Bezisterim Decreases Biological Age Acceleration in Alzheimer's Disease

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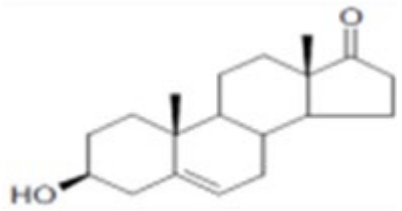
Disclosures

- Christopher Reading and Jiayan Yan are employees and shareholders of BioVie Inc.
- Varun Dwaraka is an employee and shareholder of TruDiagnostics, Inc.

Bezisterim Is a Sterol Immunomodulator

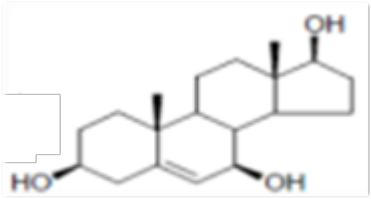
DHEA (dehydroepiandrosterone)

- Anti-inflammatory, insulin sensitizing, anti-aging, and anti-dementia activity in rodents, but not humans, due to differential metabolism



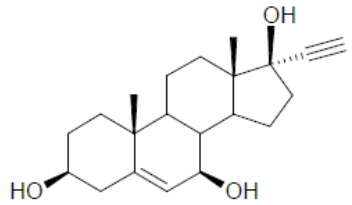
βAET (5-androstane,3β,7β,17β-triol)

- Active metabolite in rodents, anti-inflammatory activity in humans (as an injectable), neurosteroid activity, but inactivated by oxidative metabolism associated with disease



Bezisterim (17α-ethynyl-βAET)

- Pharmaceutical, metabolically stable, orally bioavailable, BBB permeable, anti-inflammatory insulin sensitizer
- Binds ERK (extracellular signal-related kinase 1/2); inhibits nuclear factor kappa B (NFκB) (master regulator of inflammation)
- In clinical development for Alzheimer's disease (AD), Parkinson's disease, and Long Covid; attractive safety profile to date
- Bezisterim and its metabolites have no sex steroid or glucocorticoid activity



Epigenetic Biomarkers of Aging and AD

- Current AD therapies: inhibiting one gene product
- Systems biology approach for AD therapy
  - There are expression changes in many genes in AD
  - Goal: Re-establish homeostasis; small changes in many genes
- Age is the #1 risk factor for AD
- Epigenetic biomarkers (DNA methylation, 850K CpGs) can identify “Epigenetic Age Acceleration” (EAA), ie, difference between observed biological age and expected biological based on the chronological age
- Bezisterim activities were analyzed in a placebo-controlled trial in subjects with mild to moderate AD
- Bezisterim decreased EAA in AD
- Curation of genes associated with EAA in AD suggests that aging and diseases of aging are driven by inflammatory signaling
- Inflammation is an innate immune response to infections and to danger associated molecular patterns
- Bezisterim may alter biological age by anti-inflammatory epigenetic modification

Bezisterim Decreases Age Acceleration in AD

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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

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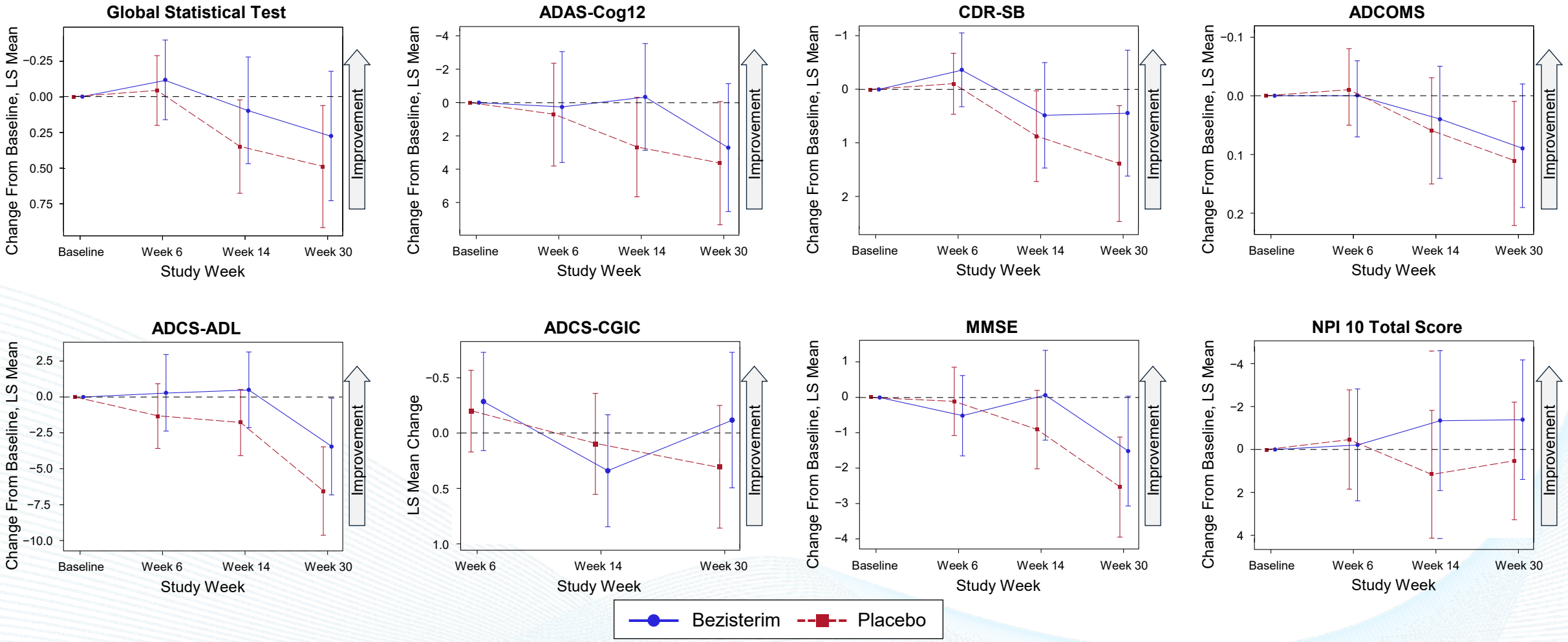
- The 7-month study was conducted during the **COVID epidemic** and there were **large numbers of exclusions due to GCP violations**, leaving the study underpowered for primary and secondary endpoints.
- Analysis was restricted to 50 participants with source-document-verified clinical measures and samples, that completed the protocol. This analysis focuses on epigenetic, metabolic, biomarker, and cognitive measures in the exploratory biomarker population that completed the protocol.

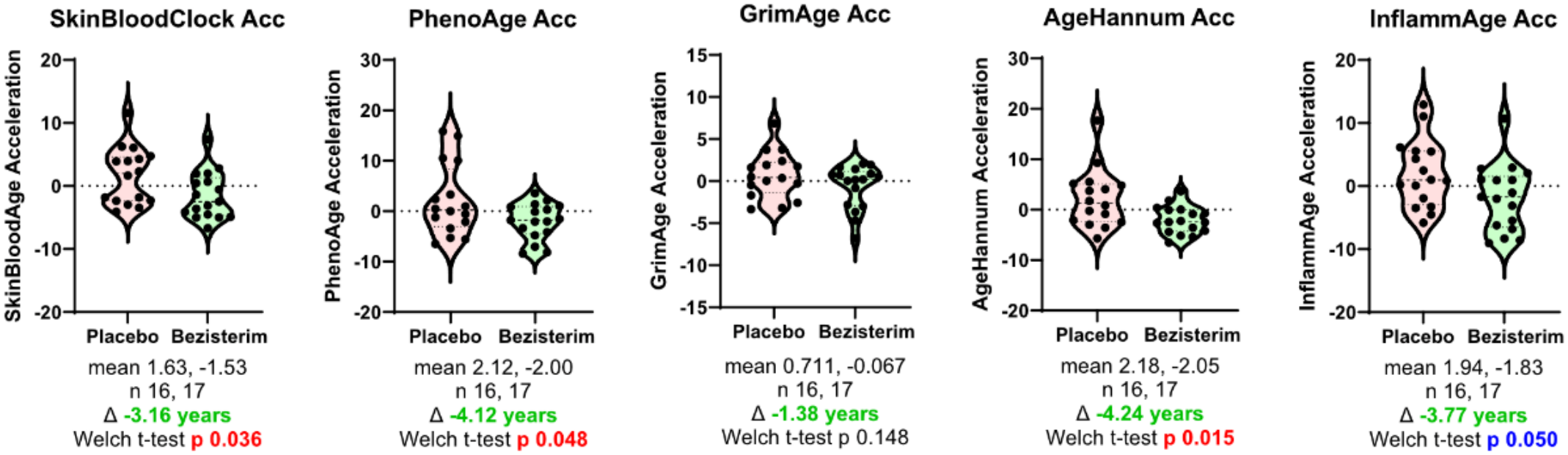
Bezisterim Showed Improvements Over Time in Primary and Secondary Endpoints[1]



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[2] and aducanumab[3]

1. Reading CL, et al. *Front Neurosci.* 2025;19:1516746; 2. van Dyck 2023 *N Engl J Med*. 388 9; 3. Budd Haeberlein 2022 *J Prev Alzheimers Dis* 9 197

Bezisterim Subjects Showed Lower EAA Than Placebo Subjects at 30 Weeks



- Age acceleration as calculated by different epigenetic aging clocks[1-5]
- Note consistent placebo acceleration, and bezisterim deceleration

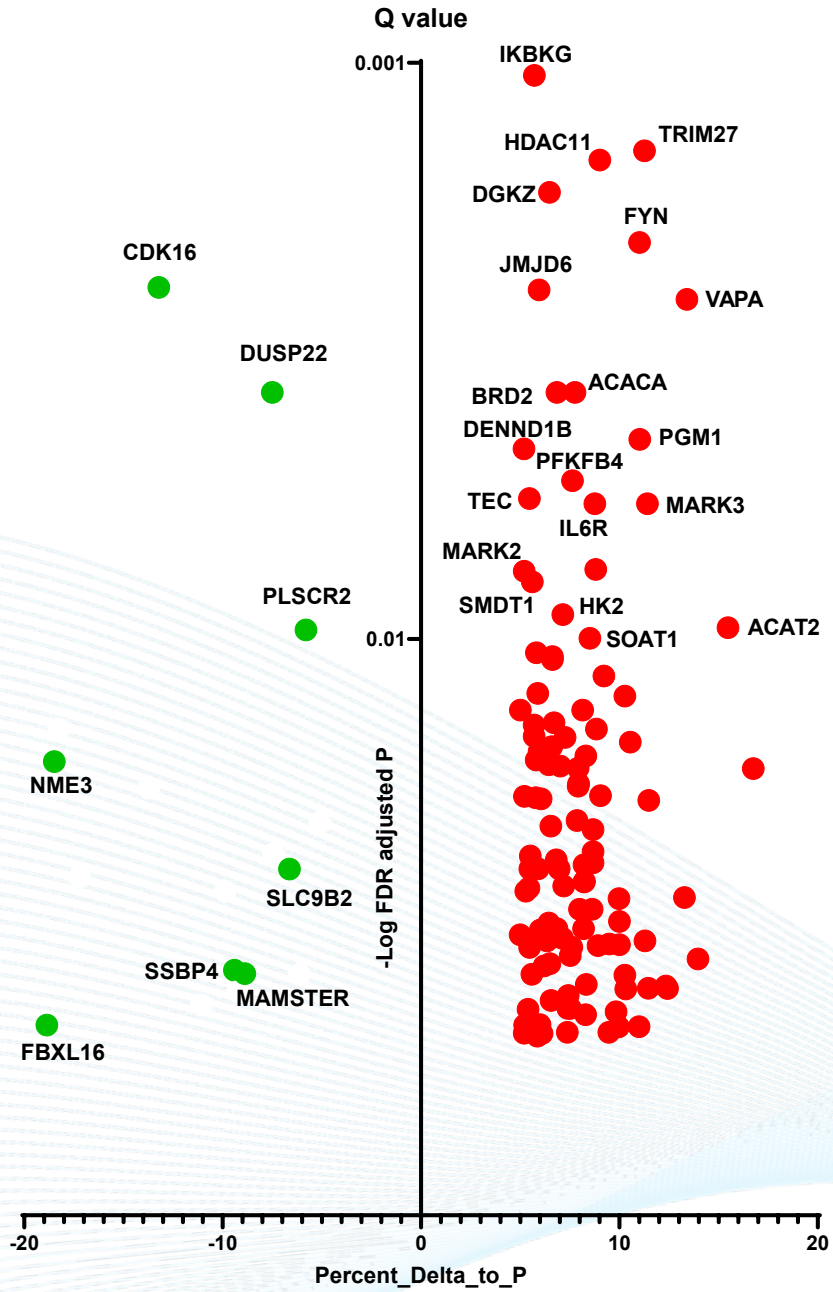
Raw data from Epigenetic Clock Development Foundation; analyses of diverse epigenetic aging clocks performed by Chronomics/Hurdle Group, including their new InflammAge Clock[5]

