NRF2 biomarkers in blood

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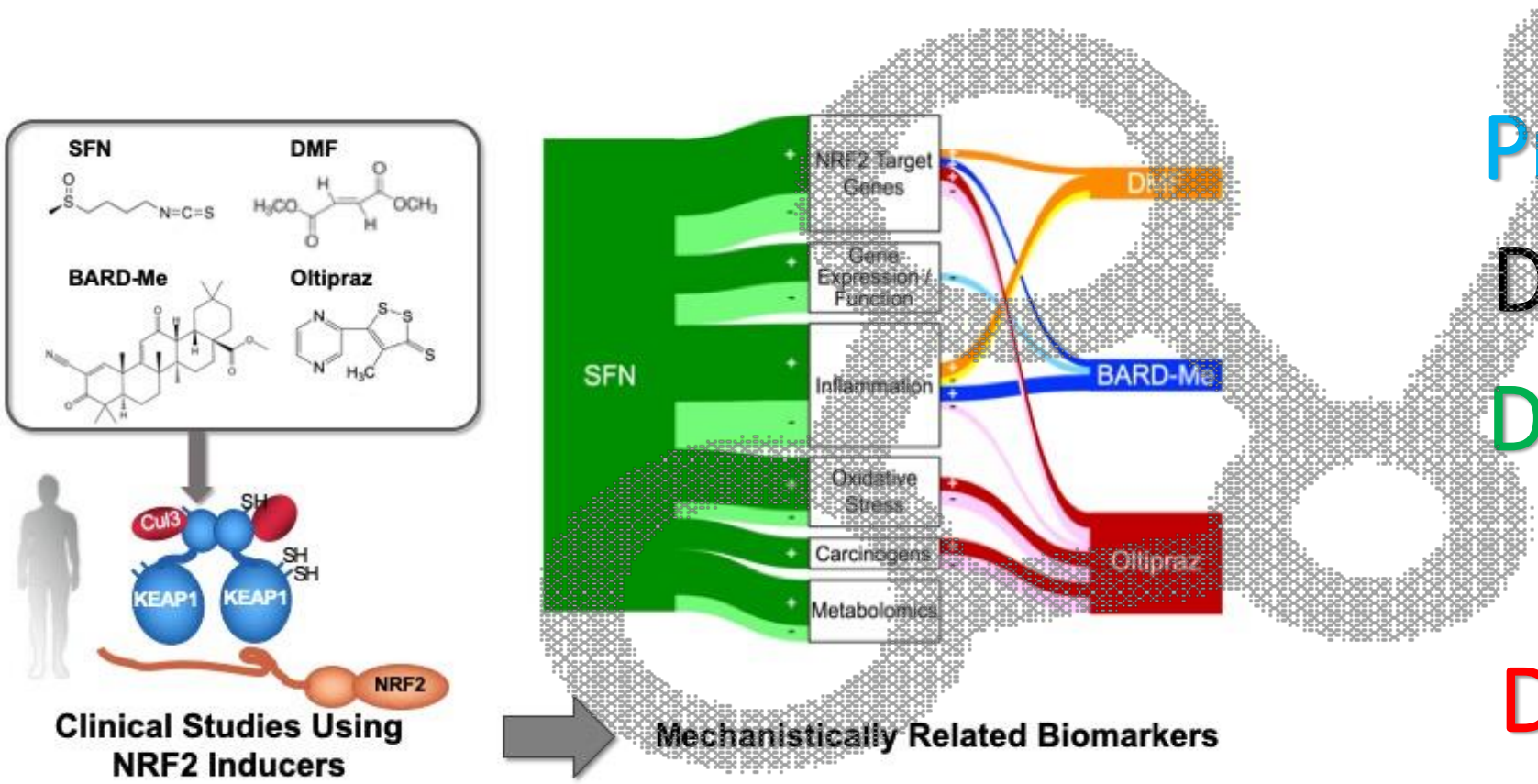
Outline

- Why NRF2 biomarkers in blood
- NRF2 target genes and the NRF2 interactome
- Blood NRF2 biomarkers in Alzheimer's disease
- NRF2 blood biomarkers in clinical trials
- NRF2 biomarkers in serum and plasma

Ia. Why NRF2 biomarkers?



