Bezisterim Epigenetic Effects on Aging and Neurodegeneration

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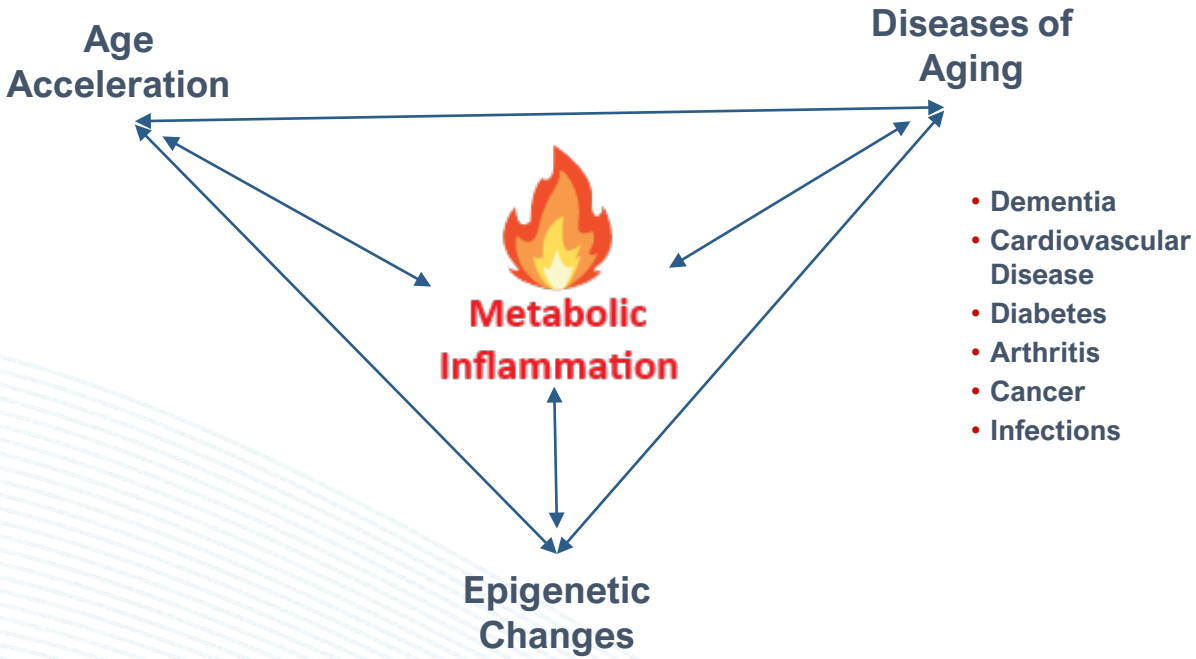
DISCLOSURES: The authors are employees and minority shareholders in BioVie or Chronomics.

7th World Aging and Rejuvenation Conference
July 9-10, 2025, Vienna, Austria

Inflammation Drives Aging and Alzheimer's



For Alzheimer's Sufferers,
Brain Inflammation
Ignites a Neuron-Killing "Forest Fire"

Sci Am March 4, 2019

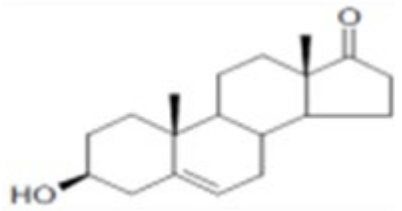
- Inflammation is an innate immune response to infections **and to tissue damage-associated molecular patterns**

Can we target epigenetic-driven age acceleration with a treatment for Alzheimer's and other neurodegenetion?

Bezisterim (Sterol Immunomodulator)

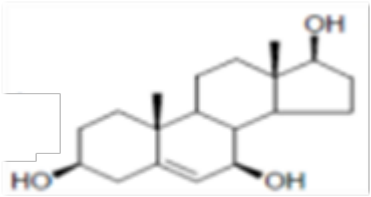
DHEA (dehydroepiandrosterone)

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



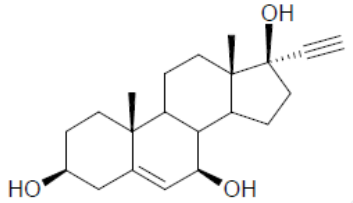
βAET (5-androstane,3β,7β,17β-triol)

- 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 ERK1/2, inhibits NFκB (master regulator of inflammation)
- In clinical development for Alzheimer's, Parkinson's, and Long Covid diseases; attractive safety profile to date
- Bezisterim and metabolites have no sex steroid or glucocorticoid activity



BBB, blood-brain barrier; ERK, extracellular signal-related kinase; NFκB, nuclear factor kappa B.

Aging and AD Epigenetic Biomarkers

- 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, difference between observed biological age and expected biological age 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 suggest that aging and diseases of aging are driven by inflammatory signaling
- Bezisterim may alter biological age by anti-inflammatory epigenetic modification

AD, Alzheimer’s disease; DNA, deoxyribonucleic acid; EAA, epigenetic age acceleration.

Bezisterim Decreases EAA in AD

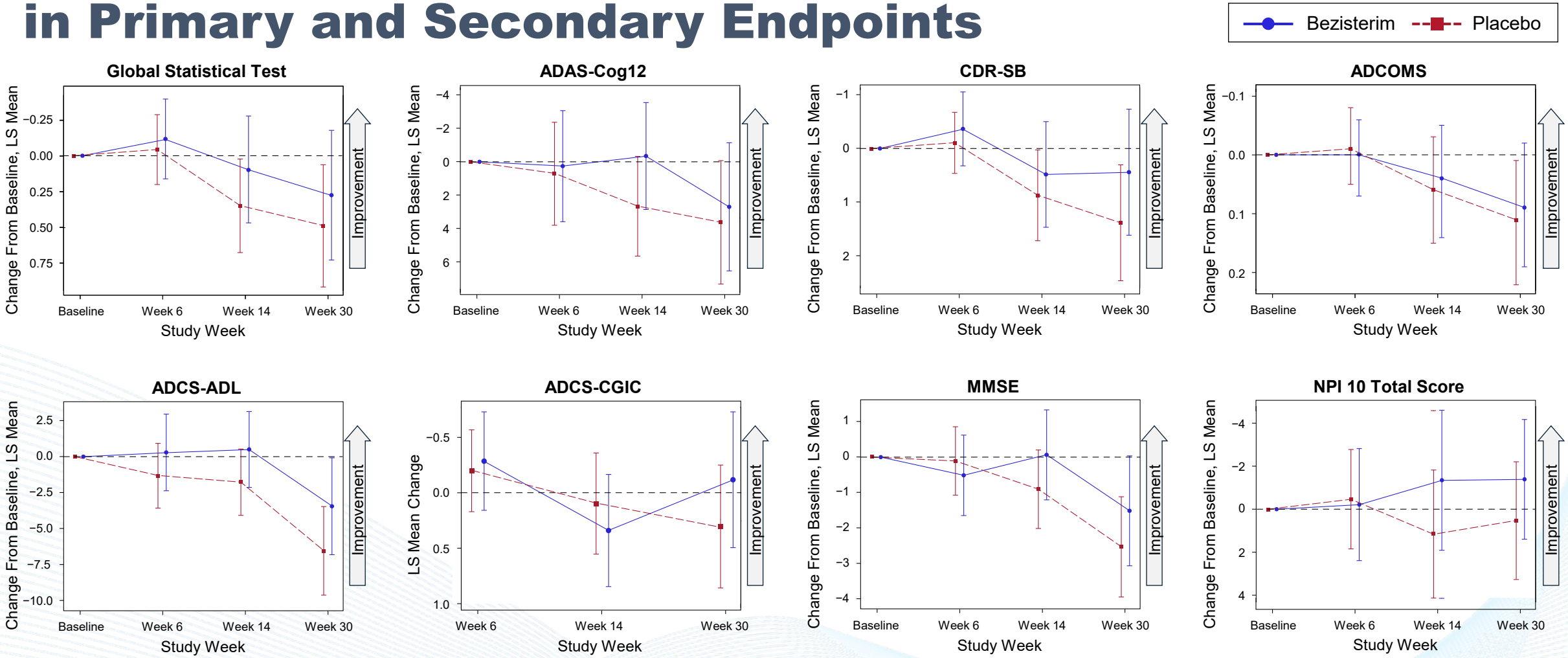
TYPE Clinical Trial
PUBLISHED 02 May 2025
DOI 10.3389/fnins.2025.1516746

- The 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. The analysis focused on epigenetic, metabolic, biomarker, and cognitive measures in the exploratory biomarker population that completed the protocol

GCP, Good Clinical Practice.
Reading CL, et al. *Front Neurosci.* 2025;19:1516746. doi: 10.3389/fnins.2025.1516746