1. Horvath 2018 *Aging* 10 1758; 2. Levine 2018 *Aging* 2018 10 573; 3. Lu 2019 *Aging*. 2019 11 303; 4. Hannum 2013 *Mol Cell* 49 359; 5. Schmunk *bioRxiv*. 2023. doi.org/10.1101/2023.12.21.572866

Hypothesis: bezisterim may modulate aging and AD risk gene expression through anti-inflammatory epigenetic modification

- TruDiagnostics identified significant trends for EAA differences between bezisterim and placebo subjects for 10 epigenetic clocks; Clock CpGs were mapped to underlying **[aging & AD risk]** genes
- We identified significant (FDR) **average gene promoter methylation differences** (bezisterim-placebo ≥ 5%) from our AD subjects **[potential biomarker genes]**
- We examined aging & AD risk gene and potential biomarker genes associations
  - Cognitive and activity clinical assessments
  - Biomarker assessments (amyloid β, phospho-Tau)
  - Dysregulated glucose metabolism; insulin resistance; Warburg effect
  - Dysregulated target phosphoproteins
  - Dysregulated lipid metabolism, lipid droplets (Alois Alzheimer, 1907)
  - Endoplasmic reticulum stress unfolded protein response, decreased autophagy
  - Inflammatory gene activation (NFκB, cytokines, inflammasome)
  - Macrophage and microglia inflammation, M1 metabolic shift
  - Mitochondrial dysfunction
  - Neuronal death, senescence apoptosis, ferroptosis, excitotoxicity
  - Synaptic dysfunction

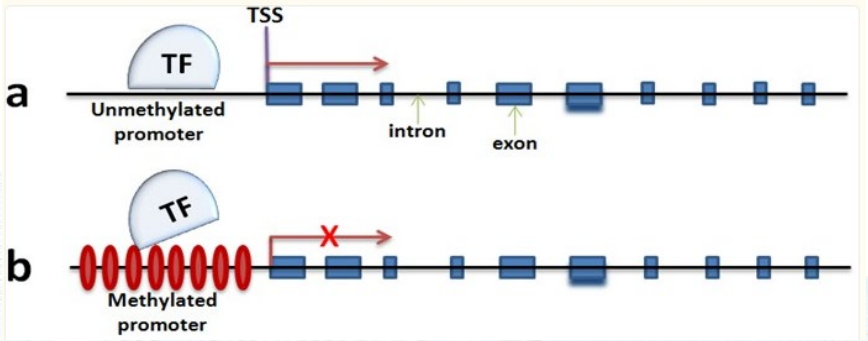
Volcano plot of genes with FDR significant 5% promoter DNAm change for bezisterim compared to placebo subjects



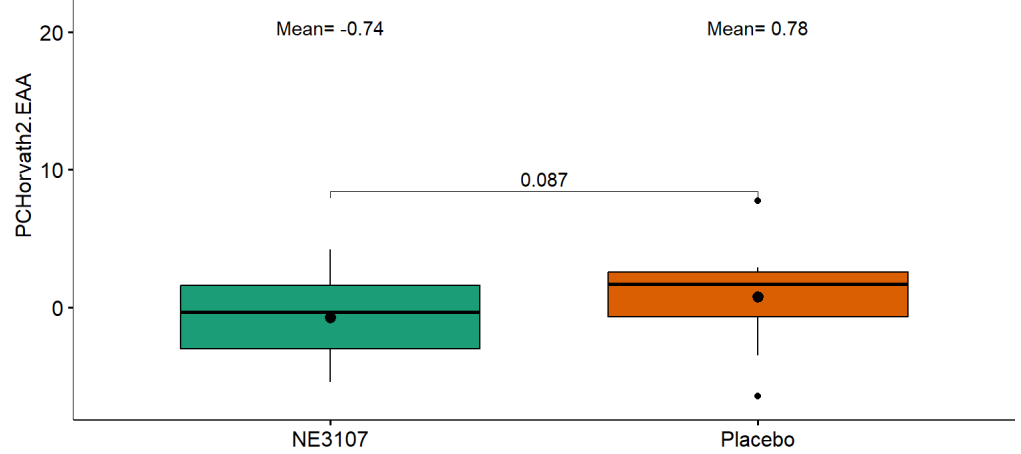
Focus on potentially beneficial changes, since bezisterim shows improvement and no SAEs associated with bezisterim to date

- If promoter DNAm is increased (decreased expression), examine genes expression associated with risk (n = 98)
- If promoter DNAm is decreased (increased expression), examine genes expression associated with benefit (n = 8)

Higher promoter DNAm is associated with lower gene expression



Modified from Xu 2015 Oncotarget 20 13922



Epigenetic age acceleration (Horvath et al., 2015 Aging 7 1159) of principal component analysis (Higgins-Chen et al., 2022 Nat Aging 2 644) of the highly accurate Skin-and-blood predictor of biological age (Horvath, et al., 2018 Aging 10 1758)

Principle Component Horvath 2 Clock

Bezisterim: Epigenetic Age Deceleration

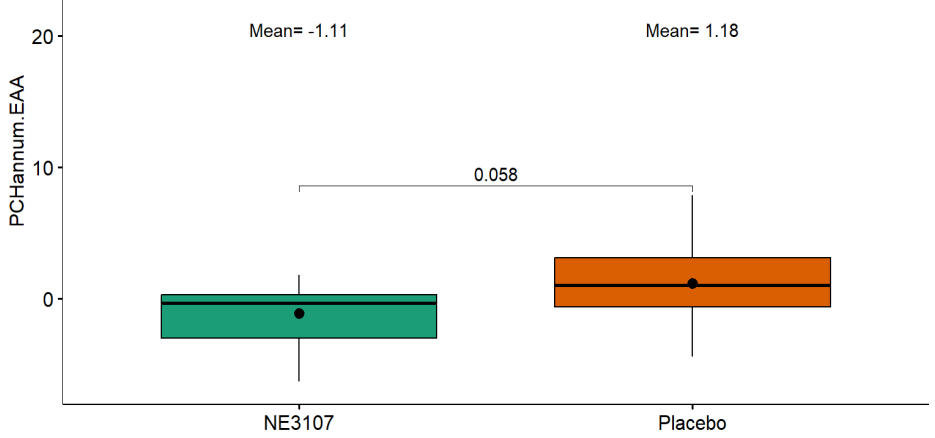
Placebo: Epigenetic Age Acceleration

Potentially beneficial changes in Aging & AD Pathophysiology

10/11 (91%) related to inflammation

Anti-inflammatory
Inflammation
Not Inflammation?

Gene	Gene Name	**PCHorvath2 EAA**	PCHannum EAA	PCGrimAge EAA	DamAge EAA	IntrinClock EAA	Stoch Zhang EAA	Stoch Horvath EAA	Stoch PhenoAge EAA	RetroClock V1 EAA	RetroClock V2 EAA	myeloid Polarization	Kinase Cascades	Carbohydrate Metab	Dysreg Phosphorylation	Nominated AD target	BMI Aging TF	Cognition aging	Epigen Inflamm score	Braak Pathology	Type 2 Diabetes
FBXL16	F-box and leucine rich repeat protein 16	●					●									●					
HDAC4	histone deacetylase 3	●							●			●		●				●		●	●
MBNL1	muscleblind like splicing regulator 1	●						●	●										●		●
PTGER2	prostaglandin E receptor 2	●						●									●				●
PTPN6	protein tyrosine phosphatase non-receptor type 6	●										●				●		●		●	
RUNX3	RUNX family transcription factor 3	●										●					●				●
SOAT1	sterol O-acyltransferase 1	●						●													
TCF12	transcription factor 12	●															●	●			●
TNFSF10	TNF superfamily member 10	●										●							●		●
UBA7	ubiquitin like modifier activating enzyme 7	●																			●
ZMYND8	zinc finger MYND-type containing 7	○												○					○		



Epigenetic age acceleration of principal component analysis of the first-generation epigenetic clock Hannum predictor of epigenetic age based on DNA methylation (Hannum et al., 2012 Mol Cell 49 359)

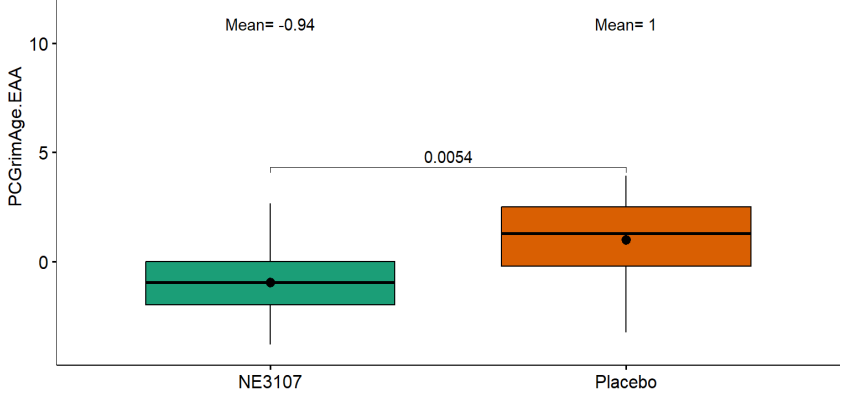
Principle Component Hannum Clock

Bezisterim: Epigenetic Age Deceleration

Placebo: Epigenetic Age Acceleration

Potentially beneficial changes in Aging & AD Pathophysiology **related to inflammation**

Gene	Gene Name	PCHorvath2 EAA	**PCHannum EAA**	PCGrimAge EAA	DamAge EAA	IntrinClock EAA	Stoch Zhang EAA	Stoch Horvath EAA	Stoch PhenoAge EAA	RetroClock V1 EAA	RetroClock V2 EAA	myeloid Polarization	Kinase Cascades	Carbohydrate Metab	Dysreg Phosphorylation	Nominated AD target	BMI Aging TF	Cognition aging	Epigen Inflamm score	Braak Pathology	Type 2 Diabetes
	Inflammation																				
AMPD3	adenosine monophosphate deaminase 3		●						●									●	●		●



Epigenetic age acceleration of principal component analysis of the GrimAge **predictor of mortality** that uses surrogate measures of plasma proteins and smoking history to predict biological age (Lu, et al., 2019 Aging 11 303)