Proof-of-concept

Diagnosis

Disease monitoring

Drug development

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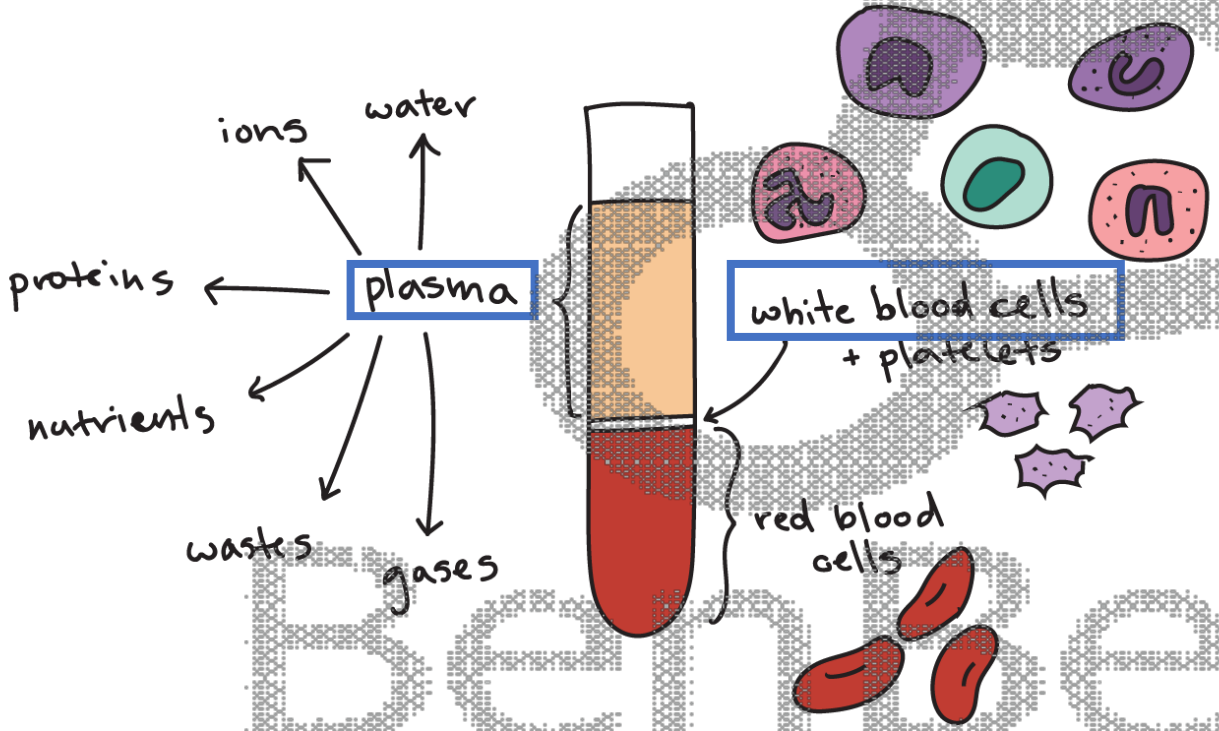
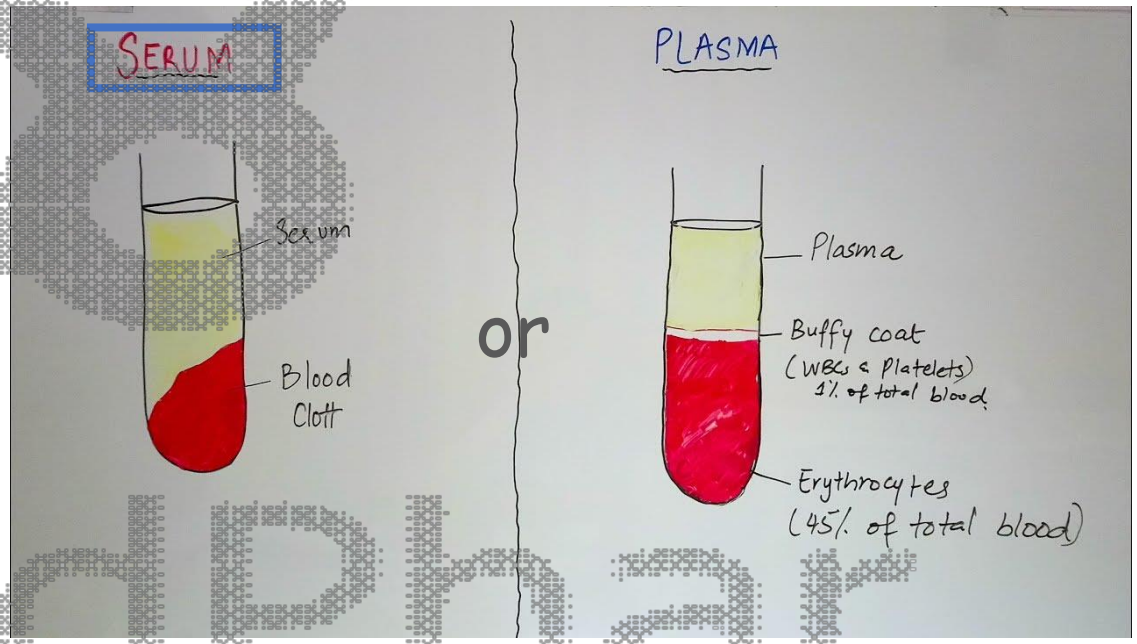
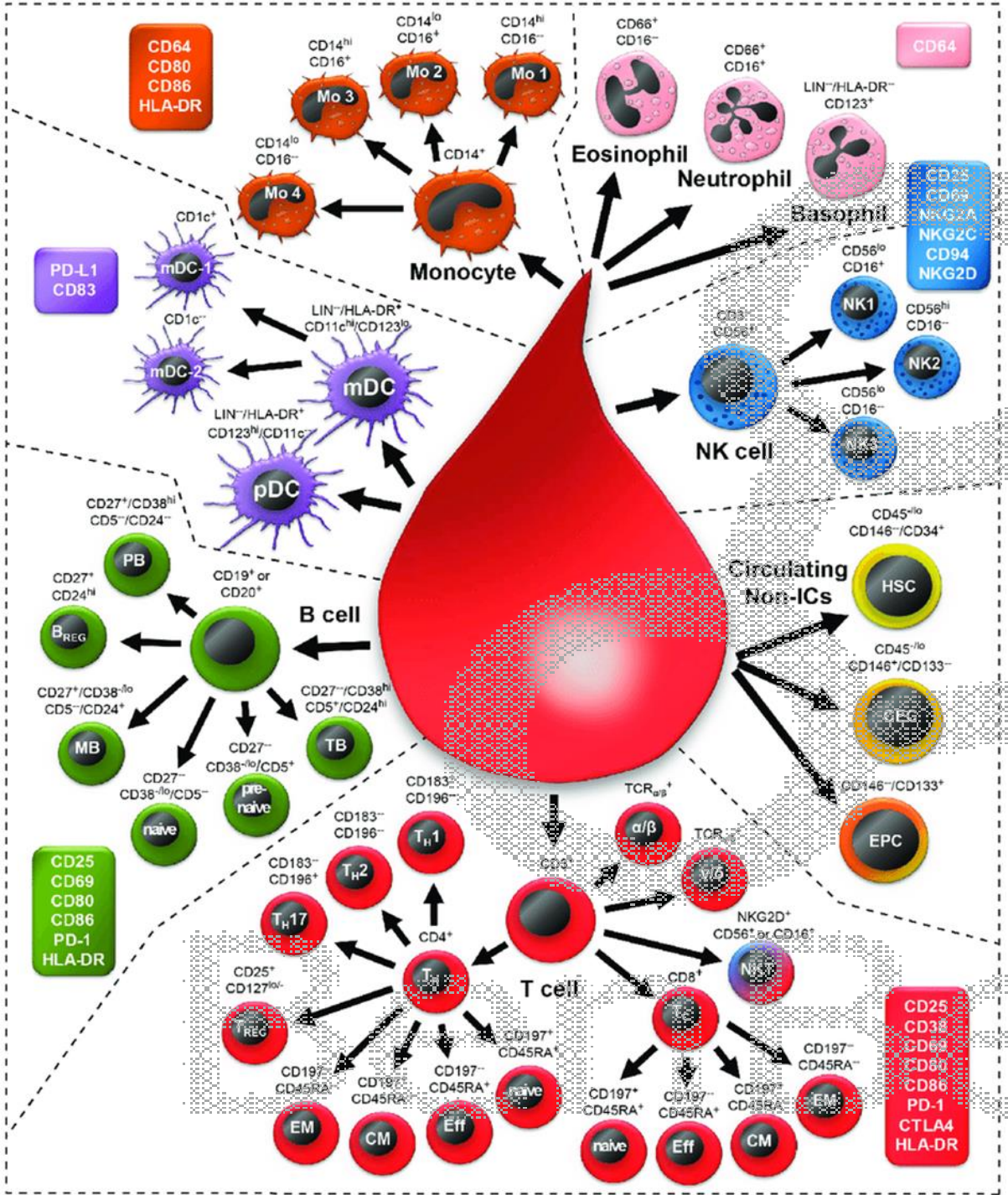
Yagishita Y, et al. Current Landscape of NRF2 Biomarkers in Clinical Trials. Antioxidants 2020, https://doi.org/10.3390/antiox9080716

Ib. Why NRF2 biomarkers in blood?

