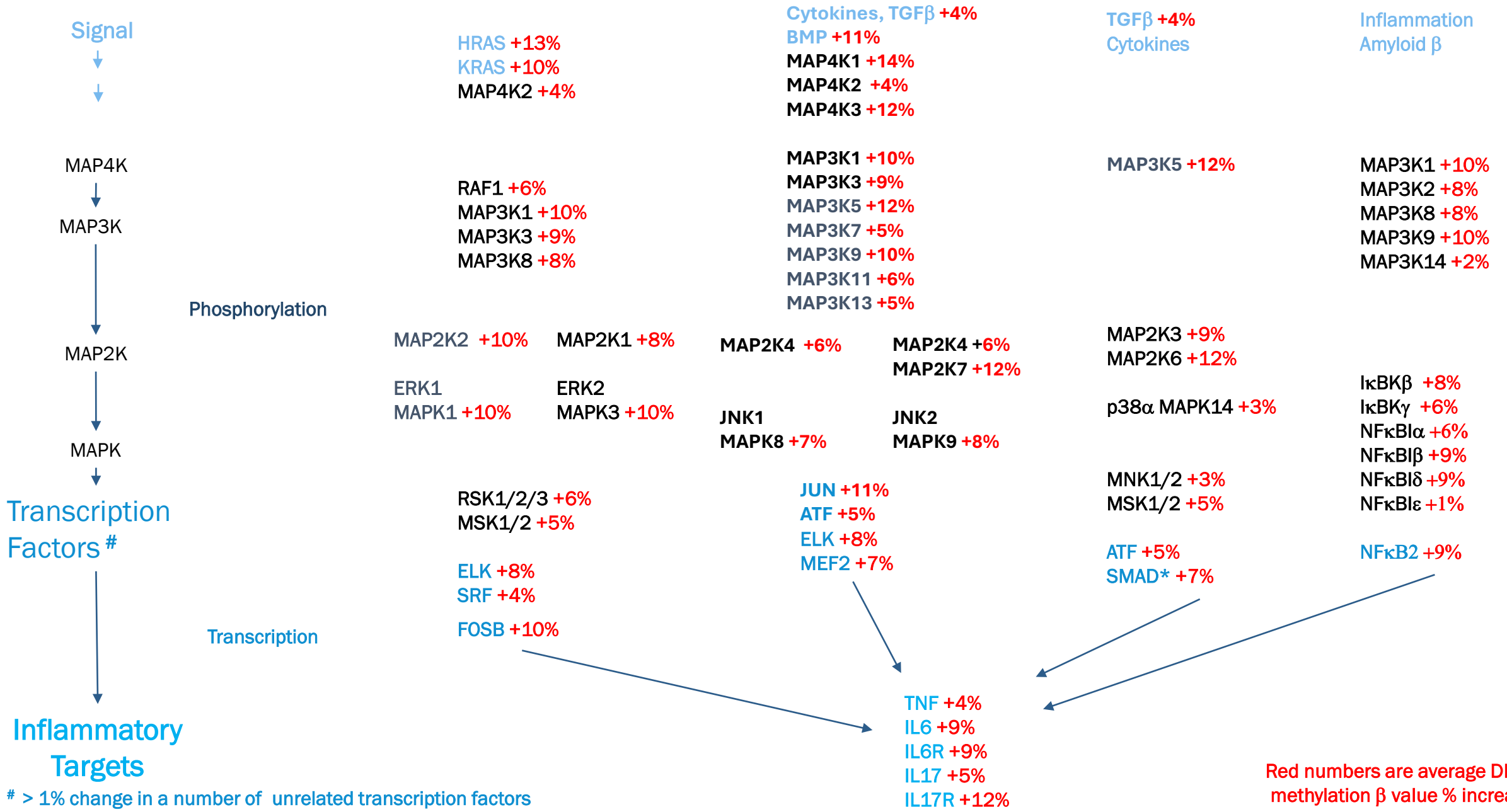


Key Genes in the AD Pathway for Promoter Methylation Increased in Bezisterim Compared to Placebo Subjects