Principle Component GrimAge Clock

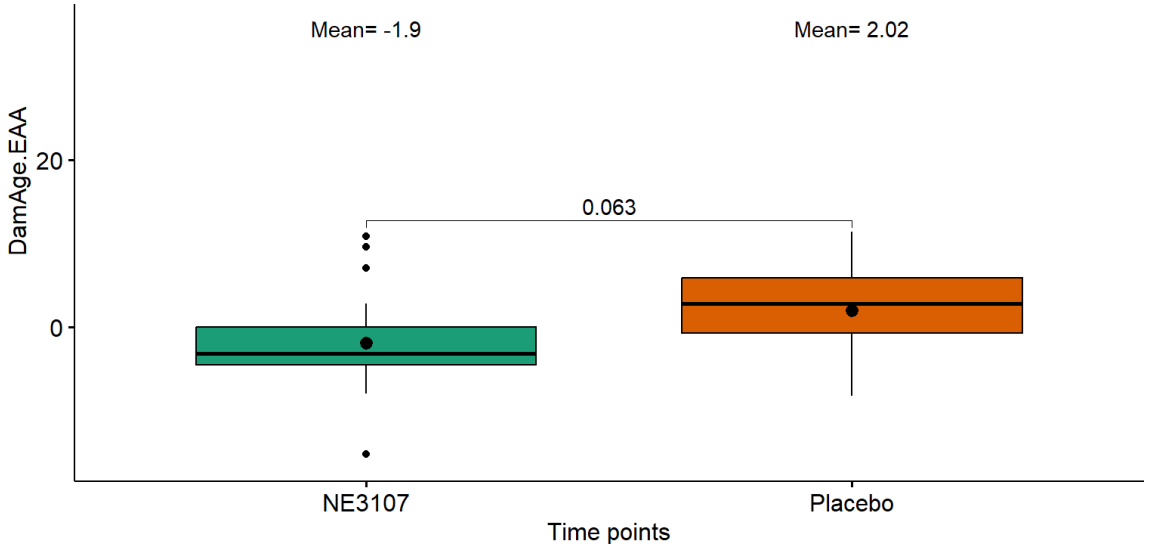
Bezisterim: Epigenetic Age Deceleration

Placebo: Epigenetic Age Acceleration

Potentially beneficial changes in Aging & AD Pathophysiology

4/5 (**80%**) **related to inflammation**

Gene	Gene Name	PCHorvath2 EAA	PCHannum EAA	**PCGrimAge EAA**	DamAge EAA	IntrinClock EAA	Stoch Zhang EAA	Stoch Horvath EAA	Stoch PhenoAge EAA	RetroClock V1 EAA	RetroClock V2 EAA	myeloid Polarization	Kinase Cascades	Carbohydrate Metab	Dysreg Phosphorylation	Nominated AD target	BMI Aging TF	Cognition aging	Epigen Inflamm score	Braak Pathology	Type 2 Diabetes
	Inflammation			●																	
	Not Inflammation?			○																	
SAT1	spermidine/spermine N1-acetyltransferase 1			●																	
TXNIP	thioredoxin interacting protein			●								●						●	●		●
VPS52	VPS52 subunit of GARP complex			●															●	●	
ZBTB4	zinc finger and BTB domain containing 4			○																	○
ZNF302	zinc finger protein 302			●																	



Epigenetic age acceleration of the DamAge predictor of detrimental causality-enriched aging and mortality biological traits (Ying, et al., 2024 Nat Aging 4 231)

DamAge Clock

Bezisterim: Epigenetic Age Deceleration

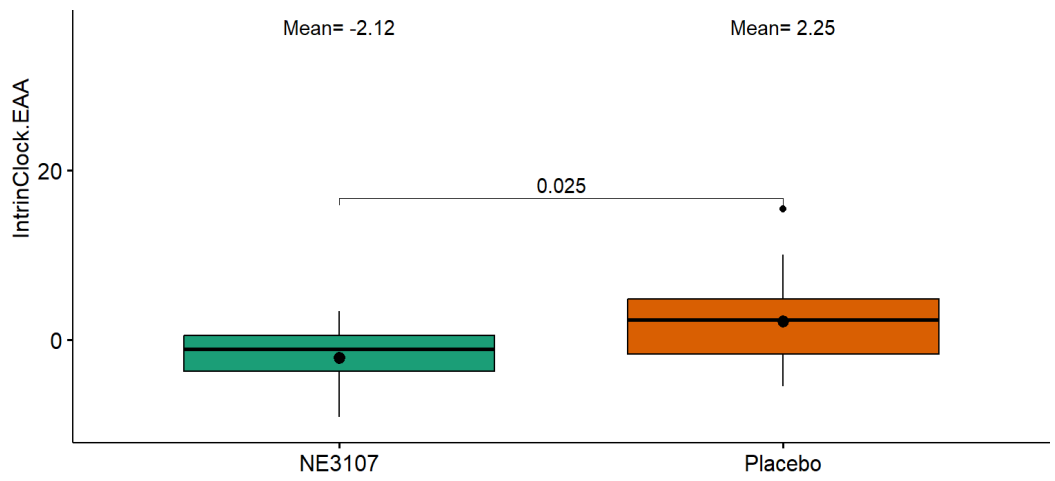
Placebo: Epigenetic Age Acceleration

Potentially beneficial changes in Aging & AD Pathophysiology

7/8 (88%) related to inflammation

Inflammation
Not Inflammation?

Gene	Gene Name	PCHorvath2 EAA	PCHannum EAA	PCGrimAge EAA	**DamAge EAA**	IntrinClock EAA	Stoch Zhang EAA	Stoch Horvath EAA	Stoch PhenoAge EAA	RetroClock V1 EAA	RetroClock V2 EAA	myeloid Polarization	Kinase Cascades	Carbohydrate Metab	Dysreg Phosphorylation	Nominated AD target	BMI Aging TF	Cognition aging	Epigen Inflamm score	Braak Pathology	Type 2 Diabetes
ACAP1	ArfGAP with coiled-coil, ankyrin repeat and PH domains 1				●							●								●	
GPX1	glutathione peroxidase 1				○									○					○		○
IRF7	interferon regulatory factor 7				●															●	
LRRC46	leucine rich repeat containing 46				●																
NRBP1	Nuclear receptor-binding protein				●								●								
PTK2B	Protein-tyrosine kinase 2-beta				●								●			●					●
SMDT1	single-pass membrane protein with aspartate rich tail 1				●																
STAT3	signal transducer and activator of transcription 2				●									●		●	●	●			●



Epigenetic age acceleration of the IntrinClock aging predictor of inherent, cell-intrinsic aging processes rather than changes in cell composition (Tomusiak, et al., 2024 Commun Biol 7 934). Immune response genes were eliminated in this clock.

Intrinsic Age Clock

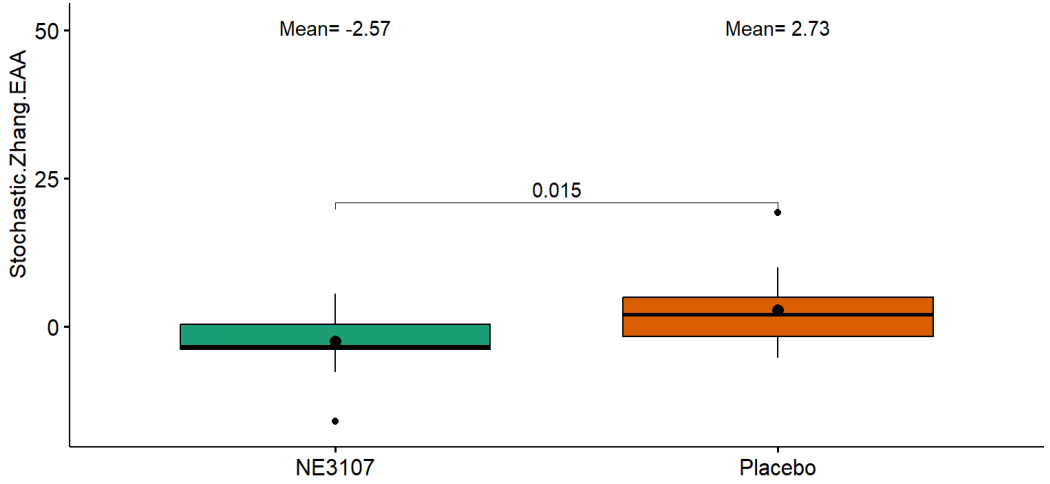
Bezisterim: Epigenetic Age Deceleration

Placebo: Epigenetic Age Acceleration

Potentially beneficial changes in Aging & AD Pathophysiology

4/8 (50%) related to inflammation

Gene	Gene Name	PCHorvath2 EAA	PCHannum EAA	PCGrimAge EAA	DamAge EAA	**IntrinClock EAA**	Stoch Zhang EAA	Stoch Horvath EAA	Stoch PhenoAge EAA	RetroClock V1 EAA	RetroClock V2 EAA	myeloid Polarization	Kinase Cascades	Carbohydrate Metab	Dysreg Phosphorylation	Nominated AD target	BMI Aging TF	Cognition aging	Epigen Inflamm score	Braak Pathology	Type 2 Diabetes
	Inflammation					●			●			●	●	●		●		●			●
	Not Inflammation?					○	○	○					○								○
ACBD4	acyl-CoA binding domain containing 4					○															
B3GALT4	beta-1,3-galactosyltransferase 4					○															
CD46	CD46 molecule					●															●
CSNK1D	Casein kinase I isoform delta					○	○	○					○								○
EFNA4	ephrin A4					○															
JMJD6	jumonji domain containing 6, arginine demethylase and lysine hydroxylase					●						●				●					
MAP2K3	mitogen-activated protein kinase kinase 3					●			●				●					●			●
MTOR	Serine/threonine-protein kinase mTOR					●							●	●				●			



Epigenetic age acceleration of the stochastic (Tong, et al., 2024 Nat Aging 4 886) Zhang computational model that simulates the accumulation of stochastic DNA methylation changes over time (Zhang, et al., 2019 Genom Med 11 54).

Stochastic Zhang Clock

Bezisterim: Epigenetic Age **Deceleration**

Placebo: Epigenetic Age **Acceleration**

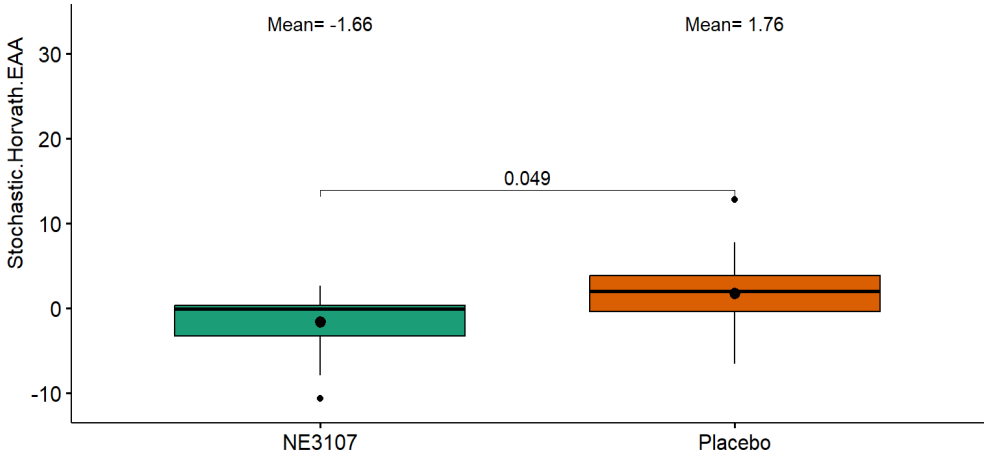
Potentially beneficial changes in Aging & AD Pathophysiology

4/5 (**80%**) **related to inflammation**

Stochastic changes are believed to be associated with random changes during normal aging

Anti-inflammatory
Inflammation
Not Inflammation?

Gene	Gene Name	PCHorvath2 EAA	PCHannum EAA	PCGrimAge EAA	DamAge EAA	IntrinClock EAA	**Stoch Zhang EAA**	Stoch Horvath EAA	Stoch PhenoAge EAA	RetroClock V1 EAA	RetroClock V2 EAA	myeloid Polarization	Kinase Cascades	Carbohydrate Metab	Dysreg Phosphorylation	Nominated AD target	BMI Aging TF	Cognition aging	Epigen Inflamm score	Braak Pathology	Type 2 Diabetes
CSNK1D	Casein kinase I isoform delta	○				○	○	○					○								○
FBXL16	F-box and leucine rich repeat protein 16	●					●									●					
HOXA4	homeobox A4						●											●			●
NEAT1	nuclear paraspeckle assembly transcript 1						●			●	●	●		●					●		
SP140	SP140 nuclear body protein						●												●		●



Epigenetic age acceleration of the stochastic computational model that simulates the accumulation of stochastic DNA methylation changes over time (Tong, et al., 2024 Nat Aging 4 886) based on the first Horvath predictor of biologcal aging (Horvath 2013 Genom Biol 14 R115).