Bezisterim Showed Improvements Over Time in Primary and Secondary Endpoints

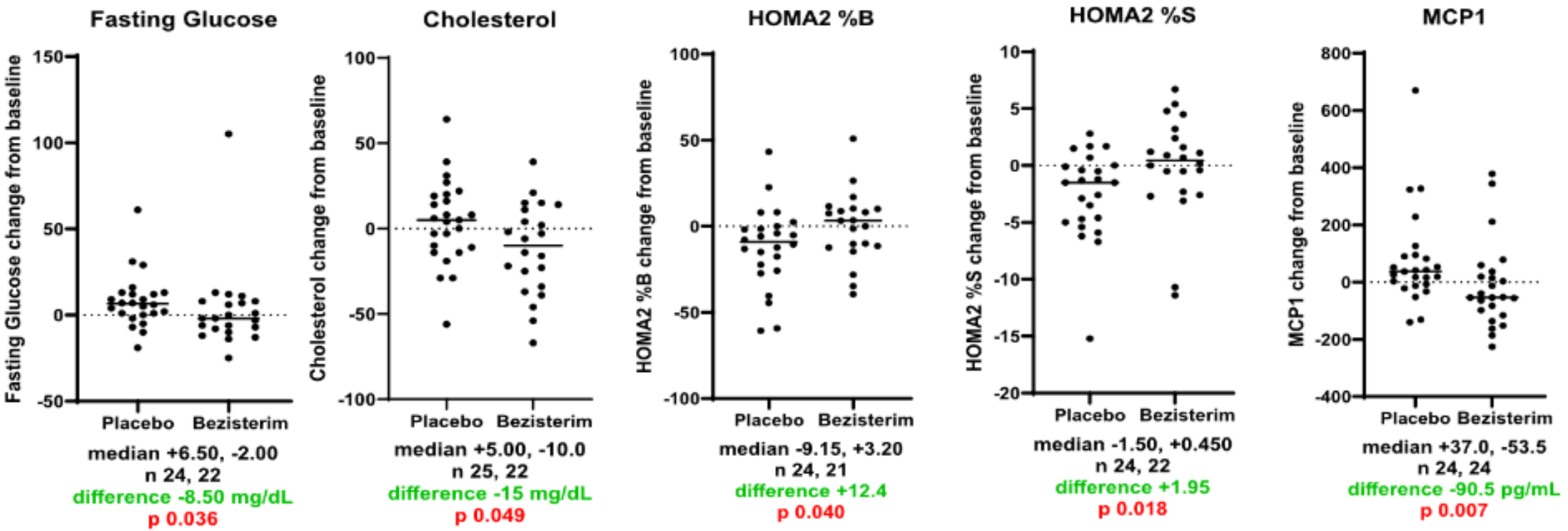


Primary and secondary efficacy endpoints. Scores for bezisterim and placebo from baseline through Week 30.[1]
Magnitude of responses was comparable to results reported for approved medications lecanemab[2] and aducanumab[3]

ADAS-Cog12, Alzheimer's Disease Assessment Scale–Cognitive Subscale, 12-item version; DCOMS, Alzheimer's Disease Composite Score; ADCS-ADL, Alzheimer's Disease Cooperative Study–Activities of Daily Living; ADCS-CGIC, Alzheimer's Dease Cooperative Study-Clinical Global Impression of Change; CDR-SB, Clinical Dementia Rating Scale Sum of Boxes; LS, least square; MMSE, Mini-Mental State Examination; NPI, Neuropsychiatric Inventory.

1. Reading CL, et al. *Front Neurosci.* 2025;19:1516746. doi: 10.3389/fnins.2025.1516746. **2.** van Dyck CH, et al. *N Engl J Med.* 2023;388(17):1631-1632. doi: 10.1056/NEJMc2301380. **3.** Budd Haeberlein S, et al. *J Prev Alzheimers Dis.* 2022;9(2):197-210. doi: 10.14283/jpad.2022.30

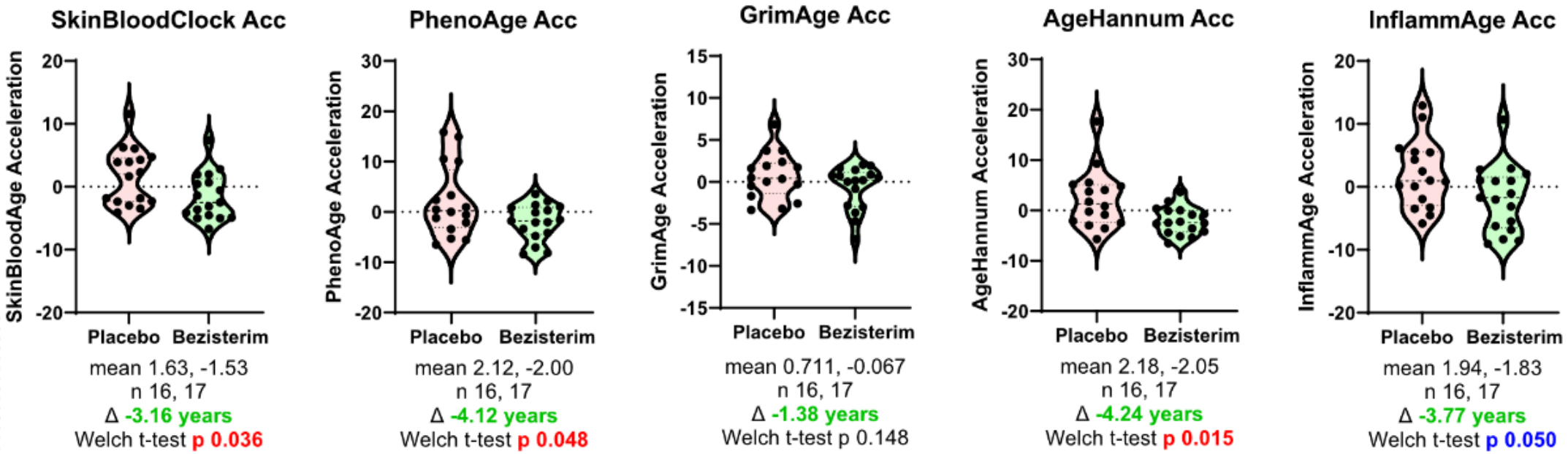
Significant Improvements From Baseline for Metabolic and Inflammatory Biomarkers With Bezisterim



GFAP, glial fibrillary acidic protein; HOMA2, Homeostatic Model Assessment 2; MCP-1, monocyte chemoattractant protein-1; NfL, neurofilament light chain; pTau, phosphorylated tau.

Reading CL, et al. *Front Neurosci.* 2025;19:1516746. doi: 10.3389/fnins.2025.1516746

Bezisterim Subjects Showed Lower EAA Than Placebo Subjects at 30 Weeks



Age acceleration as calculated by different epigenetic aging clocks

Note: consistent placebo acceleration, bezisterim deceleration

Analyses of diverse epigenetic aging clocks performed by Chronomics/Hurdle Group, including their new InflammAge Clock

Acc, acceleration.
Reading CL, et al. *Front Neurosci.* 2025;19:1516746. doi: 10.3389/fnins.2025.1516746

Could bezisterim modify aging and AD risk gene expression through epigenetic modification?

DNAm data from clinical trial 30-week blood samples

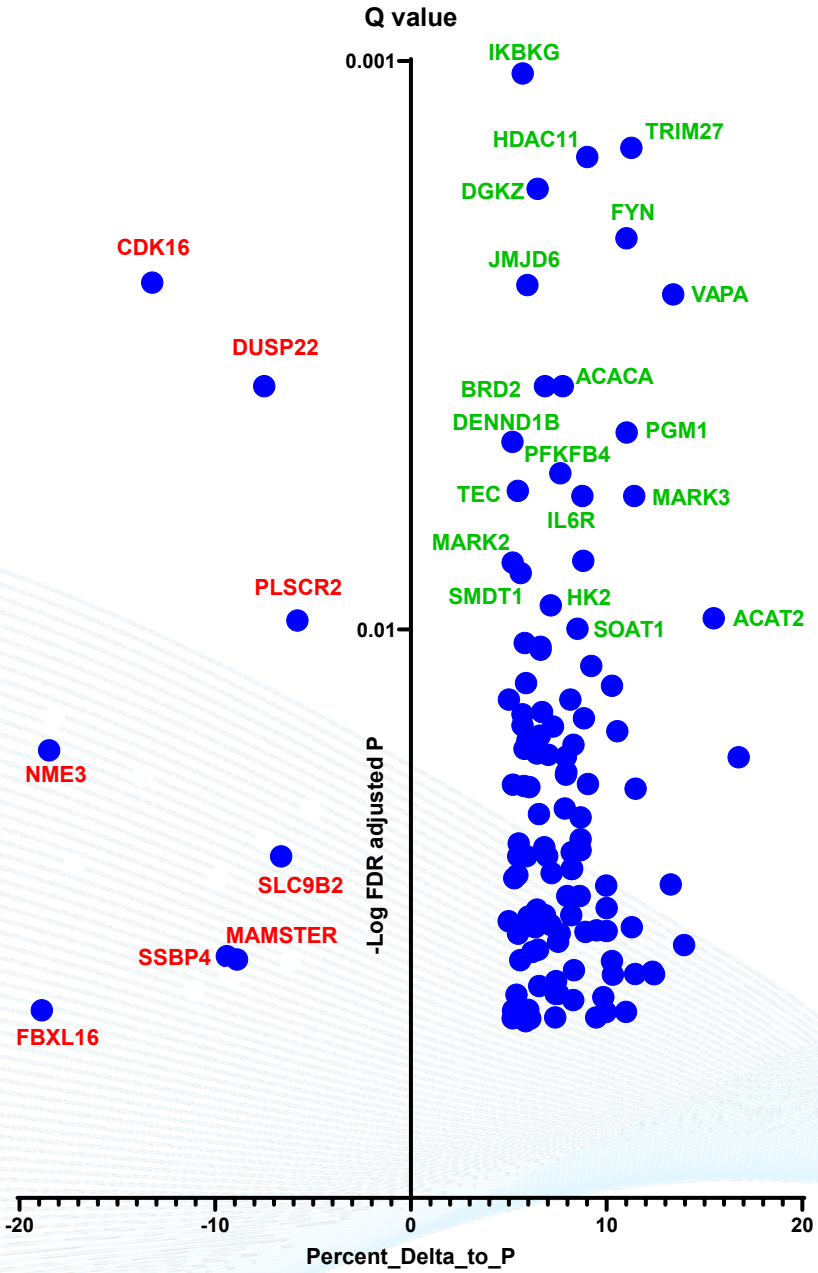
Identify significant (FDR) **average gene promoter methylation differences** (bezisterim-placebo ≥ 5%) [potential biomarker genes]

Map epigenetic clock CpGs to genes [**aging** & **AD** risk genes]

Examine associations between potential biomarker genes and published aging/AD risk parameters

- Aging and AD risk parameters include:
  - 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