Activity may reduce gene expression

- ADAM10
  - α-Secretase, decreases Aβ
- APP
  - Decreases Aβ
- Bid
  - Apoptosis death domain agonist
- ATF6
  - ER stress response decreased in Alzheimer's disease
- CASP7
  - Caspase-cleaved tau catalyzes filament formation, adopts a conformation found in early-stage tangles, and can be hyperphosphorylated
- TNF
  - Pro-inflammatory cytokine, NFκB activation, decreases LTP

Inflammatory Gene Expression Decreased by Compounded Sequential Kinase Increased Promoter Methylation with Bezisterim vs Placebo



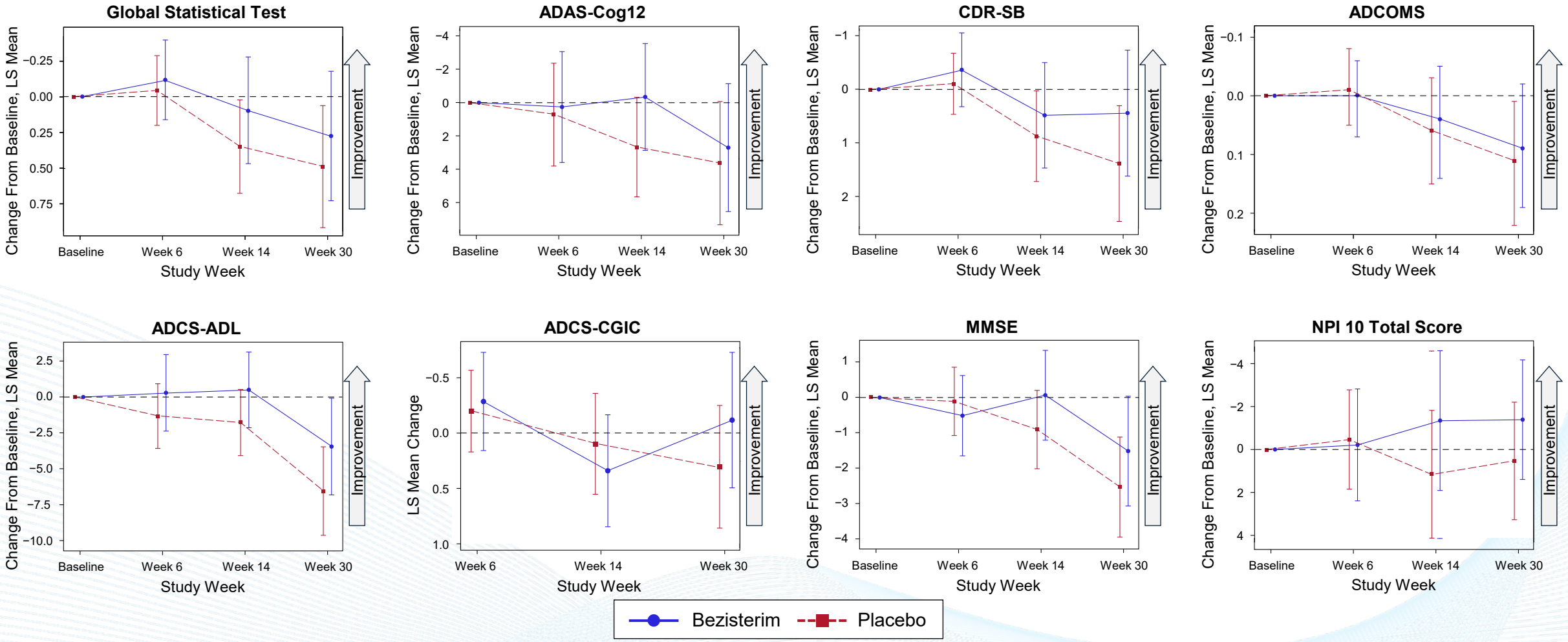
> 1% change in a number of unrelated transcription factors