Stochastic Horvath 1 Clock

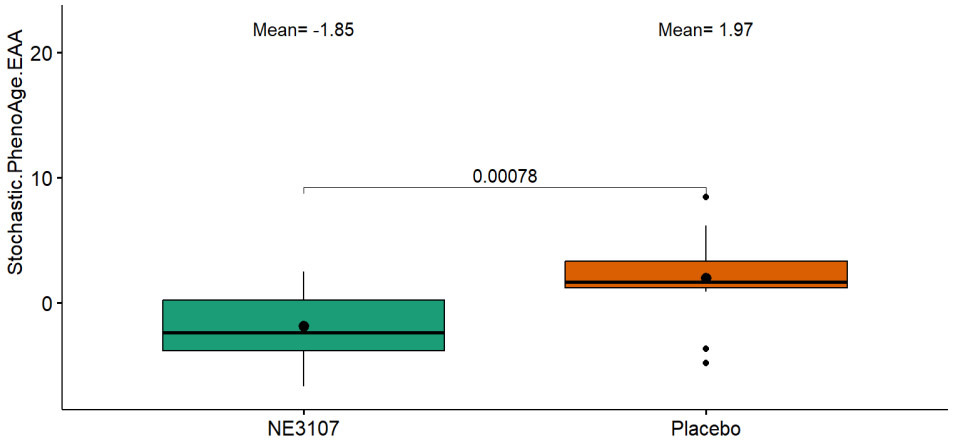
Bezisterim: Epigenetic Age Deceleration

Placebo: Epigenetic Age Acceleration

Potentially beneficial changes in Aging & AD Pathophysiology

5/9 (56%) related to inflammation

Gene	Gene Name	PCHorvath2 EAA	PCHannum EAA	PCGrimAge EAA	DamAge EAA	IntrinClock EAA	Stoch Zhang EAA	**Stoch Horvath EAA**	Stoch PhenoAge EAA	RetroClock V1 EAA	RetroClock V2 EAA	myeloid Polarization	Kinase Cascades	Carbohydrate Metab	Dysreg Phosphorylation	Nominated AD target	BMI Aging TF	Cognition aging	Epigen Inflamm score	Braak Pathology	Type 2 Diabetes
	Inflammation	●						●	●			●		●					●		●
	Not Inflammation?					○	○	○					○					○			○
ABHD14A	abhydrolase domain containing 14A							○										○			○
BAZ2A	bromodomain adjacent to zinc finger domain 2A							○													○
CSNK1D	Casein kinase I isoform delta					○	○	○					○								○
E2F2	E2F transcription factor 2							●				●		●							●
HTRA2	HtrA serine peptidase 2							●	●			●									
MBNL1	muscleblind like splicing regulator 1	●						●	●										●		●
MPI	mannose phosphate isomerase							○				○		○							
PTGER2	prostaglandin E receptor 2	●						●										●			●
SOAT1	sterol O-acyltransferase 1	●						●													



Epigenetic age acceleration of the stochastic computational model that simulates the accumulation of stochastic DNA methylation changes over time (Tong, et al., 2024 Nat Aging 4 886) based on the PhenoAge predictor of lifespan and healthspan (Levine, et al., 2018 Aging 17 573).

Stochastic PhenoAge Clock

Bezisterim: Epigenetic Age Deceleration

Placebo: Epigenetic Age Acceleration

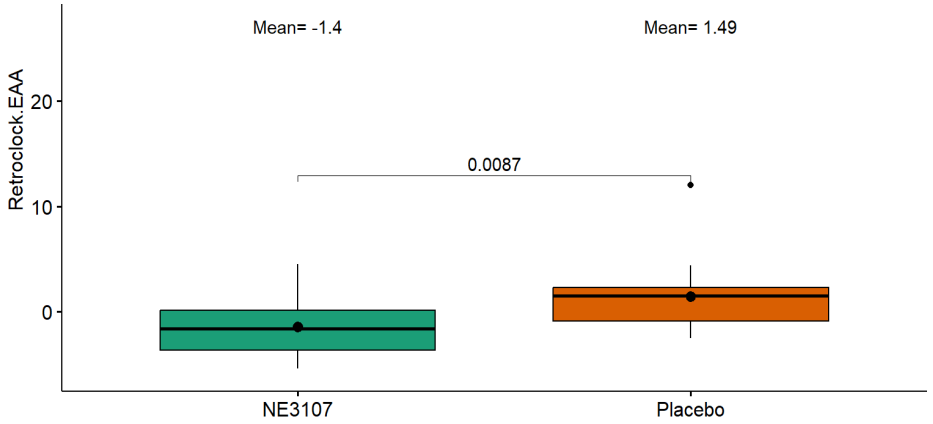
Potentially beneficial changes in Aging & AD Pathophysiology

10/12 (83%) related to inflammation

Inflammation

Not Inflammation?

Gene	Gene Name	PCHorvath2 EAA	PCHannum EAA	PCGrimAge EAA	DamAge EAA	IntrinClock EAA	Stoch Zhang EAA	Stoch Horvath EAA	**Stoch PhenoAge EAA**	RetroClock V1 EAA	RetroClock V2 EAA	myeloid Polarization	Kinase Cascades	Carbohydrate Metab	Dysreg Phosphorylation	Nominated AD target	BMI Aging TF	Cognition aging	Epigen Inflamm score	Braak Pathology	Type 2 Diabetes
AMPD3	adenosine monophosphate deaminase 3		●						●									●	●		●
CSNK1G3	Casein kinase I isoform gamma-2								○				○								○
DGKZ	diacylglycerol kinase zeta								●			●	●					●	●		●
EIF4E	eukaryotic translation initiation factor 4E								●			●		●							
FUT8	fucosyltransferase 8								●					●							
HDAC4	histone deacetylase 3	●							●			●		●				●		●	●
HEMK1	HemK methyltransferase family member 1								○												○
HSPA2	heat shock protein family A (Hsp70) member 2								●							●					
HTRA2	HtrA serine peptidase 2							●	●			●									
MAP2K3	mitogen-activated protein kinase kinase 3					●			●				●					●			●
MBNL1	muscleblind like splicing regulator 1	●						●	●										●		●
RUNX2	RUNX family transcription factor 2								●			●		●						●	●



Epigenetic age acceleration of the RetroAge V1 age predictor based on endogenous retroviral and long interspersed nuclear elements based on the Epic v1.0 array (Ndhlovu et ail., Aging Cell 10 e14288).

Retroviral Insert 1 Clock

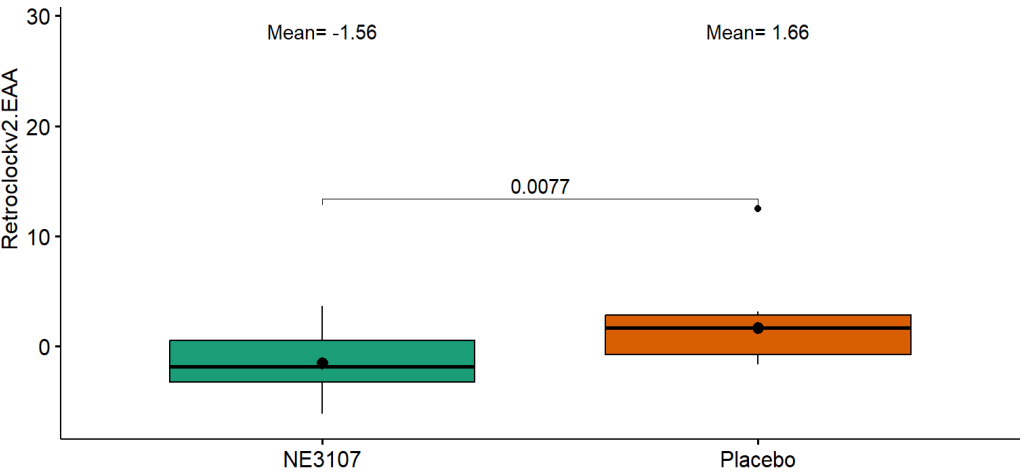
Bezisterim: Epigenetic Age **Deceleration**

Placebo: Epigenetic Age **Acceleration**

Potentially beneficial changes in Aging & AD Pathophysiology

3/3 **related to inflammation**

Gene	Gene Name	Inflammation	PCHorvath2 EAA	PCHannum EAA	PCGrimAge EAA	DamAge EAA	IntrinClock EAA	Stoch Zhang EAA	Stoch Horvath EAA	Stoch PhenoAge EAA	**RetroClock V1 EAA**	RetroClock V2 EAA	myeloid Polarization	Kinase Cascades	Carbohydrate Metab	Dysreg Phosphorylation	Nominated AD target	BMI Aging TF	Cognition aging	Epigen Inflamm score	Braak Pathology	Type 2 Diabetes
IL12RB1	interleukin 12 receptor subunit beta 1										●											●
NEAT1	nuclear paraspeckle assembly transcript 1							●			●	●	●		●					●		
PRKRIP1	PRKR interacting protein 1										●	●										



Epigenetic age acceleration of the RetroAge V2 age predictor based on endogenous retroviral and long interspersed nuclear elements based on the Epic v2.0 array (Ndhlovu et ail., Aging Cell 10 e14288).

Retroviral Insert 2 Clock

Bezisterim: Epigenetic Age Deceleration

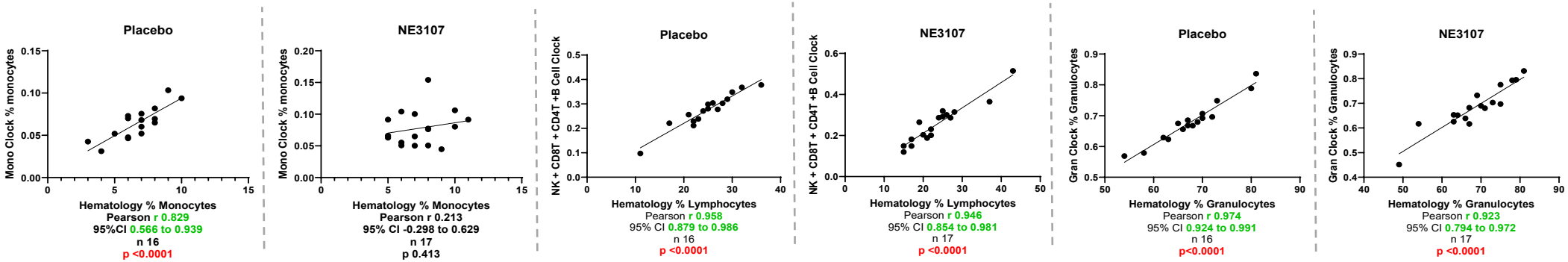
Placebo: Epigenetic Age Acceleration

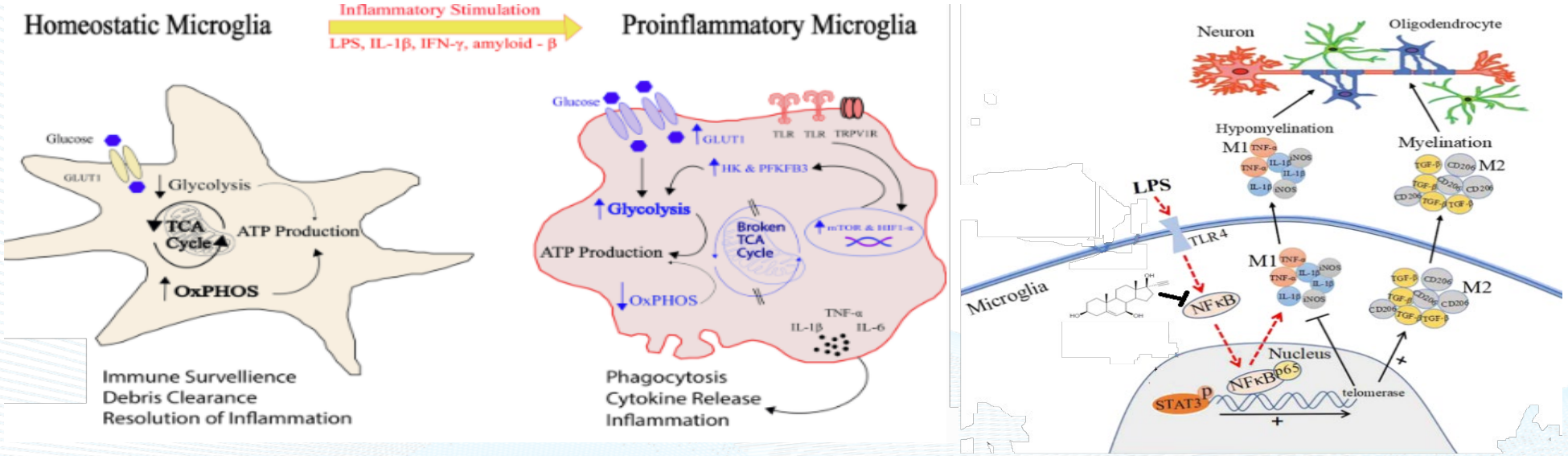
Potentially beneficial changes in Aging & AD Pathophysiology