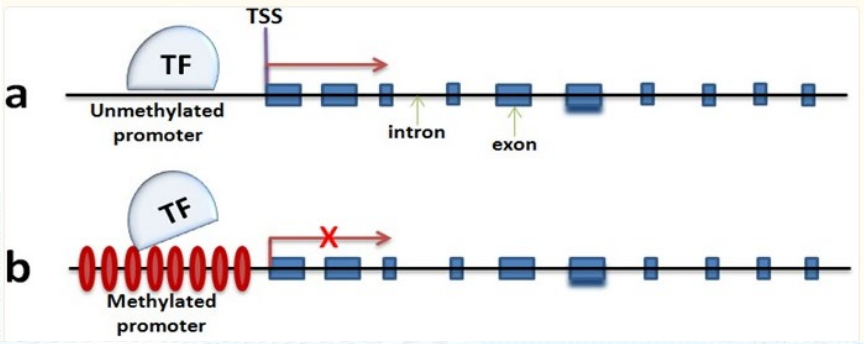
Volcano plot of genes with FDR significant 5% increased or decreased DNAm for bezisterim compared to placebo subjects



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

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

Higher promoter DNAm is associated with lower gene expression



Modified from Xu 2015 Oncotarget 20 13922

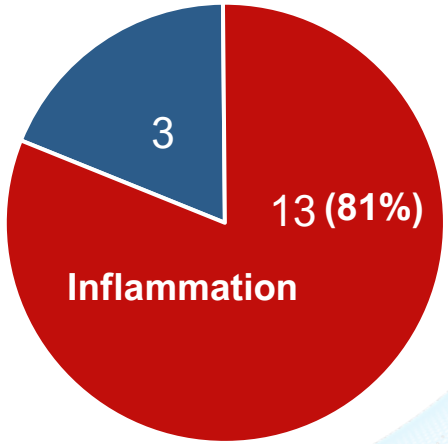
DNAm in Aging and AD Biological Clock Gene Promoters

Genes were identified for CpG residues from SkinBlood, PhenoAge, Hannum, and GrimAge clocks

- Identified known genes were analyzed for significant differences in average promoter CpG methylation between bezisterim and placebo subjects
- Increased promoter DNAm is generally associated with decreased gene expression
- Genes with significant ($P<0.05$) changes in average promoter CpG methylation in bezisterim vs placebo subjects were further selected for multiple testing significance (**FDR adjusted $P<0.05$**)
- A rounded **cutoff of 5% difference** was used

16 potentially beneficial changes for aging & AD pathophysiology,

81% associated with inflammation



Examples:

- SOAT1 (Lipid enzyme; microglial neuroinflammation, age acceleration, amyloid β, blood-brain barrier, cognition, macrophage polarization, lipid droplets [Alois Alzheimer 1907])
- MAP2K3 (p38 MAPK; microglial neuroinflammation, signal transduction, amyloid β, pTau, p38 superagers, apoptosis, hypothalamic inflammation)

CpG, cytosine-guanine; DNAm, DNA methylation; FDR, false discovery rate; MAPK, mitogen-activated protein kinase; SOAT1, sterol-O-acyl transferase 1.

DNAm Status in InflammAge Clock Genes Between Bezisterim & Placebo

The InflammAge Clock uses Epigenetic Biomarker Proxies (EBPs) derived from DNA methylation data to serve as surrogates for traditional clinical lab tests, metabolomic, and proteomic measurements associated with inflammation

15 Inflammation surrogate EBP genes from the training set* for InflammAge clock showed significant trends for differences in average DNA methylation status between bezisterim and placebo subjects

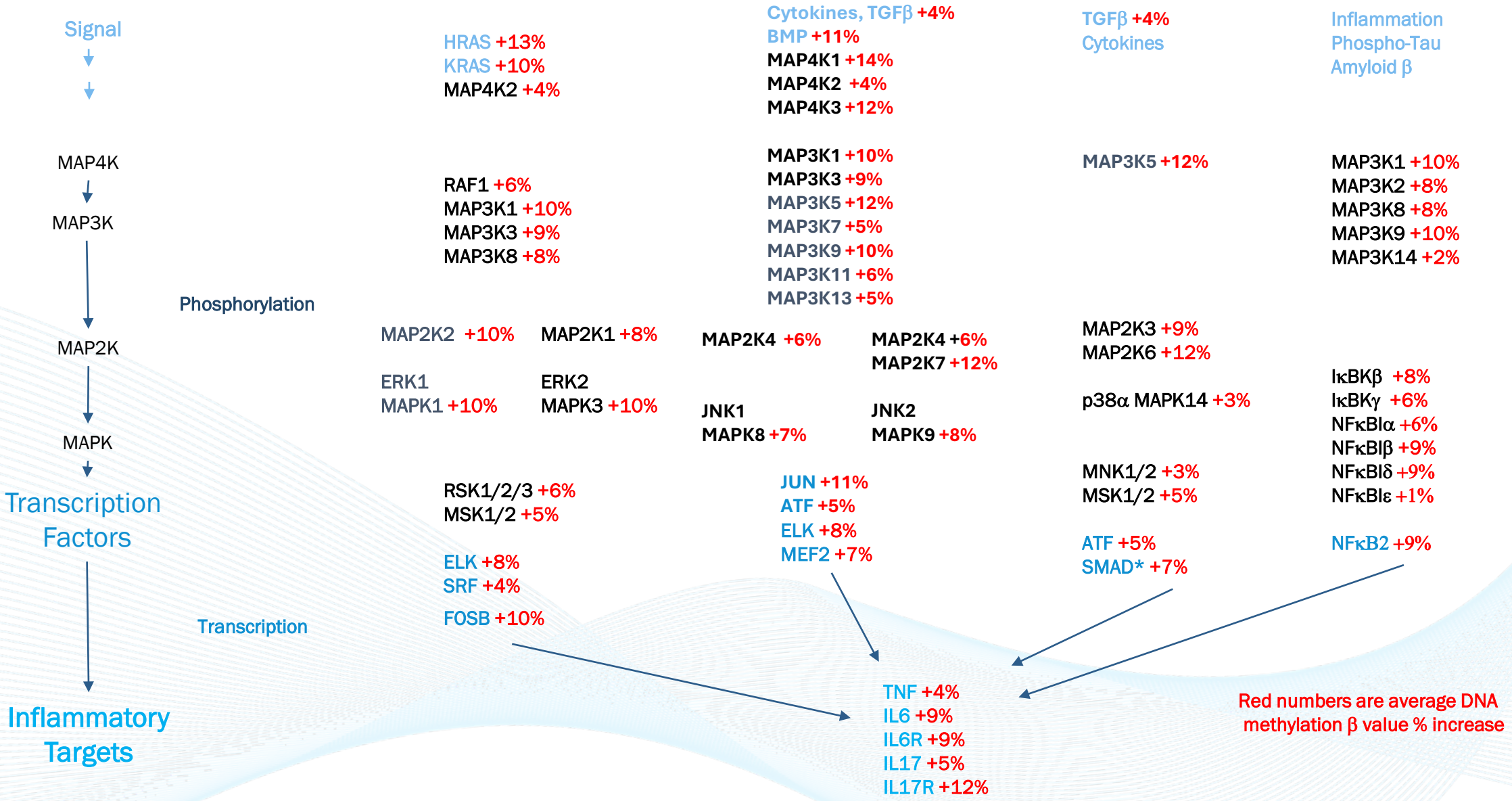
15 potentially beneficial changes in EBPs for aging and AD inflammation

gene	DNAm	p	name	expression: potential benefit
CCL22	lower	0.0032	C-C motif chemokine ligand 22	increased: monocyte chemotaxis, neuroprotective in brain?
PARP9	**higher**	0.0041	poly(ADP-ribose) polymerase family member 9	**decreased: neuroinflammation**
GP1BA	lower	0.011	glycoprotein Ib platelet subunit alpha	**increased: hemostasis**
ASAH2	**higher**	0.024	N-acylsphingosine amidohydrolase 2	**decreased: neuroinflammation**
ACVRL1	lower	0.026	activin A receptor like type 1	**increased: neuroprotective**
EZR	lower	0.032	ezrin	**increased: stress response, survival?**
MPL	lower	0.041	MPL proto-oncogene, thrombopoietin receptor	**increased: MMP9 inhibition, BBB neuroprotection**
VEGFA	lower	0.052	vascular endothelial growth factor A	**increased: neuroprotection**
PIGR	lower	0.053	polymeric immunoglobulin receptor	**increased: insulin sensitivity**
NMNAT1	lower	0.059	nicotinamide nucleotide adenylyltransferase 1	**increased: NAD, cognition, neuroprotection**
LYZ	lower	0.06	lysozyme	**increased: decreases Abeta, TNF, Neuroinflammation**
MMP12	lower	0.063	matrix metallopeptidase 12	**increased: degrades plaques?**
NLRC5	**higher**	0.074	NLR family CARD domain containing 5	**decreased: neuroinflammation, inflammasome, NFkB**
NCAM1	**higher**	0.093	neural cell adhesion molecule 1	**decreased: reduced amyloid beta production**
CD6	lower	0.095	CD6 molecule	**increased: M2 polarization of microglia?**

*Training set for InflammAge: 125 EBPs associated with inflammation; a subset was used for the saliva test for systemic chronic inflammation[1]

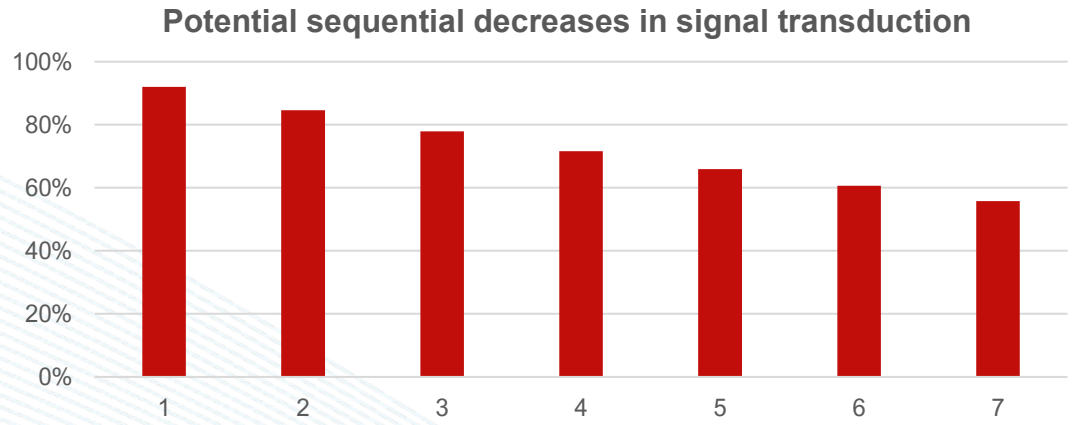
1. Schmunk LJ, et al. *Aging Cell.* 2025;24(4):e14444. doi: 10.1111/acel.14444