Red numbers are average DNA methylation β value % increase

Bezisterim KEGG Pathways Enrichment

Pathway	Fold Enrichment	FDR Adjusted P	Genes/pathway Genes
Alzheimer's disease	1.6	$6.2e^{-6}$	110/384
Amyotrophic lateral sclerosis	1.7	$1.8e^{-7}$	112/364
Neurodegeneration multiple diseases	1.6	$1.7e^{-6}$	134/476
Parkinson's disease	1.7	$7.0e^{-6}$	82/266
Huntington's disease	1.6	$1.4e^{-4}$	86/306
Cellular senescence	1.8	$1.3e^{-4}$	51/156
Apoptosis	1.8	$2.5e^{-4}$	45/136
Proteosome	2.3	$1.8e^{-3}$	19/46
TNF signaling	1.5	$2.9e^{-2}$	31/112
NFκB signaling	1.6	$3.4e^{-2}$	29/104

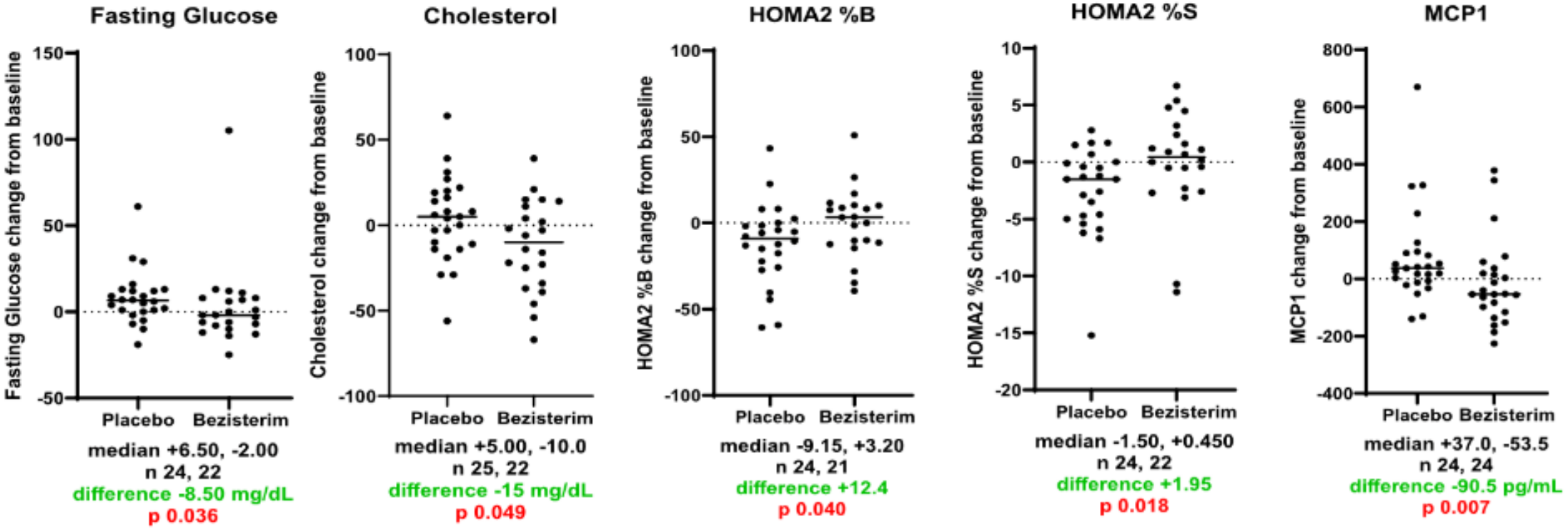
Bezisterim Showed Improvements Over Time in Primary and Secondary Endpoints