4/5 (80%) related to inflammation

Gene	Gene Name	PCHorvath2 EAA	PCHannum EAA	PCGrimAge EAA	DamAge EAA	IntrinClock EAA	Stoch Zhang EAA	Stoch Horvath EAA	Stoch PhenoAge EAA	RetroClock V1 EAA	**RetroClock V2 EAA**	myeloid Polarization	Kinase Cascades	Carbohydrate Metab	Dysreg Phosphorylation	Nominated AD target	BMI Aging TF	Cognition aging	Epigen Inflamm score	Braak Pathology	Type 2 Diabetes
	Inflammation																				
	Not Inflammation?																				
ACOT9	acyl-CoA thioesterase 9										●										
FTO	FTO alpha-ketoglutarate dependent dioxygenase										●	●						●			
HASPIN	histone H3 associated protein kinase										○		○								
NEAT1	nuclear paraspeckle assembly transcript 1						●			●	●	●		●					●		
PRKRIP1	PRKR interacting protein 1									●	●										

Bezisterim may modify myeloid polarization





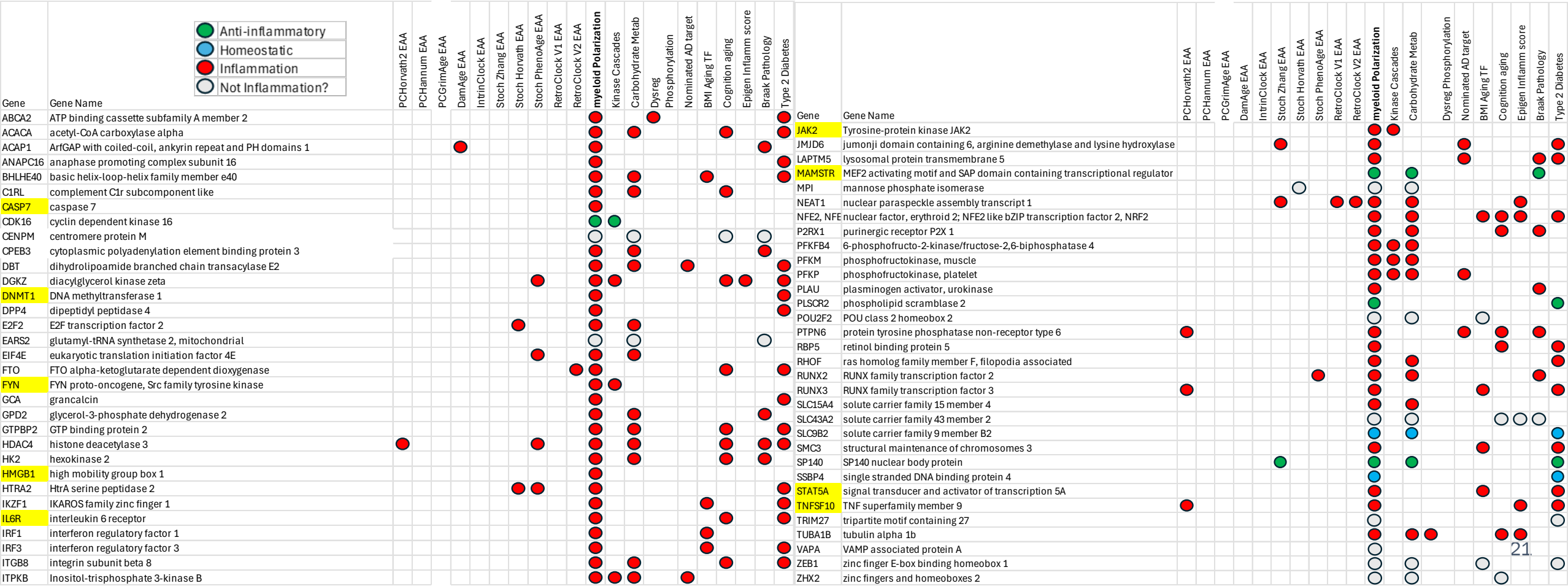
Modified from Bielanin & Sun 2023, Transl Stroke Res 14 435 and Zhou 2021 Mol Neurobiol 58 6552

Bezisterim DNAm: potential decrease in innate immune cell polarization

Dekkers, et al., 2019 Epigenetics Chromatin 12 34

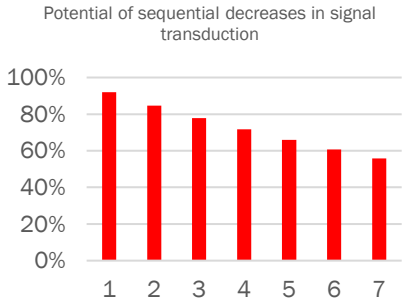
Potentially beneficial changes in Aging & AD Pathophysiology

55/64 (86%) related to inflammation



Bezisterim may decreased inflammatory gene expression *via* sequentially increased kinase promotor methylation in signal cascades