Whole peripheral blood







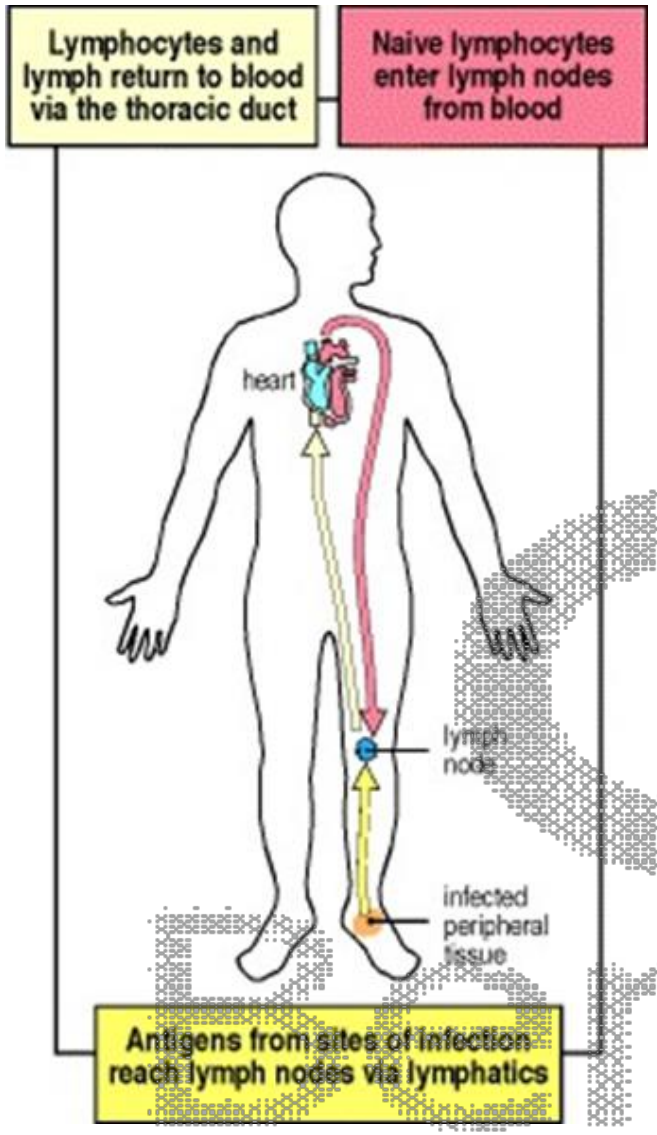
NRF2 is a transcription factor

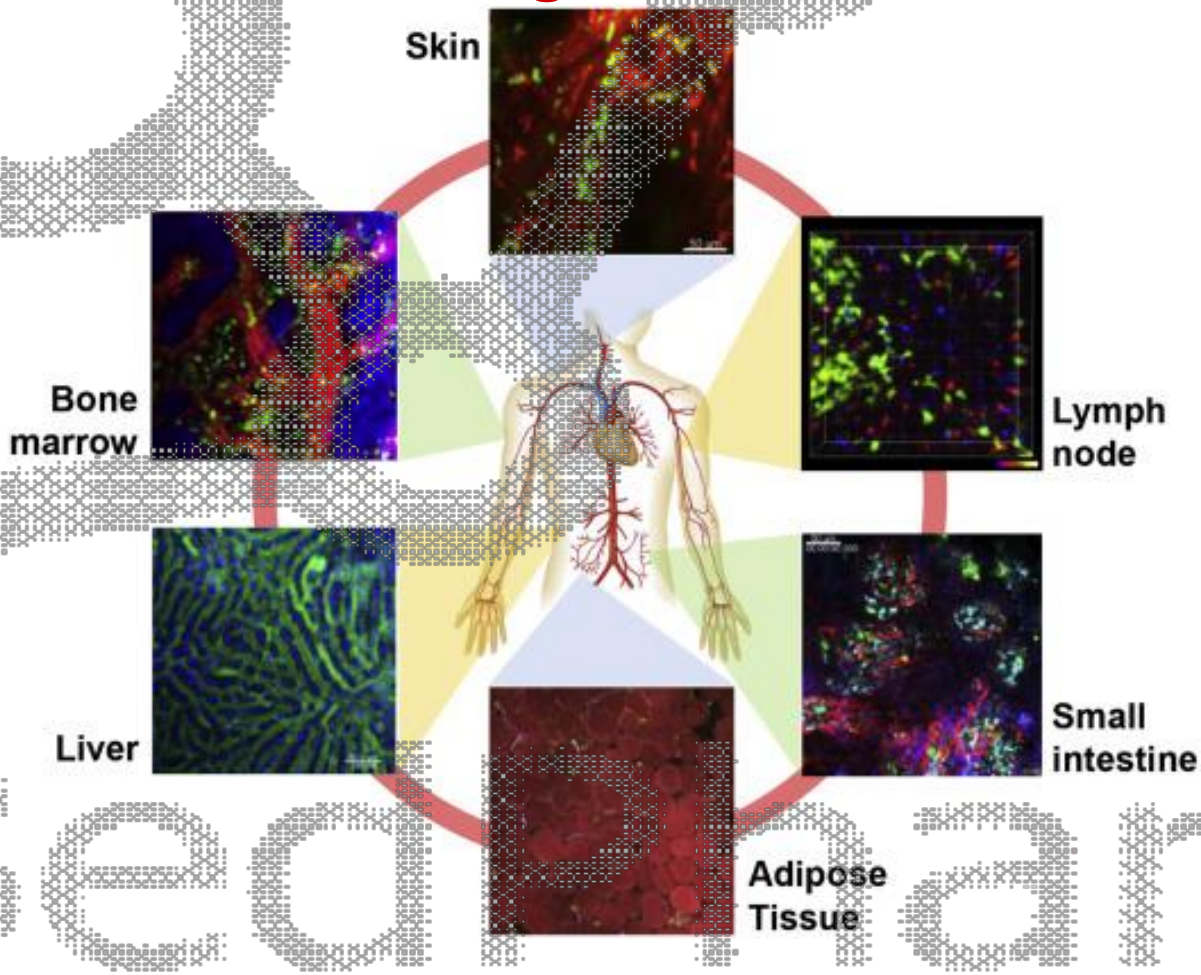
Look at the molecular NRF2 fingerprint in blood leukocytes

Ruhle PF et al. International Journal of Molecular Sciences 2016, DOI:10.3390/ijms17081316

Does the NRF2 status in peripheral immune cells reflect organ-specific pathological processes?