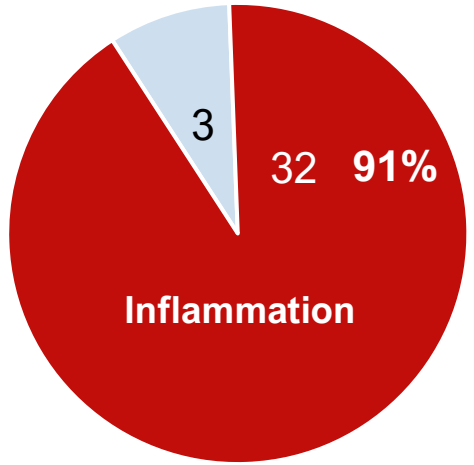
Bezisterim Induces Decrease in Inflammatory Gene Expression *via* sequential Increased Kinase Promotor Methylation



DNAm in Aging and AD Kinase Gene Promoters

- Kinase genes were identified from the human kinome
- Bezisterim had significant (FDR adj. $P<0.05$) changes (≥5%) in promoter DNAm compared to placebo for potentially beneficial changes in kinases associated with aging & AD pathophysiology (average 8%)
- 35 genes, 91% were associated with inflammation





Examples:

- FYN (microglial neuroinflammation, pTau, NFT, amyloid β, NFκB, TNF, excitotoxicity)
- PFKP (microglial neuroinflammation, glycolysis, M1 polarization, mitophagy, neurogenesis)

PFKP, phosphofructokinase, platelet; TNF, tumor necrosis factor.

Aging and AD: Dysregulated Phosphorylation

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RESEARCH ARTICLE

Dysregulated protein phosphorylation: A determining condition in the continuum of brain aging and Alzheimer's disease

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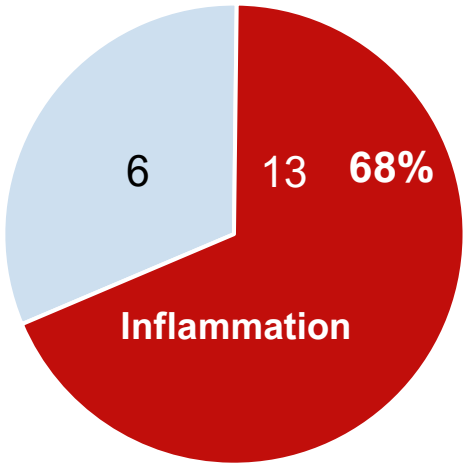
DNAm in Aging and AD Dysregulated Phosphoprotein Gene Promoters

- Potentially beneficial changes in promoter DNAm for dysregulated phosphoproteins genes associated with aging & AD pathophysiology

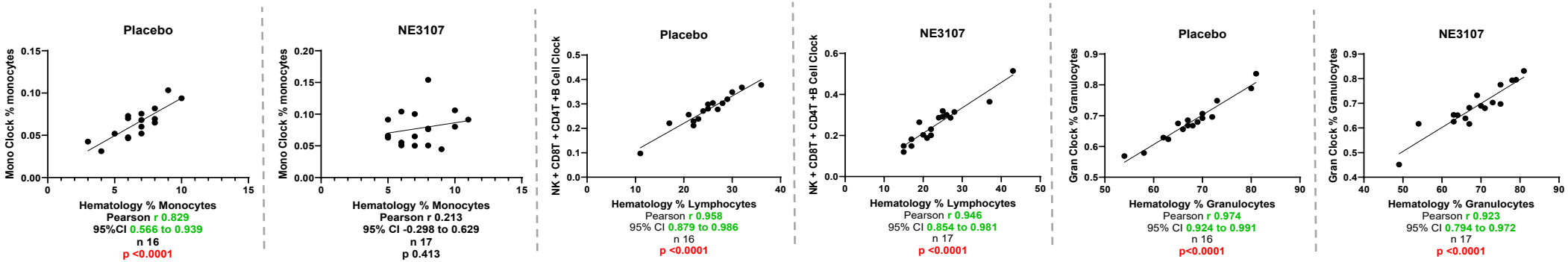
- 19 genes, 68% are associated with inflammation

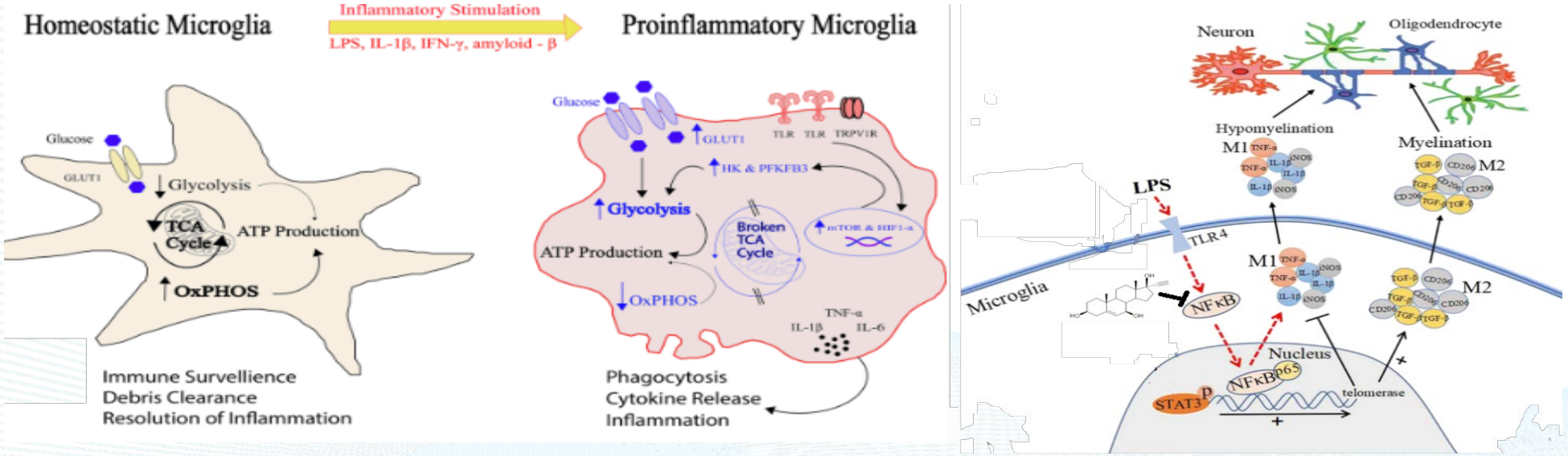


Examples:

- ATAD3A phosphorylation activates (neuroinflammation, amyloid β, Chol, synapse, cognition, senescence, aging & inhibition of mitophagy)
- CAMK2D phosphorylation activates (neuroinflammation, pTau, ROS, synapse dysfunction, AD progression, vasculopathy , insulin resistance, excititoxicity)

Bezisterim may modify myeloid polarization





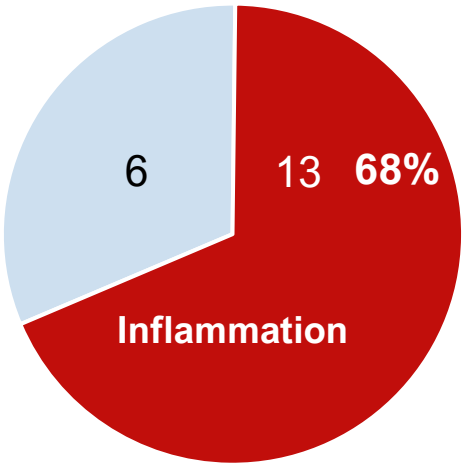
Modified from Bielanin & Sun 2023, Transl Stroke Res 14 435 and Zhou 2021 Mol Neurobiol 58 6552

DNAm in Aging and AD Myeloid Cell Polarization Genes

Polarization genes (Dekkers et al 2019 Epigenetics Chromatin 12 34).

- Potentially beneficial changes in promoter DNAm for myeloid cell polarization genes associated with aging & AD pathophysiology

- 19 genes, 68% are associated with inflammation



Examples:

- CASP7 (caspase; microglial neuroinflammation activation, apoptosis, pyroptosis),
- DPP4 (microglial neuroinflammation, insulin resistance, adipose tissue, microglia polarization, mitochondria, NFκB, ROS, amyloid β, GLP-1 cleavage)

CASP7, caspase-7; DPP4, dipeptidyl peptidase 4; GLP-1, glucagon-like peptide-1; ROS, reactive oxygen species.