Signal



MAP4K

HRAS +13%
KRAS +10%
MAP4K2 +4%

Cytokines, TGFβ +4%
BMP +11%
MAP4K1 +14%
MAP4K2 +4%
MAP4K3 +12%

TGFβ +4%
Cytokines

Inflammation
Phospho-Tau
Amyloid β

MAP3K

RAF1 +6%
MAP3K1 +10%
MAP3K3 +9%
MAP3K8 +8%

MAP3K1 +10%
MAP3K3 +9%
MAP3K5 +12%
MAP3K7 +5%
MAP3K9 +10%
MAP3K11 +6%
MAP3K13 +5%

MAP3K5 +12%

MAP3K1 +10%
MAP3K2 +8%
MAP3K8 +8%
MAP3K9 +10%
MAP3K14 +2%

Phosphorylation

MAP2K

MAP2K2 +10%
MAP2K1 +8%

MAP2K4 +6%
MAP2K4 +6%
MAP2K7 +12%

MAP2K3 +9%
MAP2K6 +12%

ERK1
MAPK1 +10%
ERK2
MAPK3 +10%

JNK1
MAPK8 +7%
JNK2
MAPK9 +8%

p38α MAPK14 +3%

MAPK

Transcription Factors #

RSK1/2/3 +6%
MSK1/2 +5%

JUN +11%
ATF +5%
ELK +8%
MEF2 +7%

MNK1/2 +3%
MSK1/2 +5%

ELK +8%
SRF +4%

ATF +5%
SMAD* +7%

IκBKβ +8%
IκBKγ +6%
NFκBIα +6%
NFκBIβ +9%
NFκBIδ +9%
NFκBIε +1%

NFκB2 +9%

FOSB +10%

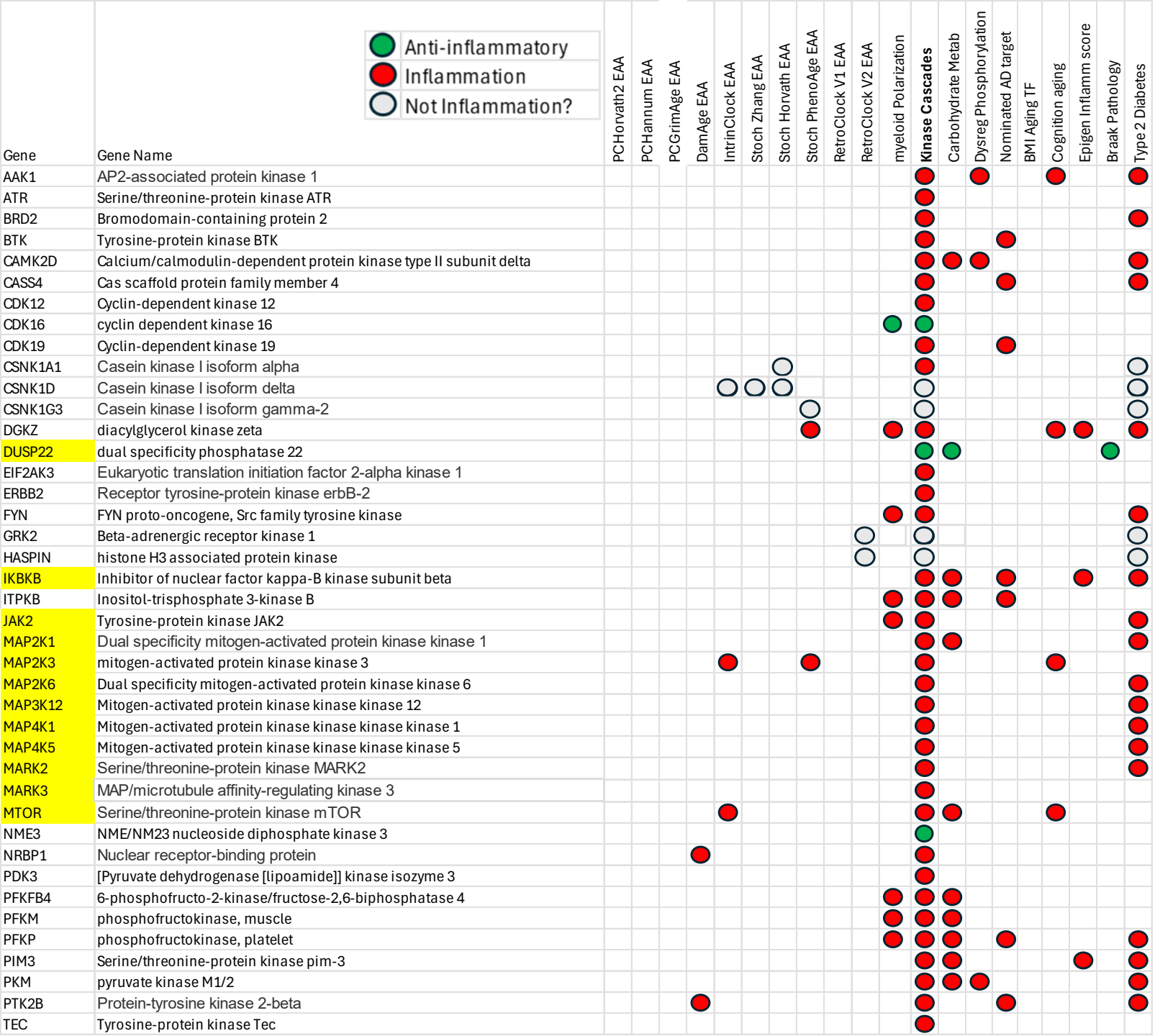
Transcription

TNF +4%
IL6 +9%
IL6R +9%
IL17 +5%
IL17R +12%

Inflammatory Targets

< 1% change in a number of unrelated transcription factors

Red numbers are average DNA methylation β value % increase



Gene	Gene Name	PCHorvath2 EAA	PCHannum EAA	PCGrimAge EAA	DamAge EAA	IntrinClock EAA	Stoch Zhang EAA	Stoch Horvath EAA	Stoch PhenoAge EAA	RetroClock V1 EAA	RetroClock V2 EAA	myeloid Polarization	**Kinase Cascades**	Carbohydrate Metab	Dysreg Phosphorylation	Nominated AD target	BMI Aging TF	Cognition aging	Epigen Inflamm score	Braak Pathology	Type 2 Diabetes
AAK1	AP2-associated protein kinase 1												🔴		🔴			🔴			🔴
ATR	Serine/threonine-protein kinase ATR												🔴								
BRD2	Bromodomain-containing protein 2												🔴								🔴
BTK	Tyrosine-protein kinase BTK												🔴			🔴					
CAMK2D	Calcium/calmodulin-dependent protein kinase type II subunit delta												🔴	🔴	🔴						🔴
CASS4	Cas scaffold protein family member 4												🔴			🔴					🔴
CDK12	Cyclin-dependent kinase 12												🔴								
CDK16	cyclin dependent kinase 16											🟢	🟢								
CDK19	Cyclin-dependent kinase 19												🔴			🔴					
CSNK1A1	Casein kinase I isoform alpha							⚪					🔴								⚪
CSNK1D	Casein kinase I isoform delta					⚪	⚪	⚪					⚪								⚪
CSNK1G3	Casein kinase I isoform gamma-2								⚪				⚪								⚪
DGKZ	diacylglycerol kinase zeta								🔴			🔴	🔴					🔴	🔴		🔴
DUSP22	dual specificity phosphatase 22												🟢	🟢						🟢	
EIF2AK3	Eukaryotic translation initiation factor 2-alpha kinase 1												🔴								
ERBB2	Receptor tyrosine-protein kinase erbB-2												🔴								
FYN	FYN proto-oncogene, Src family tyrosine kinase											🔴	🔴								🔴
GRK2	Beta-adrenergic receptor kinase 1										⚪		⚪								⚪
HASPIN	histone H3 associated protein kinase										⚪		⚪								⚪
IKBKB	Inhibitor of nuclear factor kappa-B kinase subunit beta												🔴	🔴		🔴			🔴		🔴
ITPKB	Inositol-trisphosphate 3-kinase B											🔴	🔴	🔴		🔴					
JAK2	Tyrosine-protein kinase JAK2											🔴	🔴								🔴
MAP2K1	Dual specificity mitogen-activated protein kinase kinase 1												🔴	🔴							🔴
MAP2K3	mitogen-activated protein kinase kinase 3					🔴			🔴				🔴					🔴			
MAP2K6	Dual specificity mitogen-activated protein kinase kinase 6												🔴								🔴
MAP3K12	Mitogen-activated protein kinase kinase kinase 12												🔴								🔴
MAP4K1	Mitogen-activated protein kinase kinase kinase kinase 1												🔴								🔴
MAP4K5	Mitogen-activated protein kinase kinase kinase kinase 5												🔴								🔴
MARK2	Serine/threonine-protein kinase MARK2												🔴								🔴
MARK3	MAP/microtubule affinity-regulating kinase 3												🔴								
MTOR	Serine/threonine-protein kinase mTOR					🔴							🔴	🔴				🔴			
NME3	NME/NM23 nucleoside diphosphate kinase 3												🟢								
NRBP1	Nuclear receptor-binding protein				🔴								🔴								
PDK3	[Pyruvate dehydrogenase [lipoamide]] kinase isozyme 3												🔴								
PFKFB4	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4											🔴	🔴	🔴							
PFKM	phosphofructokinase, muscle											🔴	🔴	🔴							
PFKP	phosphofructokinase, platelet											🔴	🔴	🔴		🔴					🔴
PIM3	Serine/threonine-protein kinase pim-3												🔴	🔴					🔴		🔴
PKM	pyruvate kinase M1/2												🔴	🔴	🔴						🔴
PTK2B	Protein-tyrosine kinase 2-beta				🔴								🔴			🔴					🔴
TEC	Tyrosine-protein kinase Tec												🔴								

Bezisterim DNAm: potential decrease in inflammatory kinase cascades

Potentially beneficial changes in Aging & AD Pathophysiology

37/41 (90%) related to inflammation

Bezisterim DNAm: potential decrease in aging and AD dysregulated phosphoproteins

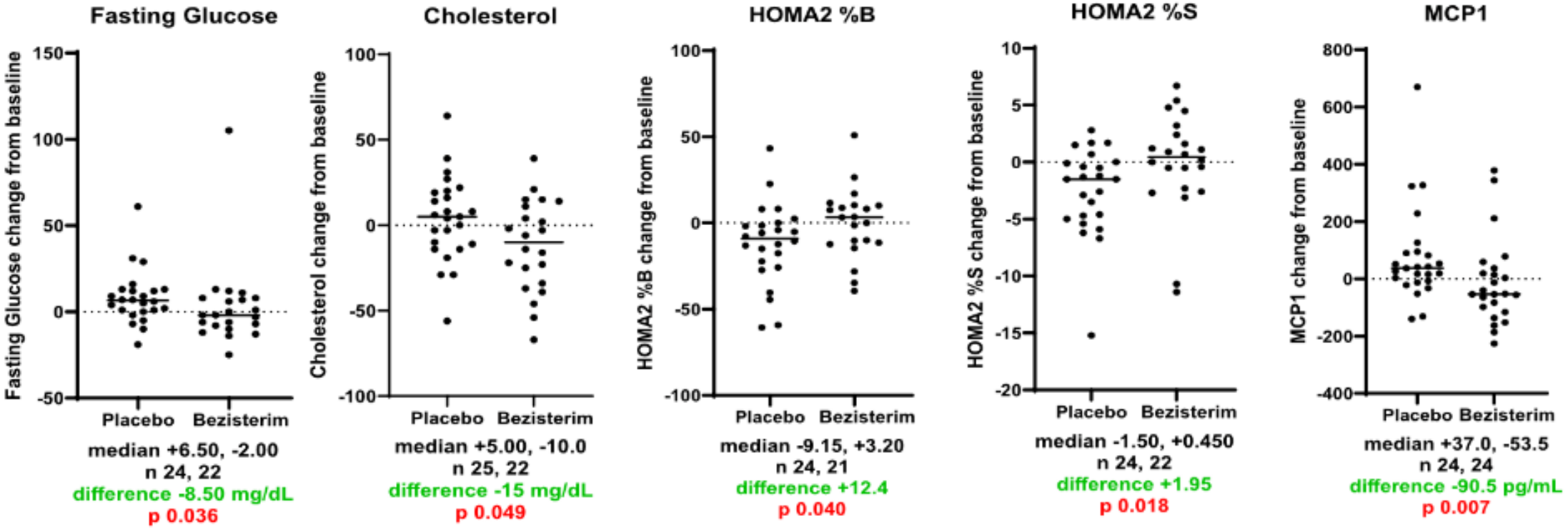
Ferrer, et al., 2021 Brain Pathol 31 e12996

Potentially beneficial changes in Aging & AD Pathophysiology
13/20 (65%) related to inflammation

Gene	Gene Name	PCHorvath2 EAA	PCHannum EAA	PCGrimAge EAA	DamAge EAA	IntrinClock EAA	Stoch Zhang EAA	Stoch Horvath EAA	Stoch PhenoAge EAA	RetroClock V1 EAA	RetroClock V2 EAA	myeloid Polarization	Kinase Cascades	Carbohydrate Metab	**Dysreg Phosphorylation**	Nominated AD target	BMI Aging TF	Cognition aging	Epigen Inflamm score	Braak Pathology	Type 2 Diabetes
AAK1	AP2-associated protein kinase 1												●		●			●			●
ABCA2	ATP binding cassette subfamily A member 2											●			●						●
ATAD3A	ATPase family AAA domain containing 3A														●			●			
ATP1A1	ATPase Na+/K+ transporting subunit alpha 1													○	○						
CAMK2D	Calcium/calmodulin-dependent protein kinase type II subunit delta												●	●	●						●
DLG2	discs large MAGUK scaffold protein 1														●	●					●
HDAC11	histone deacetylase 11														●			●			●
HOXA3	homeobox A3													○	○				○		○
MATR3	matrin 3														○						
MMAB	metabolism of cobalamin associated B														○			○			
MYL12A	myosin light chain 12A														●						
OCIAD1	OCIA domain containing 1														●						●
PGM1	phosphoglucomutase 1													○	○						
PKM	pyruvate kinase M1/2												●	●	●						●
SLU7	SLU7 homolog, splicing factor														○						
TBCCD1	TBCC domain containing 1														○						
TPI1	triosephosphate isomerase 1													●	●			●			●
TRMT112	tRNA methyltransferase activator subunit 11-2														●						
TUBA1B	tubulin alpha 1b											●		●	●		●	●	●		●
WDR13	WD repeat domain 13													●	●						

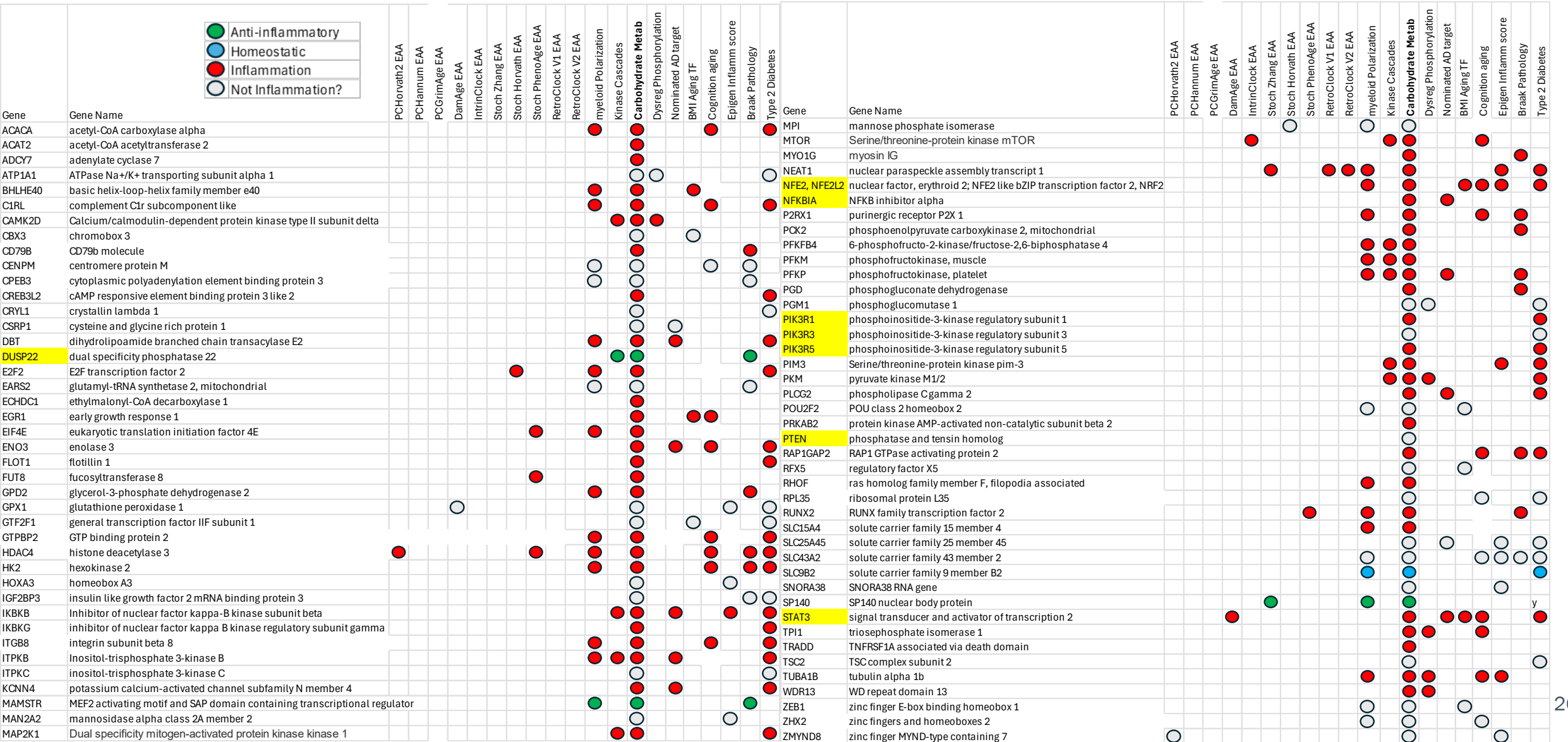
● Inflammation
○ Not Inflammation?