Organ-specific immune niches send messages into the blood

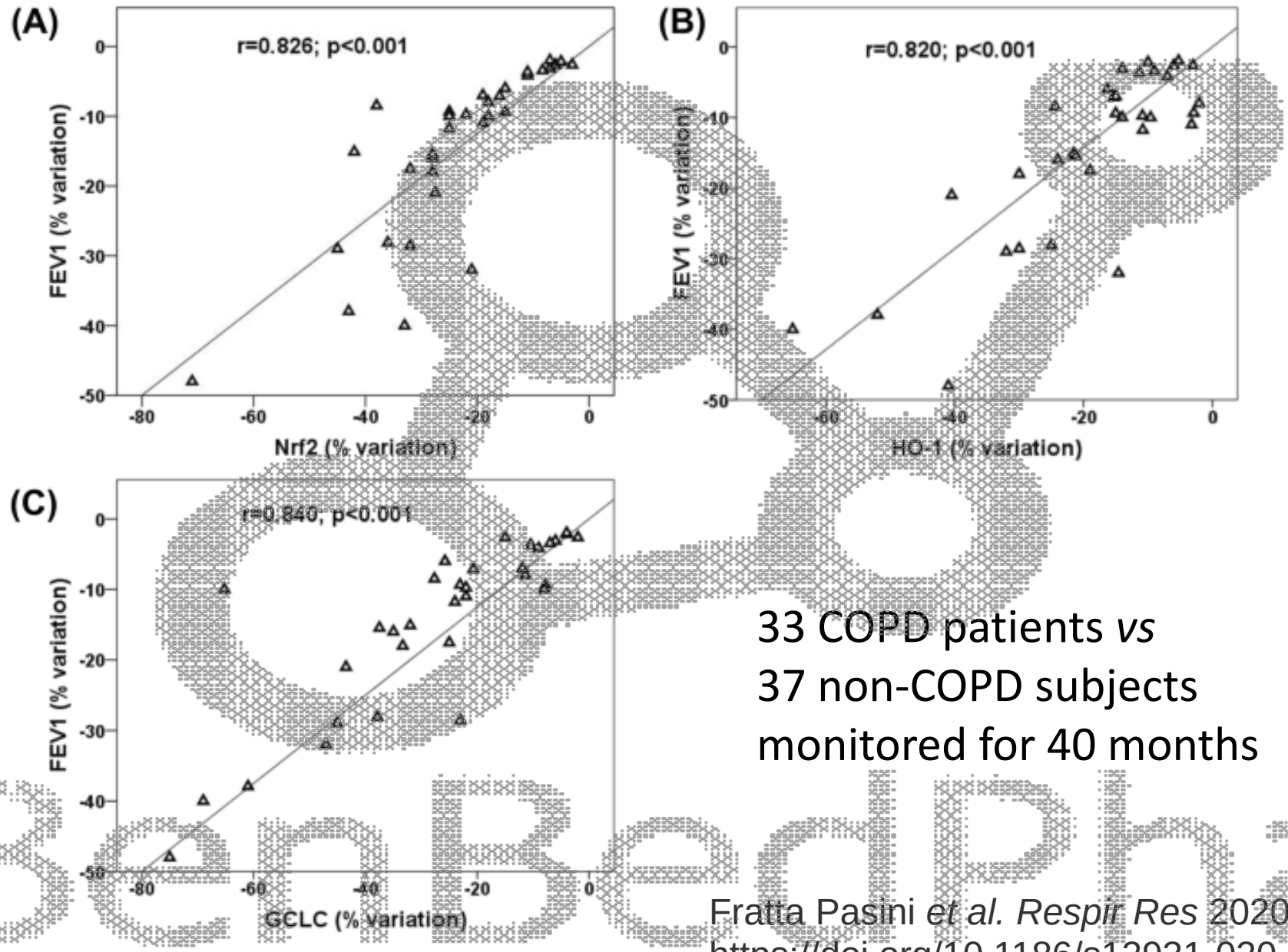




Masaru Ishii. Intravital imaging technology reveals immune system dynamics in vivo. Allergology International 2016.

Example

PBMCs in chronic obstructive pulmonary disease (COPD)