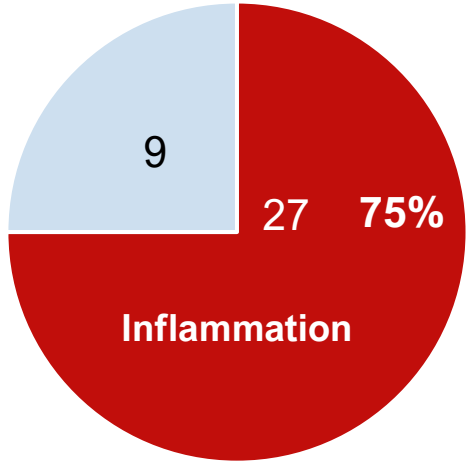
Warburg Effect: Carbohydrate Metabolism Dysregulated in AD

- Carbohydrate metabolism is dysregulated in AD
- AD reveals bias for aerobic glycolysis over oxidative phosphorylation in glucose metabolism as originally reported for cancer
- Nonprotein kinases of glucose metabolism were found to be enriched for immune modulators
- Metabolic shifts are related to macrophage and microglia polarization to an “M1” pro-inflammatory phenotype
  - Kobayashi T, et al. *Proc Natl Acad Sci U S A.* 2021; 118(33):e2100295118. doi:10.1073/pnas.2100295118
  - Haschemi A, et al. *Cell Metab.* 2012;15(6):813-826. doi:10.1016/j.cmet.2012.04.023

DNAm in Aging and AD Carbohydrate Metabolism Gene Promoters

- Potentially beneficial changes in promoter DNAm for carbohydrate metabolism genes associated with aging & AD pathophysiology

- 36 genes, 75% are associated with inflammation

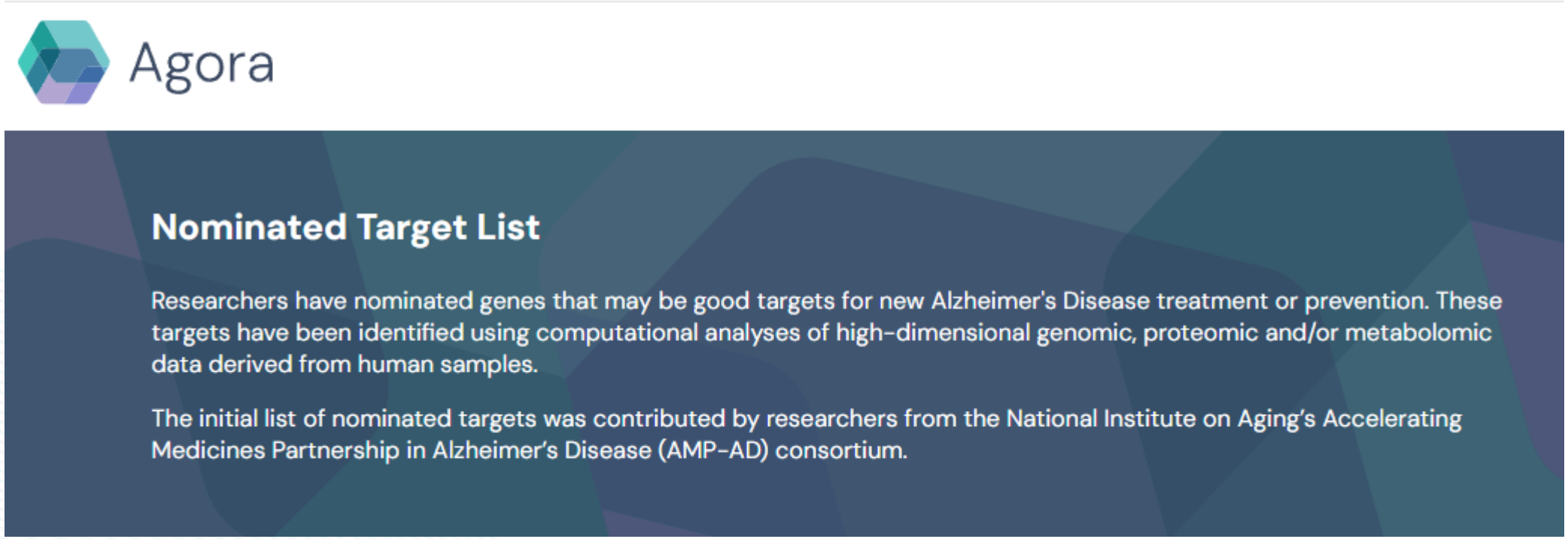


Examples:

- ACAT2 (microglial neuroinflammation, autophagy, glycolysis, M1 polarization, cholesterol metabolism, lipid droplets),
- TRADD (microglial neuroinflammation, TNFR-associated death domain; apoptosis, NFκB, IRS1, insulin resistance)

ACAT2, acetyl-CoA acetyltransferase 2; IRS1, insulin receptor substrate 1; TNFR, tumor necrosis factor receptor; TRADD, TNFR1-associated death domain protein.

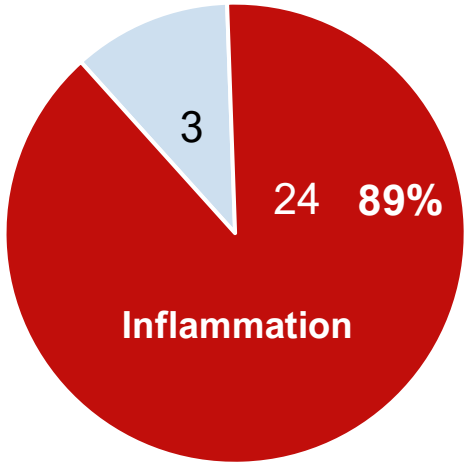
Aging Acceleration in AD Targets



DNAm in Nominated AD Target Gene Promoters

- Potentially beneficial changes in promoter DNAm for nominated targets associated with aging & AD pathophysiology

- 27 genes, 89% are associated with inflammation



Examples:

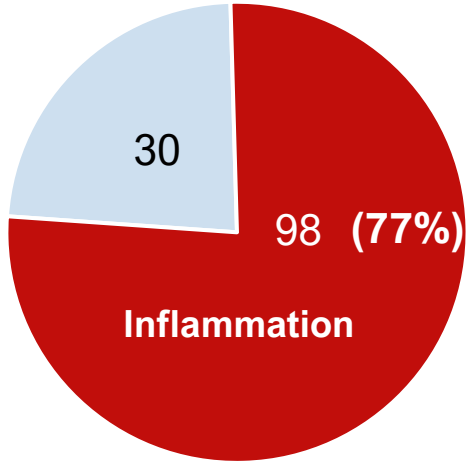
- CDK19 (cell cycle kinase; microglial neuroinflammation, senescence, neuronal loss, mitochondrial dysfunction, NFκB, CCL2)
- PFKP (platelet phosphofructokinase; microglial neuroinflammation, glycolysis, M1 astrocytes, metabolic reprogramming)

CCL2, chemoattractant protein-1; CDK19, cyclin-dependent kinase 19.

Gene Associations With Aging and AD Pathophysiology

Overall, bezisterim had significant potentially beneficial changes in promoter DNAm for 128 genes associated with aging & AD pathophysiology

- 128 genes, 77% were associated with inflammation

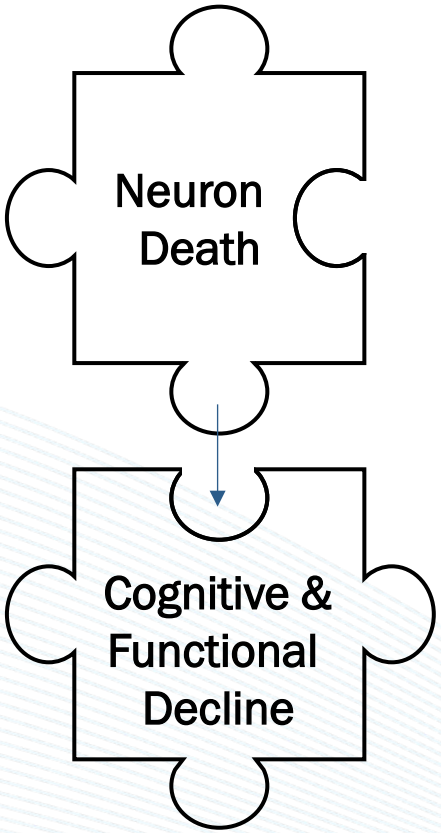


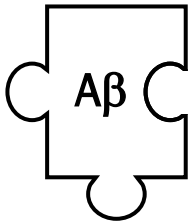
Genes with Decrease in Bezisterim/Placebo DNAm

Gene	Functions
CDK16	Increases autophagy, may induce M2 polarization, decreased IL1β & NFκB
DUSP22	JNK pathway-associated phosphatase; hypermethylation in AD, DNAm correlates positively with the Braak stages, decreases PKA activation, negative correlation with fasting glucose, decreases insulin resistance, inactivates JNK and ERK through dephosphorylation
PLSCR2	Decreases inflammatory signaling, cytokines and chemokines, non-inflammatory clearance of apoptotic cells, decreases M1 polarization
NME3	Mitochondrial health, mitophagy, protection from ROS DNA damage
SLC9B2	Cellular homeostasis, drug and toxin efflux, BP homeostasis, insulin secretion, loss exacerbates aging and obesity IGT
FBXL16	Inhibits microglial activation, enhances APP degradation
MAMSTER	Transcriptional coregulator, associated with decreased BRAAK pathology
SSBP4	Homeostatic transcriptional regulator

APP, amyloid precursor protein; BP, blood pressure; IGT, impaired glucose tolerance.

Puzzle Pieces of Alzheimer's Disease

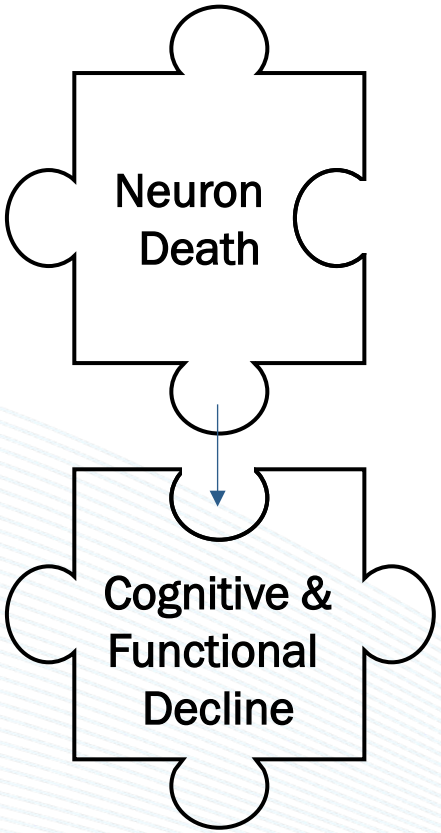


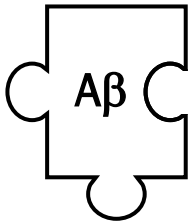


Amyloid
beta

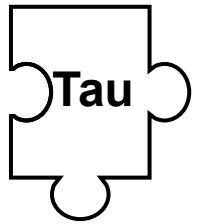
Amyloid Hypothesis
(based on familial AD disease genetics)
Pieces do not seem to fit for Late Onset AD
(over 100 genes identified)