- **Primary and secondary efficacy endpoints.** Scores for bezisterim and placebo from baseline through week 30. Magnitude of responses was comparable to results reported for approved medications lecanemab[24] and aducanumab[25]

24. van Dyck 2023 *N Engl J Med*. 388 9; 25. Budd Haeberlein 2022 *J Prev Alzheimers Dis* 9 197

Directional Improvements From Baseline for Metabolic and Inflammatory Biomarkers with Bezisterim



There were non-significant directional improvements in fasting insulin, HOMA2 insulin resistance, triglycerides, fructosamine, adiponectin, leptin, systolic and diastolic blood pressure, and weight (data not shown) with bezisterim. There were no significant differences in pTau, GFAP, NfL or Aβ42/40 ratio.

Biomarker Correlations With Neurological Assessments

Measure	Biomarker	Placebo		Bezisterim	
		Pearson R	P<0.1	Pearson R	P<0.1
GST	GFAP	0.671	$1.7e^{-4}$	0.253	
Global Statistical Test	pTau	0.444	**0.023**	0.174	
	Systolic BP	0.431	**0.028**	0.313	
	Cholesterol	−0.060		0.495	**0.014**
	NfL	0.169		0.480	**0.018**
	RANTES	0.165		−0.567	**0.004**
	SBC dAge	0.209		0.473	**0.055**
CDR-SB	GFAP	0.511	**0.008**	0.272	
Clinical Demential Rating	RANTES	0.398	**0.044**	−0.445	**0.004**
	Systolic BP	0.435	**0.026**	0.388	
Cog12	GFAP	0.589	**0.002**	0.272	
ADAS-Cognition	pTau	0.410	**0.037**	0.133	
	Cholesterol	−0.082		0.550	**0.005**
	NfL	0.083		0.458	**0.024**
	RANTES	−0.033		−0.637	**0.001**
	SBC dAge	0.167		0.455	**0.067**
MMSE	Cholesterol	0.020		−0.468	**0.021**
Mini-Mental State Exam	RANTES	0.003		0.516	**0.010**
	SBC dAge	−0.011		−0.580	**0.015**
CGIC	C1q	−0.395	**0.046**	0.294	
Clinician Global Impression of change	pTau	0.414	**0.036**	0.208	
	SBC dAge	0.017		0.467	**0.059**
ADL	CRP	−0.458	**0.019**	0.322	
Activities of Daily Living	Fructosamine	0.391	**0.048**	−0.459	**0.024**
	HOMA2 %B	−0.395	**0.046**	−0.014	
	NfL	−0.039		−0.642	**0.001**
ADCOMS	GFAP	0.497	**0.010**	0.294	
AD Composite Score	pTau 217	0.393	**0.047**	0.169	
	Systolic BP	0.525	**0.006**	0.370	
	NfL	0.120		0.454	**0.026**
	RANTES	0.227		−0.575	**0.003**
	SBC dAge	0.134		0.469	**0.058**

- Changes from baseline in biomarkers in **placebo, but not bezisterim** subjects correlated with **neurological assessment decline**
  - For example, **GFAP, pTau 217 and CRP**
- Directions of improvement in scales
  - Increased MMSE and ADL = improvement
  - Decreased GST, CDR-SB, CGIC, & ADCOMS = improvement

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	pTau	0.444	**0.023**	0.174	
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- Changes from baseline in biomarkers in **bezisterim but not placebo** subjects correlated with **neurological assessment improvement**
  - For example, **decreases in neurofilament light chain (NfL)** and **cholesterol**
- Directions of improvement in scales
  - Increased MMSE and ADL = improvement
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- The **chemokine RANTES** can alter astrocyte driven **microglial M1>M2 transition**
  - For **placebo** subjects, **increased RANTES** change from baseline was associated with **decline** in Clinical Dementia Rating
  - For **bezisterim** subjects, **increased RANTES** was associated with **improvements in neurological assessments**
- Directions of improvement in scales
  - Increased MMSE and ADL = improvement
  - Decreased GST, CDR-SB, CGIC, & ADCOMS = improvement