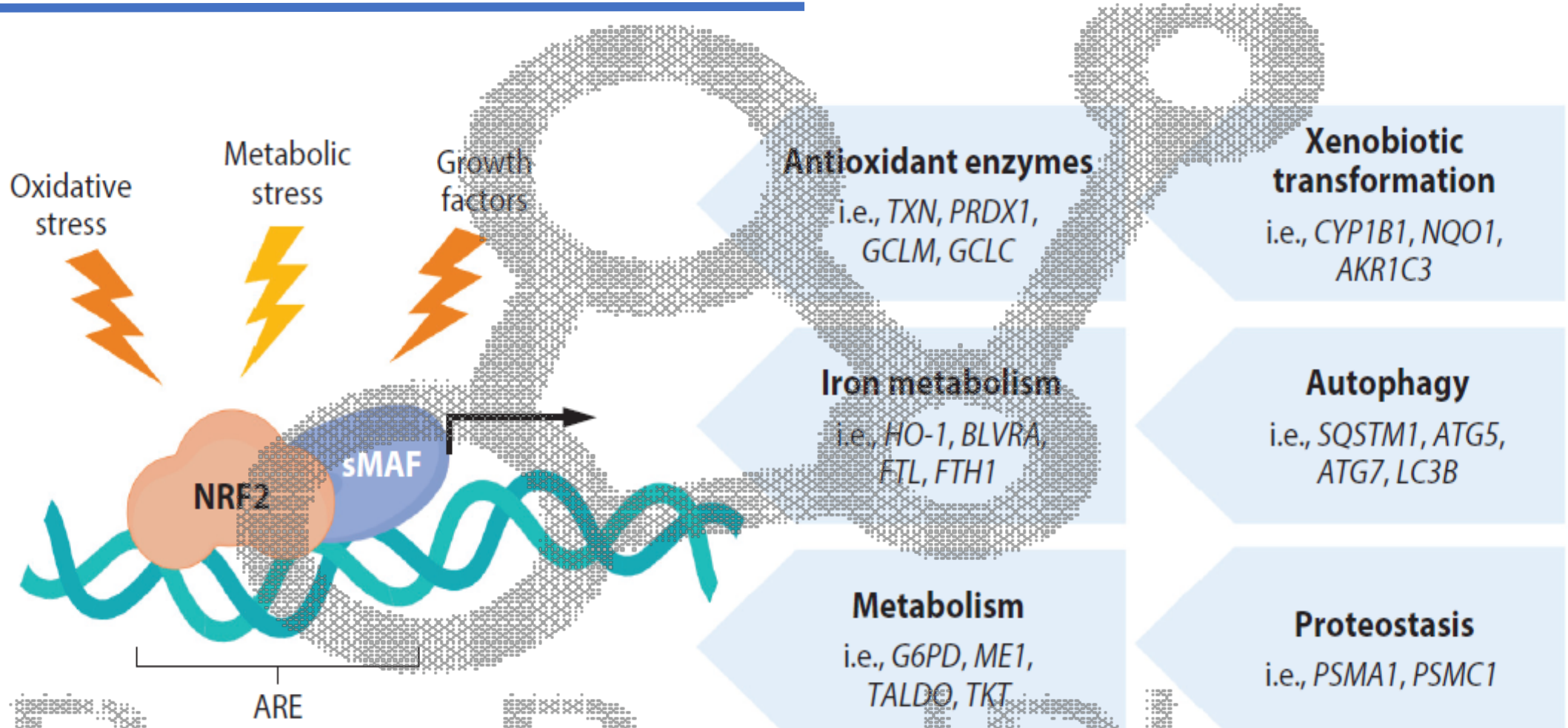


33 COPD patients *vs*
37 non-COPD subjects
monitored for 40 months

Fratta Pasini *et al. Respir Res* 2020, 21: 37.
https://doi.org/10.1186/s12931-020-1292-7

II. NRF2 target genes

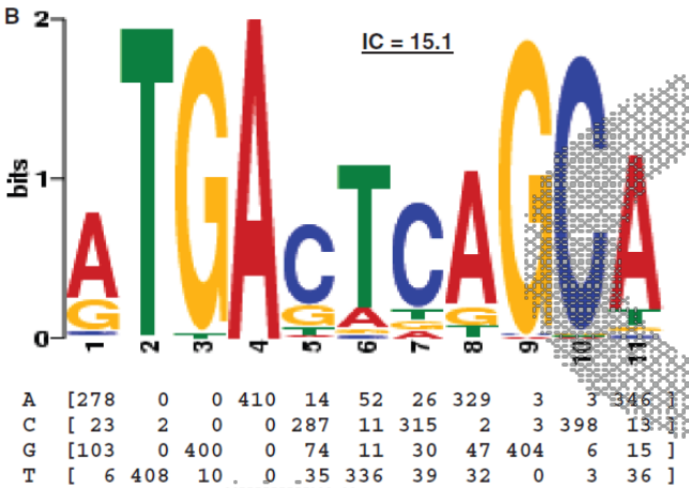
Validated NRF2 target genes



… and many more other target genes that are under validation

Many genes have NRF2 binding sites in their promoter

NRF2 binding profile



MEME motif discovery algorithm
(http://meme.sdsc.edu)
IC=information content

Mouse embryonal fibroblasts from Keap1-/-, Nrf2-/- and WT mice

Integration of ChIP-Seq and microarray data:
NRF2-binding sites in **645 basal** and **654 inducible genes**
(244 genes at the intersection)

Malhotra D et al. Nucleic Acids Res. 2010, doi: 10.1093/nar/gkq212.

Are all the NRF2 inducible gene targets transcribed simultaneously?

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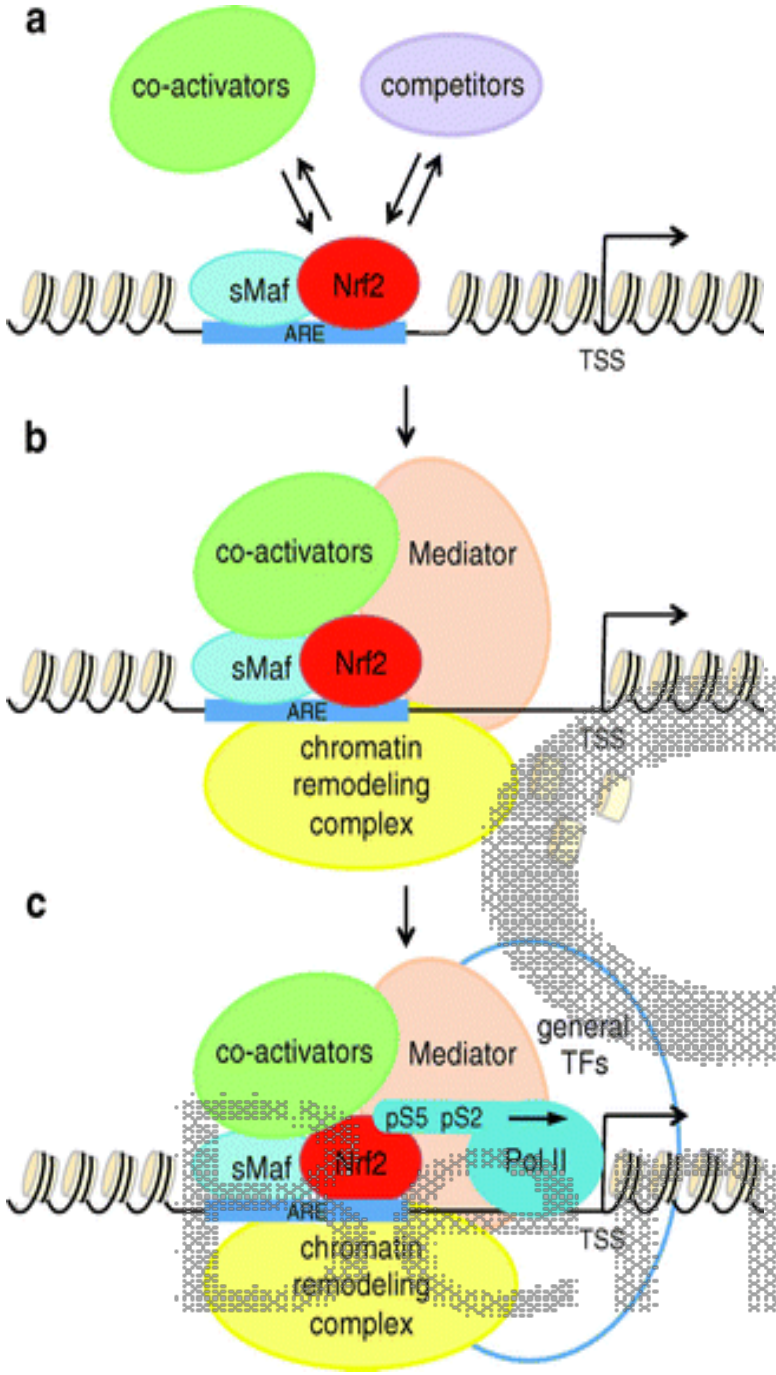