Puzzle Pieces of Alzheimer's Disease



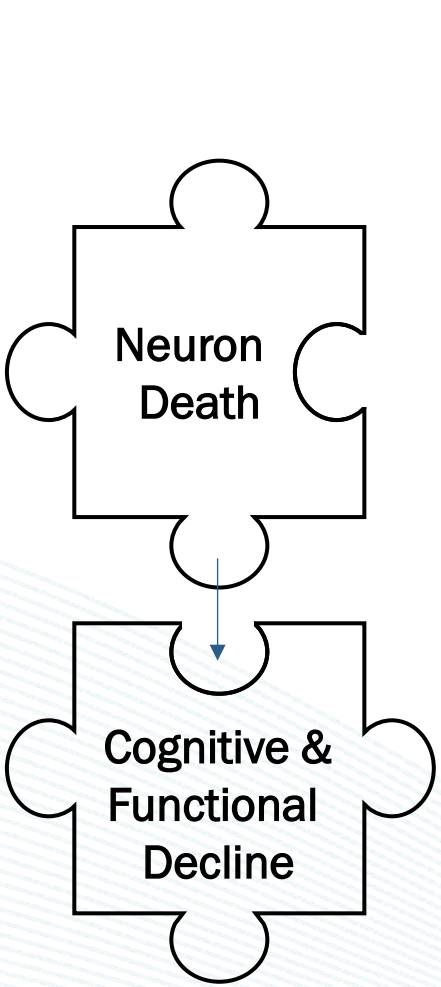


Add pTau
Still not clear



Microtubule Associated
Protein Tau

Puzzle Pieces of Alzheimer's Disease

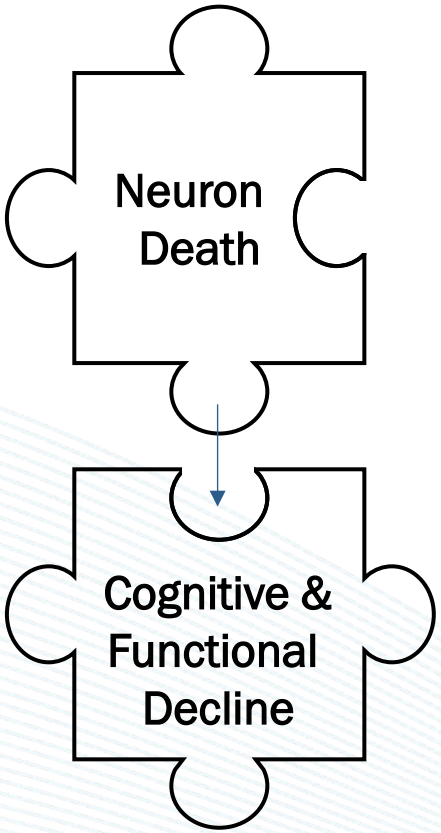


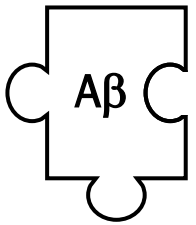
Alois Alzheimer, 1907

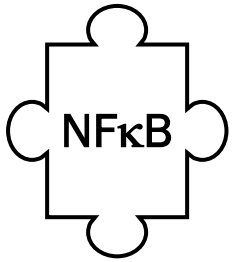
Familial Alzheimer's

Clue to metabolic inflammation

Puzzle Pieces of Alzheimer's Disease



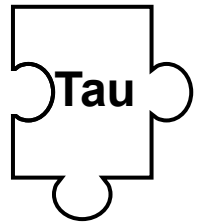


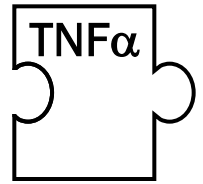


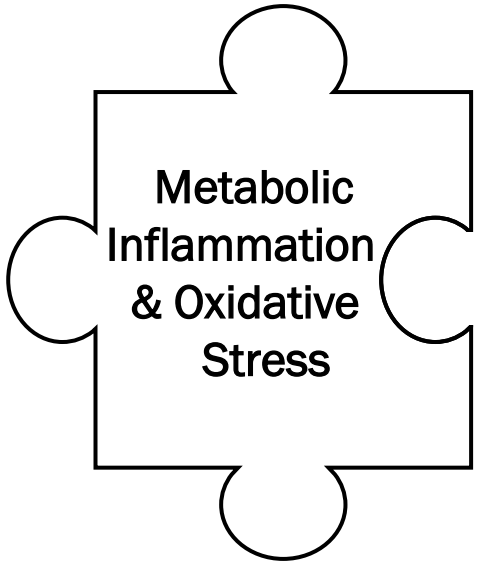
Nuclear Factor kappa B

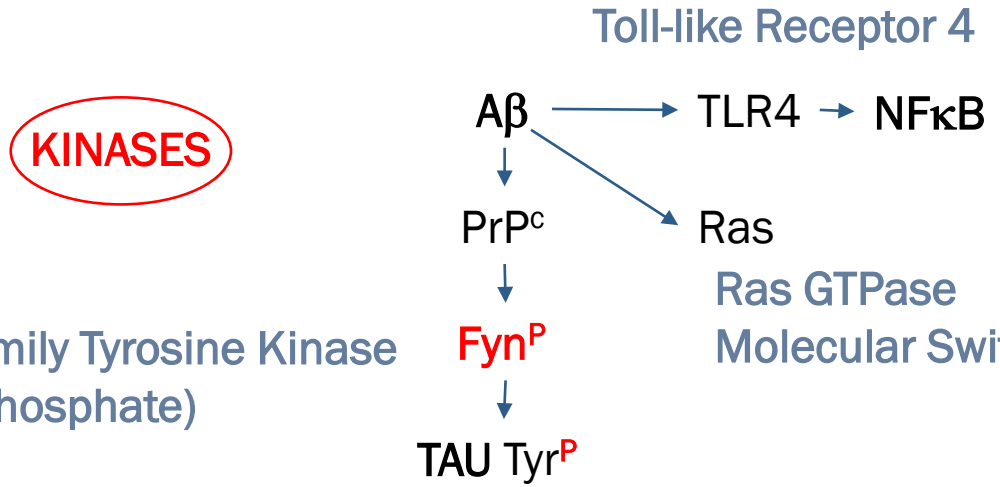
INFLAMMATION

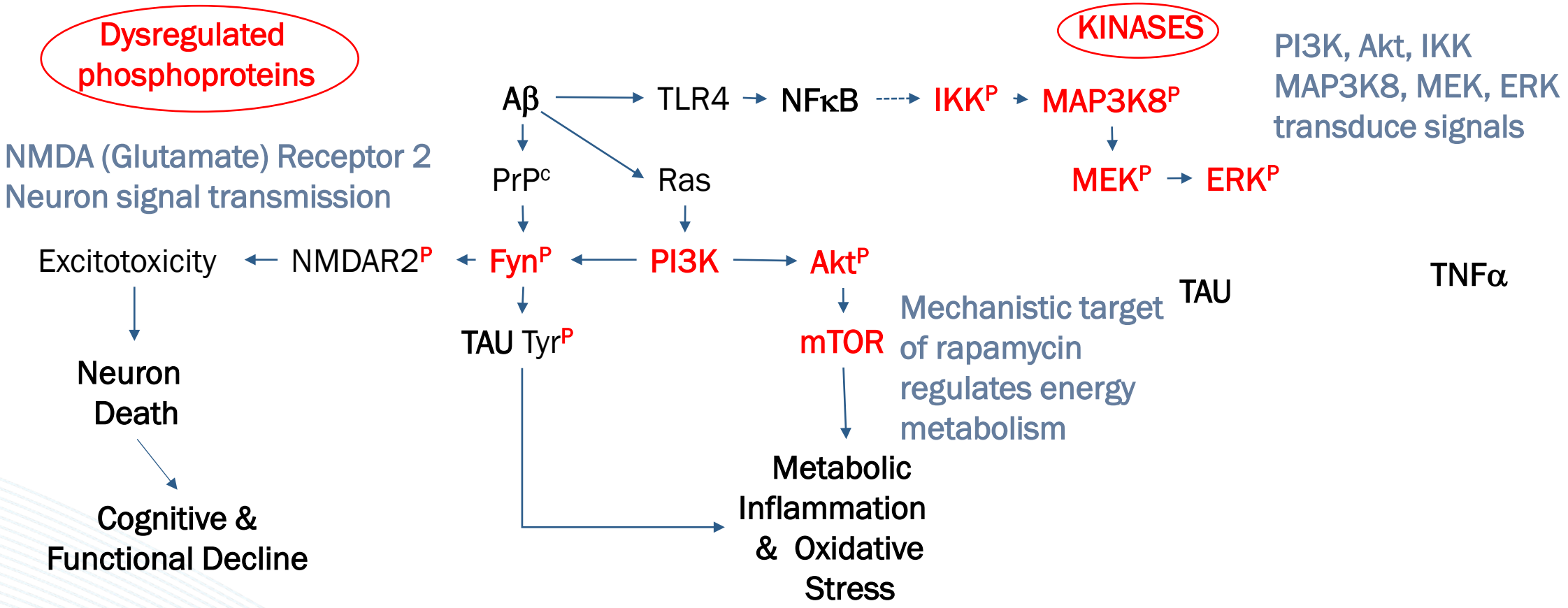
Tumor Necrosis Factor alpha

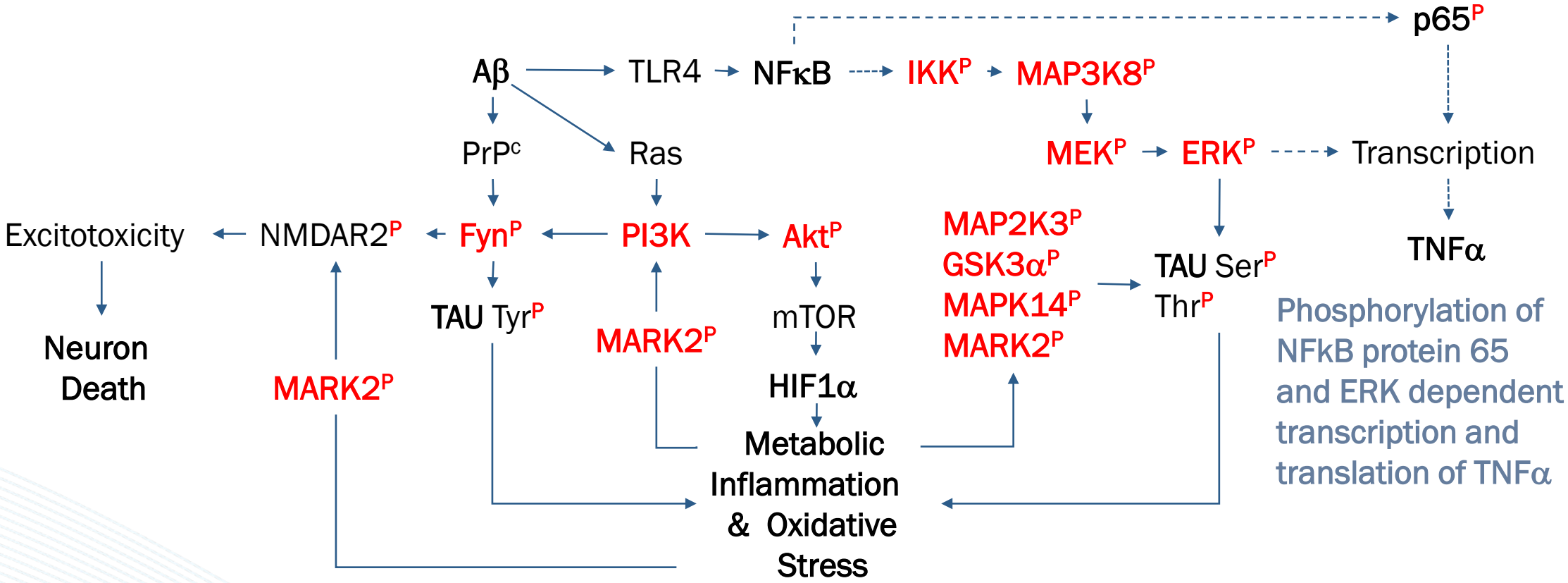







Toll-like Receptor 4
KINASES
Aβ