Significant Improvements From Baseline for Metabolic and Inflammatory Biomarkers with Bezisterim



Bezisterim DNAm: potential decrease in Carbohydrate Metabolism, glycolysis (Warburg effect)

Potentially beneficial changes in Aging & AD Pathophysiology
56/83 (67%) related to inflammation



Bezisterim DNAm: potential decrease in Type 2 Diabetes pathophysiology

Potentially beneficial changes in Aging & AD Pathophysiology

75/101 (**75%**) **related to inflammation**

Bezisterim: Potential decrease in aging & AD nominated targets

Gene	Gene Name	PCHorvath2 EAA	PCHannum EAA	PCGrimAge EAA	DamAge EAA	IntrinClock EAA	Stoch Zhang EAA	Stoch Horvath EAA	Stoch PhenoAge EAA	RetroClock V1 EAA	RetroClock V2 EAA	myeloid Polarization	Kinase Cascades	Carbohydrate Metab	Dysreg Phosphorylation	**Nominated AD target**	BMI Aging TF	Cognition aging	Epigen Inflamm score	Braak Pathology	Type 2 Diabetes
ANKRD31	ankyrin repeat domain 31															○					
BTK	Tyrosine-protein kinase BTK												●			●					●
CAPN2	calpain 2															●					●
CASS4	Cas scaffold protein family member 4												●			●					
CDK19	Cyclin-dependent kinase 19												●			●					●
CSRP1	cysteine and glycine rich protein 1													○		○					○
DBT	dihydrolipoamide branched chain transacylase E2											●		●		●					●
DENND1B	DENN domain containing 1B															●		●			●
DLG2	discs large MAGUK scaffold protein 1														●	●					
ENO3	enolase 3													●		●		●			
FAM163A	family with sequence similarity 163 member A															●		●			
FBXL16	F-box and leucine rich repeat protein 16	●					●									●					●
HEBP2	heme binding protein 2															○					
HSPA2	heat shock protein family A (Hsp70) member 2								●							●					●
IKBKB	Inhibitor of nuclear factor kappa-B kinase subunit beta												●	●		●			●		●
IL15	interleukin 15															●		●			●
ITPKB	Inositol-trisphosphate 3-kinase B											●	●	●		●					
JMJD6	jumonji domain containing 6, arginine demethylase and lysine hydroxylase					●						●				●					●
KCNN4	potassium calcium-activated channel subfamily N member 4													●		●					●
LAPTM5	lysosomal protein transmembrane 5											●				●				●	
NFKBIA	NFKB inhibitor alpha													●		●					●
NFKBIZ	NFKB inhibitor zeta															●			●		●
NR3C1	nuclear receptor subfamily 3 group C member 1															○	○				
PFKP	phosphofructokinase, platelet											●	●	●		●					●
PLCG2	phospholipase C gamma 2													●		●					●
PTK2B	Protein-tyrosine kinase 2-beta				●								●			●		●		●	
PTPN6	protein tyrosine phosphatase non-receptor type 6	●										●				●					
SH3GLB2	SH3 domain containing GRB2 like, endophilin B2															●		●			
SLC25A45	solute carrier family 25 member 45													○		○			○		○
STAT3	signal transducer and activator of transcription 2				●									●		●	●	●			
STAU1	staufen double-stranded RNA binding protein 1															●					

Anti-inflammatory
Inflammation
Not Inflammation?

The initial list of nominated targets was contributed by researchers from the **National Institute on Aging's Accelerating Medicines Partnership in Alzheimer's Disease (AMP-AD) Consortium.**

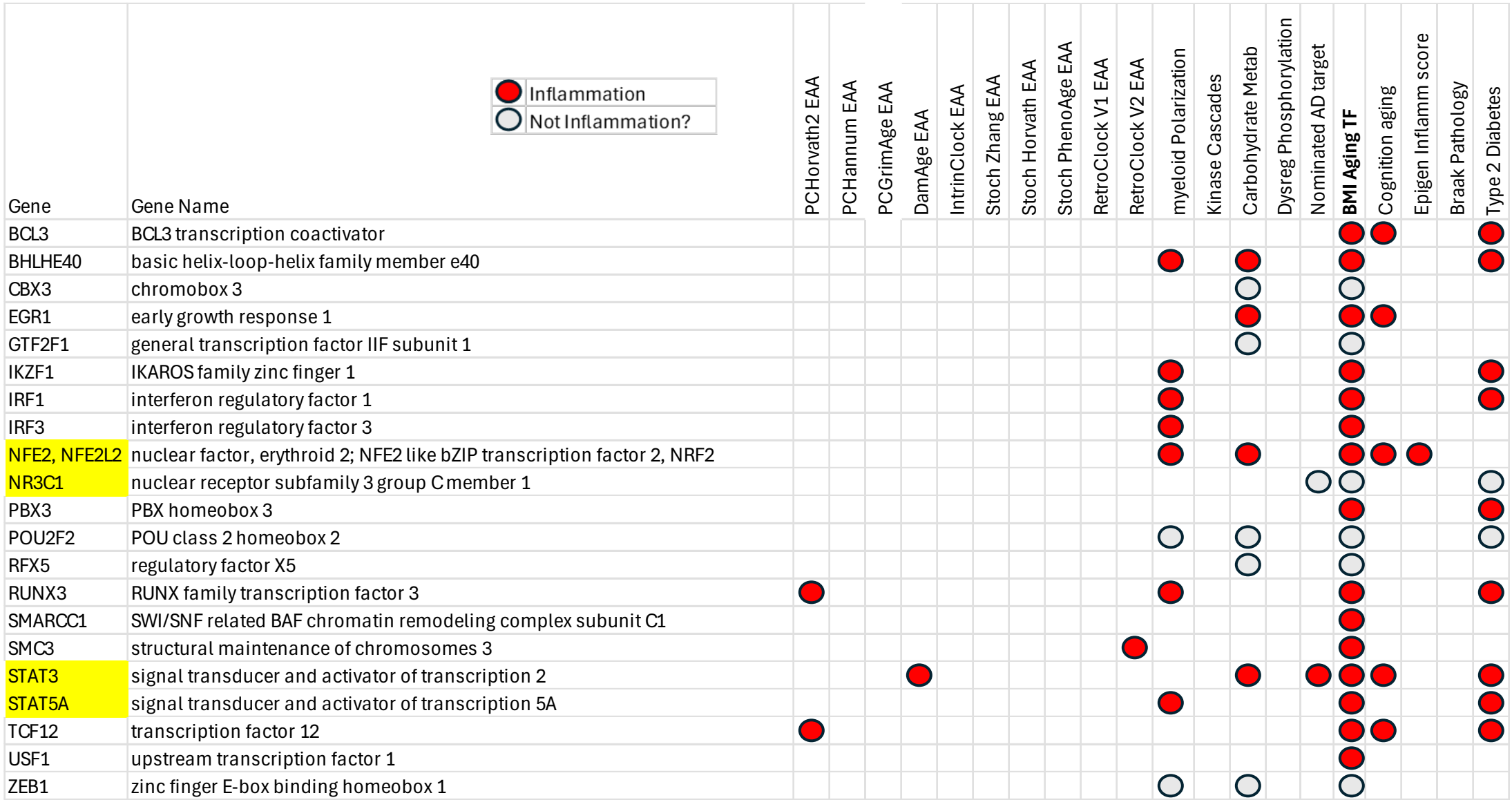
Potentially beneficial changes in Aging & AD Pathophysiology

26/31 (**84%**)
related to inflammation

Bezisterim: Potential decrease in aging & AD BMI-associated Transcription Factors

Marioni et al., 2018 Mol Psychiatry 23 2133; Bahado-Singh et al., 2021 PLoS One 16 e0248375

Potentially beneficial changes in Aging & AD Pathophysiology
15/21 (91%) related to inflammation



Gene	Gene Name	PCHorvath2 EAA	PCHannum EAA	PCGrimAge EAA	DamAge EAA	IntrinClock EAA	Stoch Zhang EAA	Stoch Horvath EAA	Stoch PhenoAge EAA	RetroClock V1 EAA	RetroClock V2 EAA	myeloid Polarization	Kinase Cascades	Carbohydrate Metab	Dysreg Phosphorylation	Nominated AD target	**BMI Aging TF**	Cognition aging	Epigen Inflamm score	Braak Pathology	Type 2 Diabetes
BCL3	BCL3 transcription coactivator																●	●			●
BHLHE40	basic helix-loop-helix family member e40											●		●			●				●
CBX3	chromobox 3													○			○				
EGR1	early growth response 1													●			●	●			
GTF2F1	general transcription factor IIF subunit 1													○			○				
IKZF1	IKAROS family zinc finger 1											●					●				●
IRF1	interferon regulatory factor 1											●					●				●
IRF3	interferon regulatory factor 3											●					●				
NFE2, NFE2L2	nuclear factor, erythroid 2; NFE2 like bZIP transcription factor 2, NRF2											●		●			●	●	●		
NR3C1	nuclear receptor subfamily 3 group C member 1															○	○				○
PBX3	PBX homeobox 3																●				●
POU2F2	POU class 2 homeobox 2											○		○			○				○
RFX5	regulatory factor X5													○			○				
RUNX3	RUNX family transcription factor 3	●										●					●				●
SMARCC1	SWI/SNF related BAF chromatin remodeling complex subunit C1																●				
SMC3	structural maintenance of chromosomes 3										●						●				
STAT3	signal transducer and activator of transcription 2				●									●		●	●	●			●
STAT5A	signal transducer and activator of transcription 5A											●					●				●
TCF12	transcription factor 12	●															●	●			●
USF1	upstream transcription factor 1																●				
ZEB1	zinc finger E-box binding homeobox 1											○		○			○				

Bezisterim: Potential decrease in aging & AD Cognition-associated genes

Marioni et al., 2018 Mol Psychiatry 23 2133;
Bahado-Singh et al., 2021 PLoS One 16 e0248375