- NRF2 selects the genes to be activated by binding as a heterodimer with sMaf to the ARE in promoter regions of the target genes;
- NRF2 recruits co-activators, components of the transcription machinery and nucleosome-remodelling complexes that make the chromatin structure accessible to the Pol II machinery;
- General transcription factors and Pol II are recruited;
- Pol II is phosphorylated, and transcription starts.

Tonelli C, Chio IIC and Tuveson DA. Antioxid Redox Signal 2018 doi: 10.1089/ars.2017.7342.

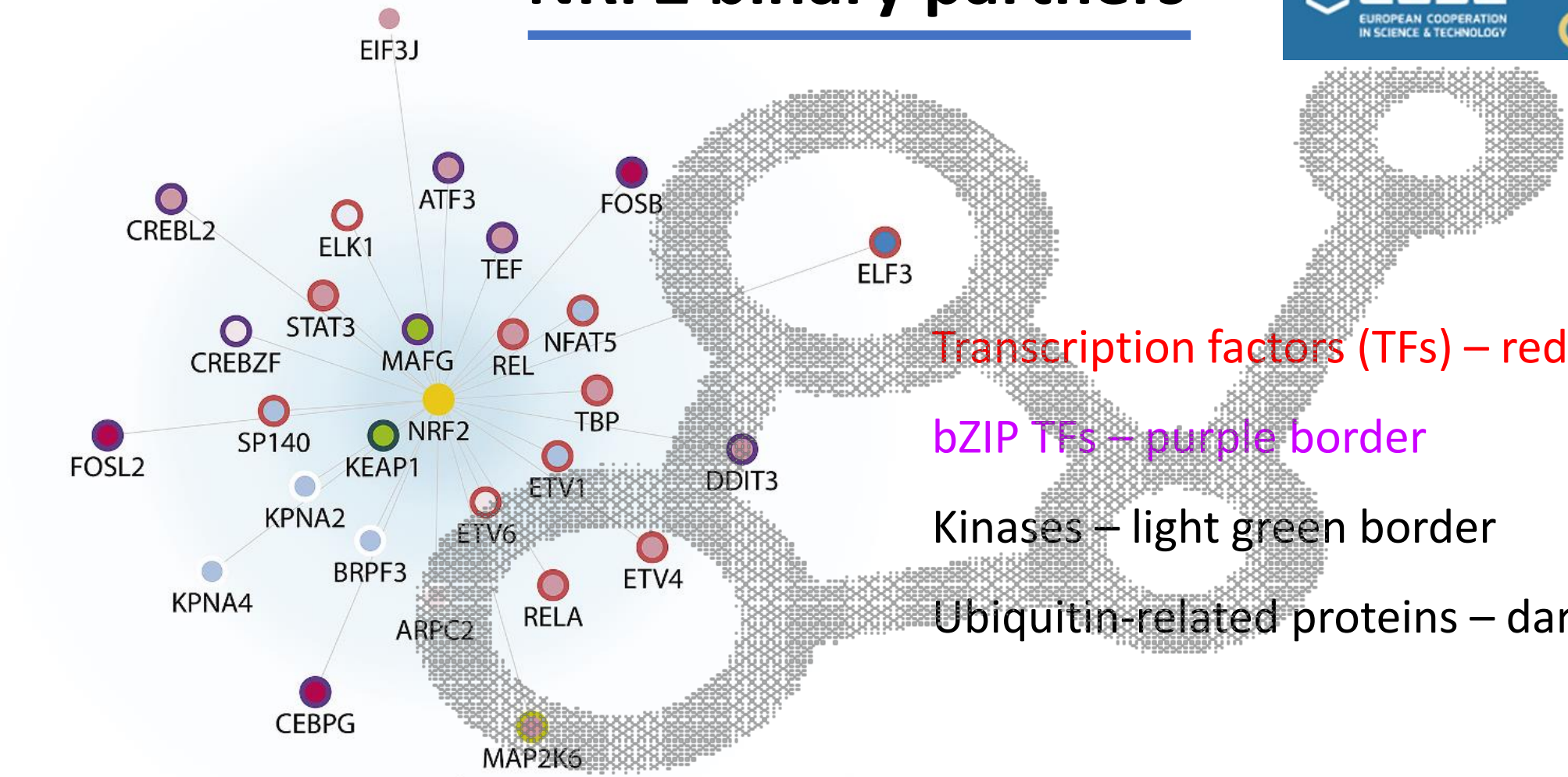
Other transcription factors may regulate NRF2 target genes

Examples:

The HMOX1 gene is at the interference of several
redox-sensitive transcription factors such as NRF2, NFkB, HIF1α and AP1.

The bZip transcription factors NRF2 and AP1 regulate each other
as the AP1-binding site 12-O-tetradecanoylphorbol-13-acetate response element
(TRE, TGA(C/G)TCA) is often embedded into the NRF2-binding ARE.