TLR4
NFκB
PrPc
Ras
Ras GTPase
Molecular Switch
Fyn SRC Family Tyrosine Kinase
(transfers phosphate)
FynP
TAU TyrP
TAU
TNFα
Neuron
Death
Cognitive &
Functional Decline
Metabolic
Inflammation
& Oxidative
Stress

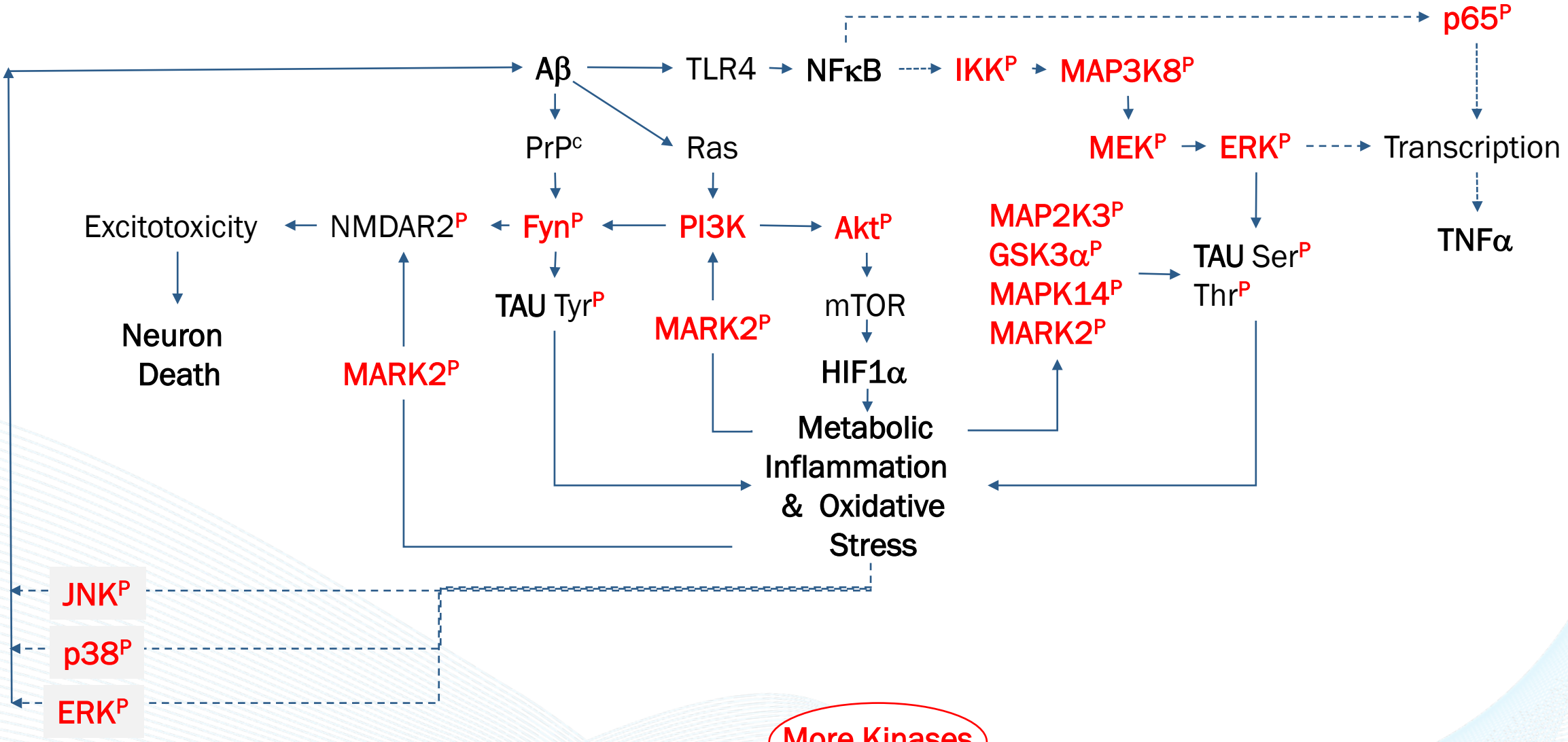

Dysregulated phosphoproteins
KINASES
PI3K, Akt, IKK
MAP3K8, MEK, ERK
transduce signals
NMDA (Glutamate) Receptor 2
Neuron signal transmission
Aβ
TLR4
NFκB
IKKP
MAP3K8P
PrPc
Ras
MEKP
ERKP
Excitotoxicity
NMDAR2P
FynP
PI3K
AktP
TAU
TNFα
Neuron Death
TAU TyrP
mTOR
Mechanistic target of rapamycin regulates energy metabolism
Cognitive & Functional Decline
Metabolic Inflammation & Oxidative Stress



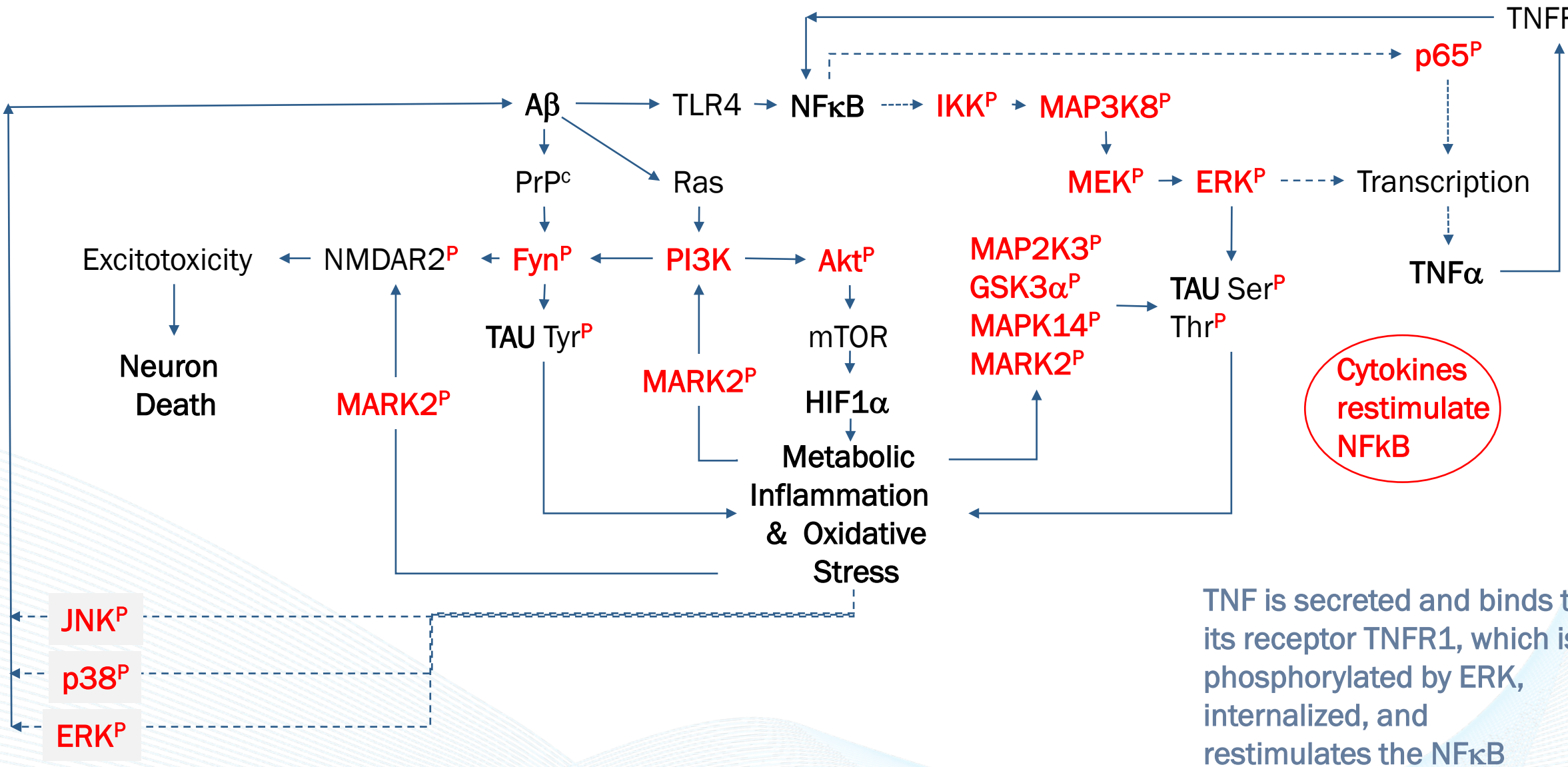
Phosphorylation of NFkB protein 65 and ERK dependent transcription and translation of TNFα