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	Cholesterol	−0.060		0.495	**0.014**
	NfL	0.169		0.480	**0.018**
	RANTES	0.165		−0.567	**0.004**
	SBC dAge	0.209		0.473	**0.055**
CDR-SB	GFAP	0.511	**0.008**	0.272	
	RANTES	0.398	**0.044**	−0.445	**0.004**
	Systolic BP	0.435	**0.026**	0.388	
Cog12	GFAP	0.589	**0.002**	0.272	
	pTau	0.410	**0.037**	0.133	
	Cholesterol	−0.082		0.550	**0.005**
	NfL	0.083		0.458	**0.024**
	RANTES	−0.033		−0.637	**0.001**
	SBC dAge	0.167		0.455	**0.067**
MMSE	Cholesterol	0.020		−0.468	**0.021**
	RANTES	0.003		0.516	**0.010**
	SBC dAge	−0.011		−0.580	**0.015**
CGIC	C1q	−0.395	**0.046**	0.294	
	pTau	0.414	**0.036**	0.208	
	SBC dAge	0.017		0.467	**0.059**
ADL	CRP	−0.458	**0.019**	0.322	
	Fructosamine	0.391	**0.048**	−0.459	**0.024**
	HOMA2 %B	−0.395	**0.046**	−0.014	
	NfL	−0.039		−0.642	**0.001**
ADCOMS	GFAP	0.497	**0.010**	0.294	
	pTau 217	0.393	**0.047**	0.169	
	Systolic BP	0.525	**0.006**	0.370	
	NfL	0.120		0.454	**0.026**
	RANTES	0.227		−0.575	**0.003**
	SBC dAge	0.134		0.469	**0.058**

- SkinBloodClock (SBC) dAge at completion correlated with neurological improvements in bezisterim subjects. There were no correlations in placebo subjects.
- Directions of improvement in scales
  - Increased MMSE and ADL = improvement
  - Decreased GST, CDR-SB, CGIC, & ADCOMS = improvement

Bezisterim Increased Methylation of Gene Promoters may be Associated with Clinical Improvements

- We identified 18 significant (FDR $p<0.05$) **bezisterim** subject clinical measure changes from baseline correlations with individual CpGs of genes that may be **beneficial** for AD subjects
- There were 6 significant (FDR $p<0.05$) **placebo** subject clinical measure changes from baseline correlations with CpGs of metabolic, inflammatory and dementia biomarker genes that may be **detrimental** for AD subjects
- The data suggest that bezisterim might be altering methylation of AD related genes in concert with **improvements in cognition and function**

Bezisterim Summary

- Analog of the neurosteroid βAET and may recapitulate, in humans, the beneficial effects on diseases of aging seen with DHEA in rodents
- Decreases inflammatory signal transduction and restores insulin sensitivity
- Well-tolerated safety profile to date
- Decreases biological age acceleration
- Bezisterim may act predominantly in myeloid innate immune cells (monocytes, macrophages, astrocytes, microglia)
- May act through RANTES transition of macrophage and microglia (M1) inflammatory to (M2) restorative programs through modification of the monocyte DNA methylome
- Bezisterim subjects had higher DNAm of genes enriched in neurodegeneration pathways
- Alters correlations with inflammatory metabolic disease, and biologic age biomarkers
- May improves Parkinson's disease motoric activity and Alzheimer's disease metabolic inflammation, cognition, function, imaging and biomarkers in clinical studies
- Bezisterim may improve systems dysregulation to restore homeostasis through expression changes of key neurodegeneration gene products
- This may be the first study to demonstrate improvement in longevity and clinical benefit in a disease of aging

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