NRF2 binary partners



Transcription factors (TFs) – red border

bZIP TFs – purple border

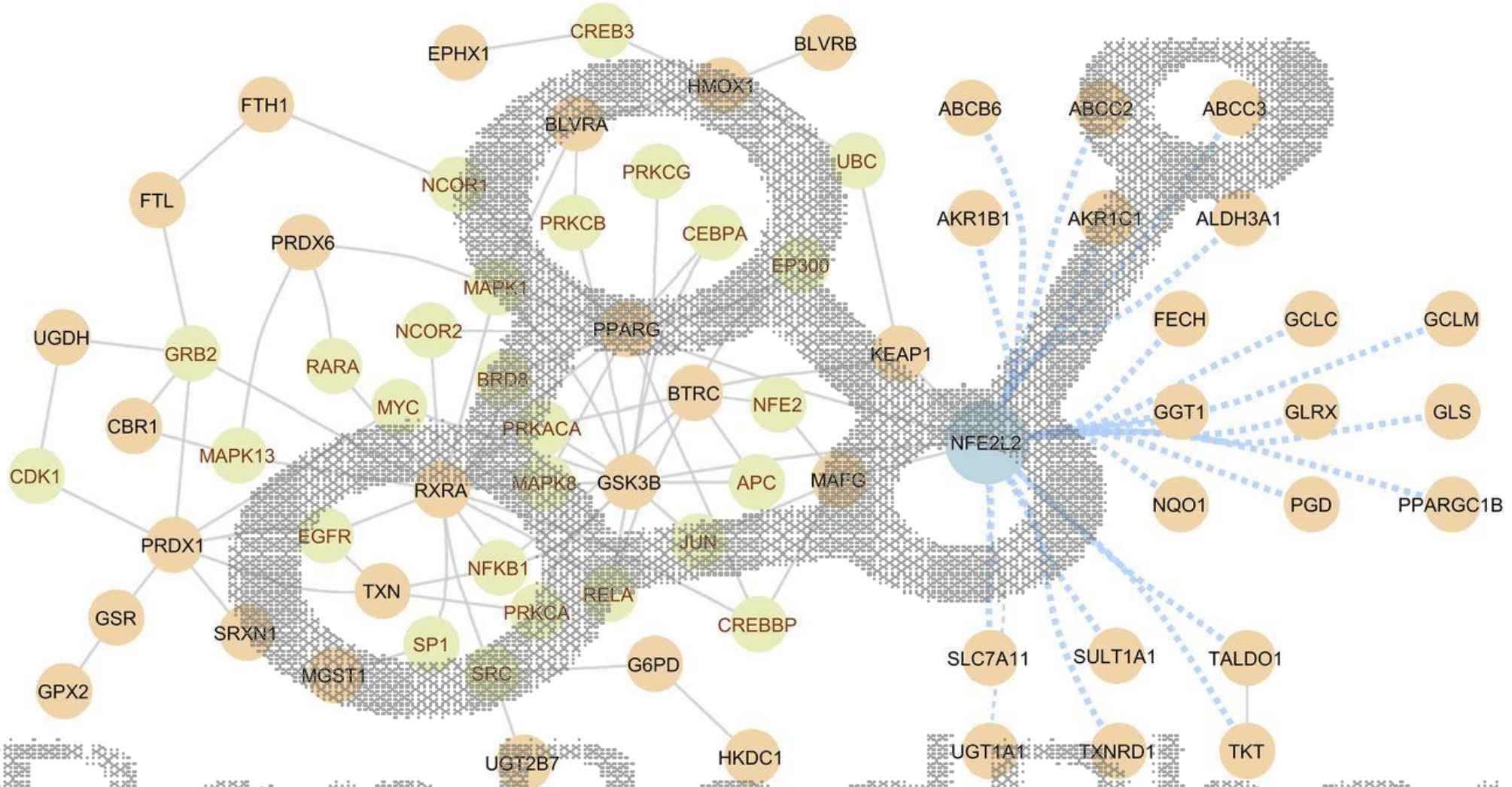
Kinases – light green border

Ubiquitin-related proteins – dark green border

Poh J et al. Redox Biol. 2020. 37:101686. doi: 10.1016/j.redox.2020.101686.

Locating the NRF2 regulatory pathway in the human interactome

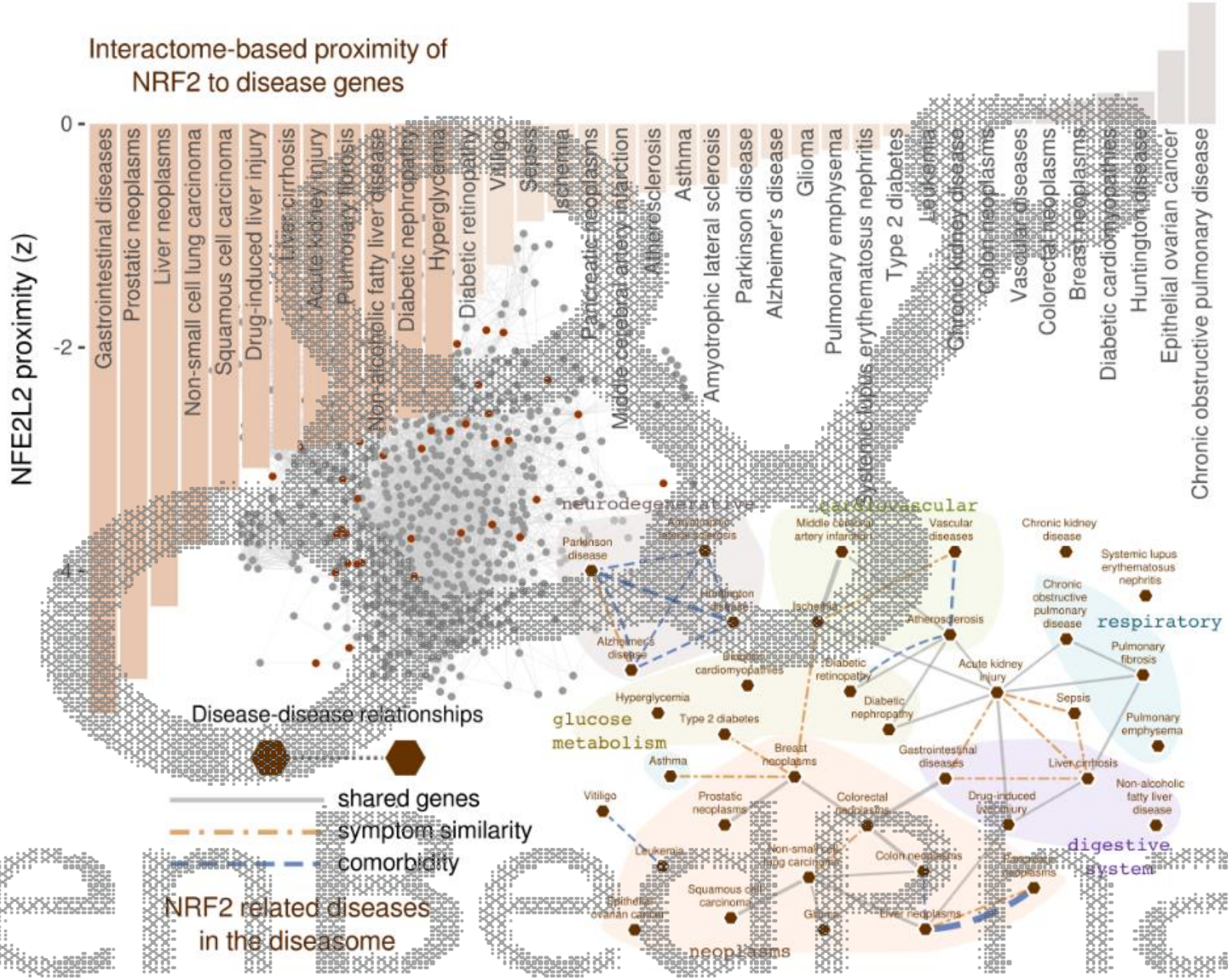
NRF2 - Systems medicine-based analysis
https://sbi.imim.es/data/nrf2/.