Pro-inflammatory kinases increase TAU phosphorylation, mTOR, and neuron death

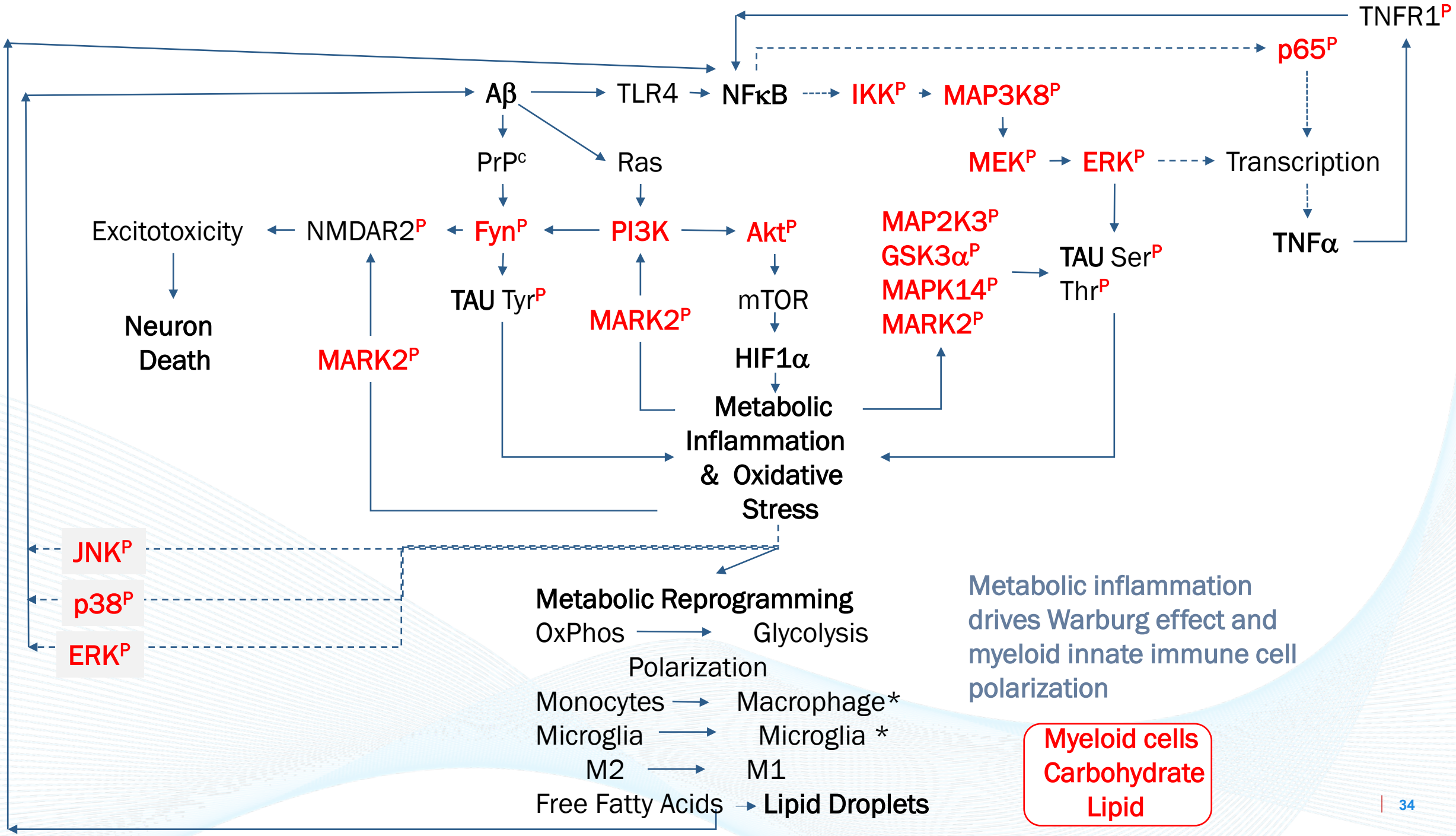
More Kinases



Metabolic inflammation activation of kinases JNK, p38, and ERK Increase amyloid β formation

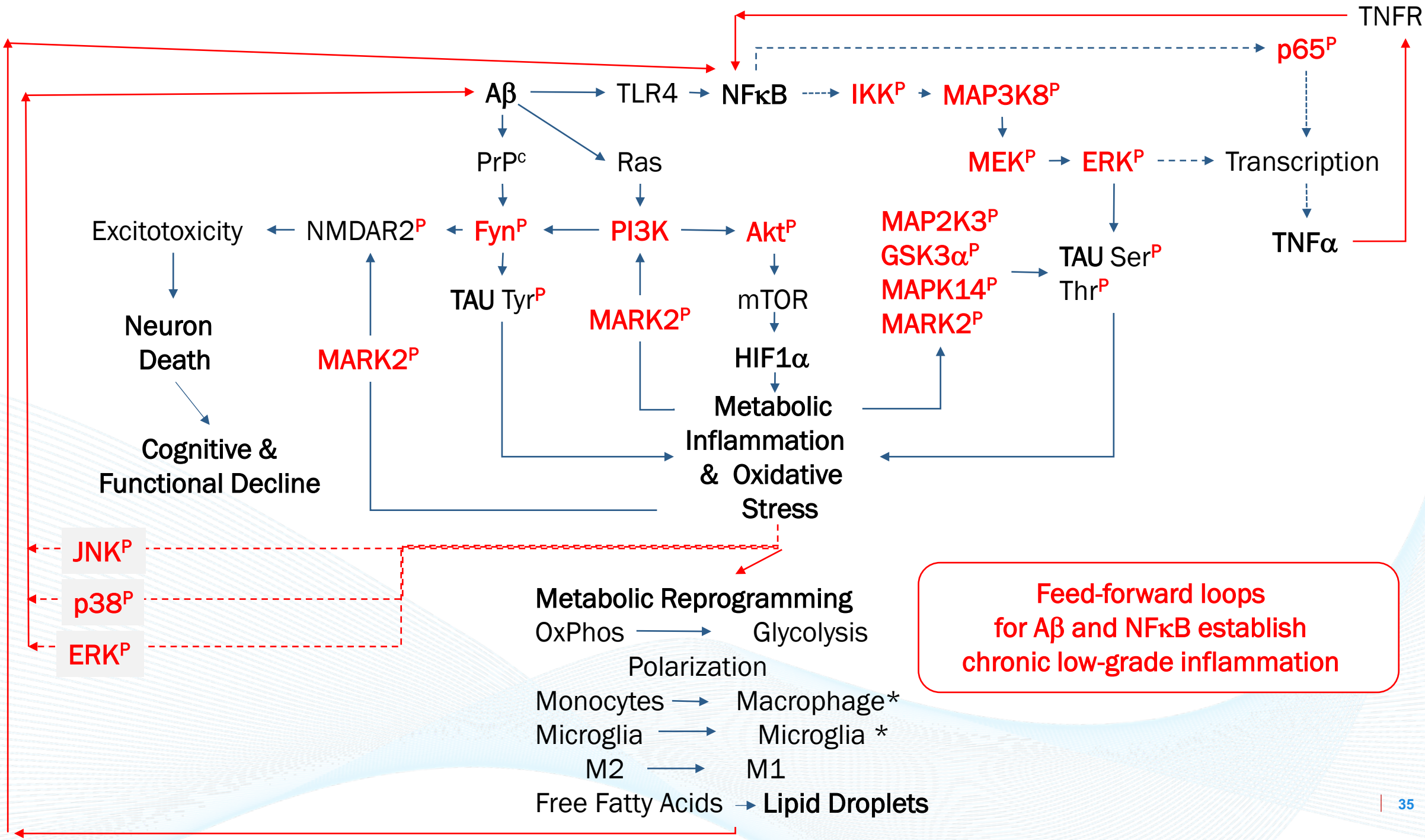


TNF is secreted and binds to its receptor TNFR1, which is phosphorylated by ERK, internalized, and restimulates the NFκB pathway



Metabolic inflammation drives Warburg effect and myeloid innate immune cell polarization

Myeloid cells
Carbohydrate
Lipid


TNFR1P
p65P
Aβ
TLR4
NFκB
IKKP
MAP3K8P
PrPc
Ras
MEKP
ERKP
Transcription
Excitotoxicity
NMDAR2P
FynP
PI3K
AktP
MAP2K3P
GSK3αP
MAPK14P
MARK2P
TAU SerP ThrP
TNFα
Neuron Death
MARK2P
TAU TyrP
MARK2P
mTOR
HIF1α
Metabolic Inflammation & Oxidative Stress
Cognitive & Functional Decline
JNKP
p38P
ERKP
Metabolic Reprogramming
OxPhos → Glycolysis
Polarization
Monocytes → Macrophage*
Microglia → Microglia *
M2 → M1
Free Fatty Acids → Lipid Droplets
Feed-forward loops for Aβ and NFκB establish chronic low-grade inflammation

Conclusions

- Bezisterim may decrease inflammatory mechanisms of **aging** and **AD** *via* changes in DNAm in key inflammation networks
  - Biological **age acceleration**
  - Inflammatory **kinase signaling**
  - Dysregulated **protein phosphorylation**
  - Innate immune **cell polarization**
  - **Glycolysis** and Warburg effect
  - Nominated **aging** and **AD** targets
- Bezisterim effects may be related to improvements in cognition and function
- Small bezisterim associated changes in a multiple genes in inflammatory pathways may have a compounding anti-inflammatory effect
- Small DNAm changes in other genes not associated with **aging** & **AD** pathophysiology are unlikely to cause toxicities
- 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