Potentially beneficial changes in Aging & AD Cognition

35/43 (**81%**) **related to inflammation**

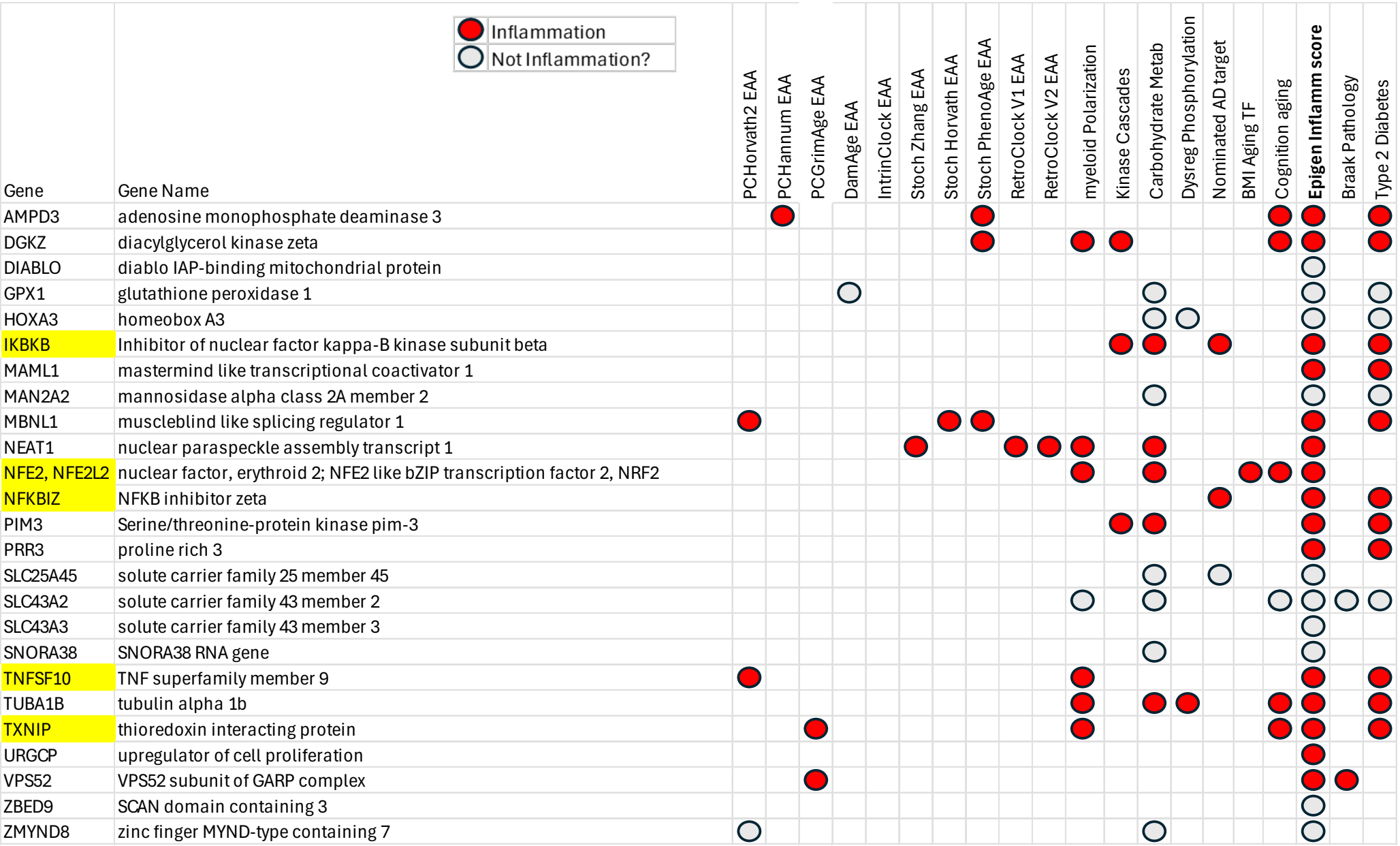
Gene	Gene Name	PC Horvath2 EAA	PC Hannum EAA	PC GrimAge EAA	DamAge EAA	IntrinClock EAA	Stoch Zhang EAA	Stoch Horvath EAA	Stoch PhenoAge EAA	RetroClock V1 EAA	RetroClock V2 EAA	myeloid Polarization	Kinase Cascades	Carbohydrate Metab	Dysreg Phosphorylation	Nominated AD target	BMI Aging TF	**Cognition aging**	Epigen Inflamm score	Braak Pathology	Type 2 Diabetes
AAK1	AP2-associated protein kinase 1												●		●			●			●
ABHD14A	abhydrolase domain containing 14A							○										○			○
ACACA	acetyl-CoA carboxylase alpha											●		●				●			●
AMPD3	adenosine monophosphate deaminase 3		●						●									●	●		●
ATAD3A	ATPase family AAA domain containing 3A														●			●			
BCL3	BCL3 transcription coactivator																●	●			●
C1RL	complement C1r subcomponent like											●		●				●			
CENPM	centromere protein M											○		○				○		○	
CLSTN3	calsyntenin 3																	●			●
DENND1B	DENN domain containing 1B															●		●			●
DGKZ	diacylglycerol kinase zeta								●			●	●					●	●		
EGR1	early growth response 1													●			●	●			
ENO3	enolase 3													●		●		●			
FAM163A	family with sequence similarity 163 member A															●		●			
FTO	FTO alpha-ketoglutarate dependent dioxygenase										●	●						●			
G2E3	G2/M-phase specific E3 ubiquitin protein ligase																	○			
GTPBP2	GTP binding protein 2											●		●				●			●
HDAC11	histone deacetylase 11														●			●			●
HDAC4	histone deacetylase 3	●							●		●			●				●		●	●
HK2	hexokinase 2											●		●				●		●	●
HOXA4	homeobox A4				○													○			○
IL15	interleukin 15															●		●			●
IL6R	interleukin 6 receptor											●						●			●
ITGB8	integrin subunit beta 8											●		●				●			●
MAP2K3	mitogen-activated protein kinase kinase 3					●			●			●						●			
MMAB	metabolism of cobalamin associated B														○			○			
MTOR	Serine/threonine-protein kinase mTOR					●							●	●				●			
NFE2, NFE2L2	nuclear factor, erythroid 2; NFE2 like bZIP transcription factor 2, NRF2											●		●			●	●	●		●
P2RX1	purinergic receptor P2X 1											●		●				●		●	●
PSMB1	proteasome 20S subunit beta 1																	●			●
PTGER2	prostaglandin E receptor 2	●						●										●			
PTPN6	protein tyrosine phosphatase non-receptor type 6	●										●				●		●		●	●
RAP1GAP2	RAP1 GTPase activating protein 2													●				●		●	
RBP5	retinol binding protein 5											●						●			
RPL35	ribosomal protein L35													○				○			○
SH3GLB2	SH3 domain containing GRB2 like, endophilin B2															●		●			●
SLC43A2	solute carrier family 43 member 2											○		○				○	○	○	○
STAT3	signal transducer and activator of transcription 2													●		●	●	●			●
TCF12	transcription factor 12	●															●	●			●
TPI1	triosephosphate isomerase 1													●	●			●			●
TUBA1B	tubulin alpha 1b											●		●	●			●	●		●
TXNIP	thioredoxin interacting protein			●								●						●	●		
ZHX2	zinc fingers and homeoboxes 2											○		○				○			

Epigenetic Inflammation Score (EIS)

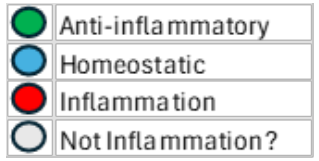
Verschoor, et al., Aging Cell 22 e13863

Potentially beneficial changes in Aging & AD Pathophysiology

15/25 (67%) **related to inflammation**



Gene	Gene Name	PCHorvath2 EAA	PCHannum EAA	PCGrimAge EAA	DamAge EAA	IntrinClock EAA	Stoch Zhang EAA	Stoch Horvath EAA	Stoch PhenoAge EAA	RetroClock V1 EAA	RetroClock V2 EAA	myeloid Polarization	Kinase Cascades	Carbohydrate Metab	Dysreg Phosphorylation	Nominated AD target	BMI Aging TF	Cognition aging	**Epigen Inflamm score**	Braak Pathology	Type 2 Diabetes
AMPD3	adenosine monophosphate deaminase 3		●						●									●	●		●
DGKZ	diacylglycerol kinase zeta								●			●	●					●	●		●
DIABLO	diablo IAP-binding mitochondrial protein																		○		
GPX1	glutathione peroxidase 1				○									○					○		○
HOXA3	homeobox A3													○	○				○		○
IKBKB	Inhibitor of nuclear factor kappa-B kinase subunit beta												●	●		●			●		●
MAML1	mastermind like transcriptional coactivator 1																		●		●
MAN2A2	mannosidase alpha class 2A member 2													○					○		○
MBNL1	muscleblind like splicing regulator 1	●						●	●										●		●
NEAT1	nuclear paraspeckle assembly transcript 1						●			●	●	●		●					●		
NFE2, NFE2L2	nuclear factor, erythroid 2; NFE2 like bZIP transcription factor 2, NRF2											●		●			●	●	●		
NFKBIZ	NFKB inhibitor zeta															●			●		●
PIM3	Serine/threonine-protein kinase pim-3												●	●					●		●
PRR3	proline rich 3																		●		●
SLC25A45	solute carrier family 25 member 45													○		○			○		
SLC43A2	solute carrier family 43 member 2											○		○				○	○	○	○
SLC43A3	solute carrier family 43 member 3																		○		
SNORA38	SNORA38 RNA gene													○					○		
TNFSF10	TNF superfamily member 9	●										●							●		●
TUBA1B	tubulin alpha 1b											●		●	●			●	●		●
TXNIP	thioredoxin interacting protein			●								●						●	●		●
URGCP	upregulator of cell proliferation																		●		
VPS52	VPS52 subunit of GARP complex			●															●	●	
ZBED9	SCAN domain containing 3																		○		
ZMYND8	zinc finger MYND-type containing 7	○												○					○		



Gene	Gene Name	PCHorvath2 EAA	PCHannum EAA	PCGrimAge EAA	DamAge EAA	IntrinClock EAA	Stoch Zhang EAA	Stoch Horvath EAA	Stoch PhenoAge EAA	RetroClock V1 EAA	RetroClock V2 EAA	myeloid Polarization	Kinase Cascades	Carbohydrate Metab	Dysreg Phosphorylation	Nominated AD target	BMI Aging TF	Cognition aging	Epigen Inflamm score	**Braak Pathology**	Type 2 Diabetes
ACAP1	ArfGAP with coiled-coil, ankyrin repeat and PH domains 1				🔴							🔴								🔴	
CD79B	CD79b molecule													🔴						🔴	
CENPM	centromere protein M											⚪		⚪				⚪		⚪	
CPEB3	cytoplasmic polyadenylation element binding protein 3											🔴		🔴						🔴	
DUSP22	dual specificity phosphatase 22												🟢	🟢						🟢	
EARS2	glutamyl-tRNA synthetase 2, mitochondrial											⚪		⚪						⚪	
GPD2	glycerol-3-phosphate dehydrogenase 2											🔴		🔴						🔴	🔴
HDAC4	histone deacetylase 3	🔴							🔴			🔴		🔴				🔴		🔴	🔴
HK2	hexokinase 2											🔴		🔴				🔴		🔴	🔴
IGF2BP3	insulin like growth factor 2 mRNA binding protein 3													⚪						⚪	⚪
IRF7	interferon regulatory factor 7				🔴															🔴	
ISOC2	isochorismatase domain containing 2																			🔵	
KDM2B	lysine demethylase 2B																			🔴	🔴
LAPTM5	lysosomal protein transmembrane 5											🔴				🔴				🔴	🔴
MAMSTR	MEF2 activating motif and SAP domain containing transcriptional regulator											🟢		🟢						🟢	🟢
MGAT1	alpha-1,3-mannosyl-glycoprotein 2-beta-N-acetylglucosaminyltransferase																			⚪	
MYO1G	myosin IG													🔴						🔴	🔴
P2RX1	purinergic receptor P2X 1											🔴		🔴				🔴		🔴	🔴
PCK2	phosphoenolpyruvate carboxykinase 2, mitochondrial													🔴						🔴	🔴
PHACTR1	phosphatase and actin regulator 1																			🔴	🔴
PLAU	plasminogen activator, urokinase											🔴								🔴	
PTPN6	protein tyrosine phosphatase non-receptor type 6	🔴										🔴				🔴		🔴		🔴	
RAB23	RAB23, member RAS oncogene family																			🔴	🔴
RAP1GAP2	RAP1 GTPase activating protein 2													🔴				🔴		🔴	🔴
RELT	RELT TNF receptor																			🔴	🔴
RUNX2	RUNX family transcription factor 2								🔴			🔴		🔴						🔴	🔴
SLC43A2	solute carrier family 43 member 2											⚪		⚪				⚪	⚪	⚪	⚪
TPD52L2	TPD52 like 2																			🔴	🔴
TRAF6	TNF receptor associated factor 6																			🔴	
VPS52	VPS52 subunit of GARP complex			🔴															🔴	🔴	
ZDHHC14	zinc finger DHHC-type palmitoyltransferase 14																			⚪	⚪

Braak Brain Pathology

https://ewascatalog.org/

Potentially beneficial changes in Aging & AD Pathology

25/31 (**81%**) **related to inflammation**

Conclusions

- Potentially beneficial changes in DNAm were observed in AD subjects treated for 7 months with bezisterim compared to placebo
- Significant, potentially beneficial changes were observed in promoter DNAm of genes related to multiple aspects of aging and AD pathophysiology
  - Age acceleration
  - Myeloid cell polarization
  - Inflammatory kinase cascades. Phosphoproteins
  - Glucose, Insulin resistance, Obesity, T2D
  - Cognition, Braak Stage Pathology
- Changes of 5% or more in large numbers of genes in kinase cascades may result in significantly decreased inflammatory signaling due to compounding effects
- An overwhelming portion of these changes were associated with potentially beneficial changes in inflammatory programs
- Changes in many individual genes were shared with multiple aging & AD traits, suggesting their potential as biomarkers
- We may be able to target epigenetic-driven age acceleration as a treatment for Alzheimer's, and other diseases of aging, and to improve normal aging healthspan