Gray link: physical interactions between proteins involved in the NRF2 regulatory pathway (regulatory proteins, brown circles) and the proteins through which they are connected (mediator proteins, gray circles).
Blue link: regulator proteins involving more than one mediator protein to connect to NRF2.

Cuadrado A et al. Pharmacol Rev. 2018,
doi: 10.1124/pr.117.014753.

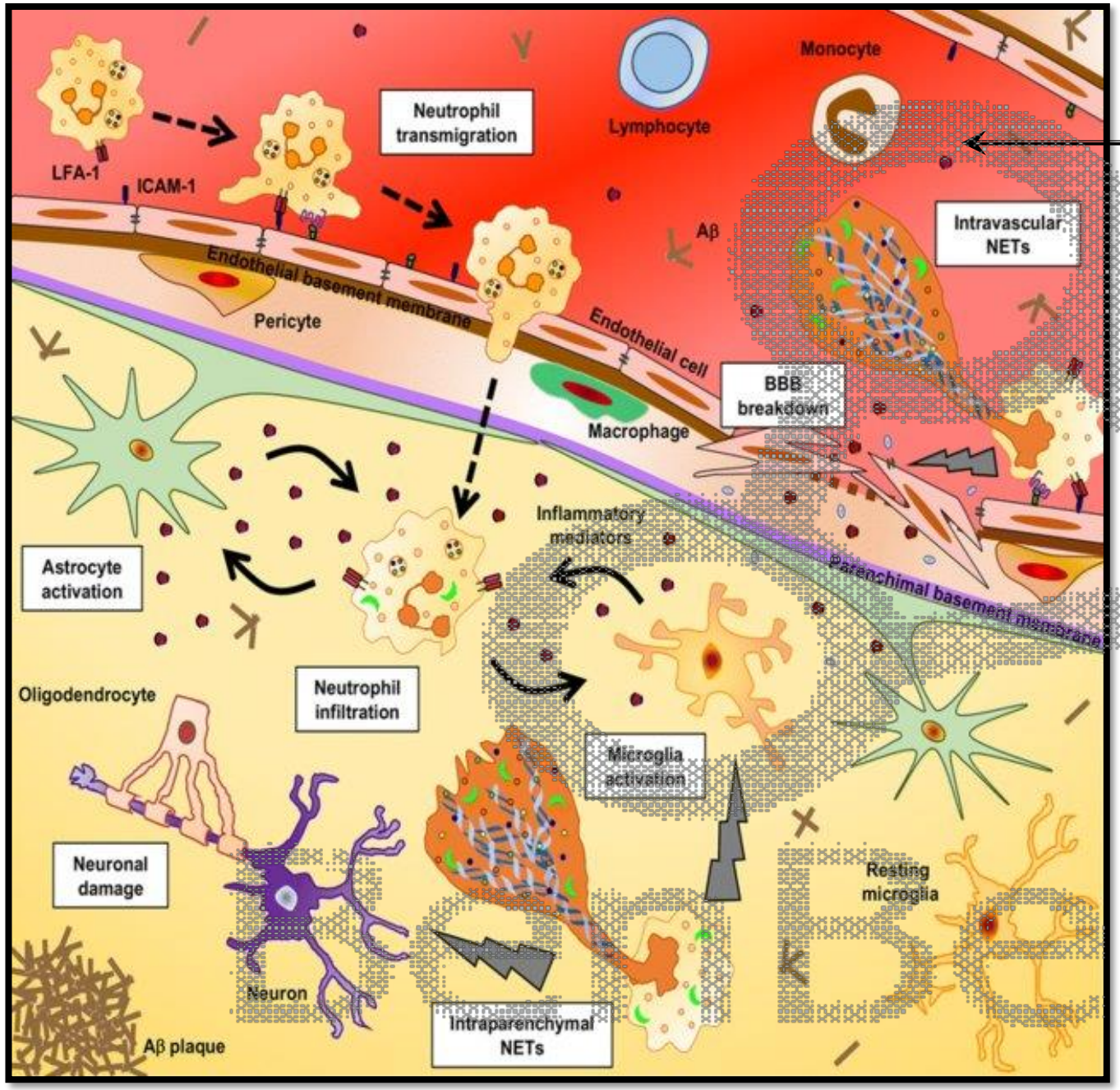
The NRF2-related diseasome



Cuadrado A et al. Pharmacol Rev. 2018, doi: 10.1124/pr.117.014753.

III. NRF2 blood biomarkers in Alzheimer's disease

Are blood leukocytes "primed" in AD?



BBB alterations and leukocyte extravasation in postcapillary venules in the AD brain

- Liebner S et al. Functional morphology of the blood-brain barrier in health and disease. Acta Neuropathol. 2018, 135(3):311-336.
- Ramos-Cejudo J et al. The Neutrophil to Lymphocyte Ratio Is Associated With the Risk of Subsequent Dementia in the Framingham Heart Study. Front Aging Neurosci. 2021; 13: 773984.

Case-control study design

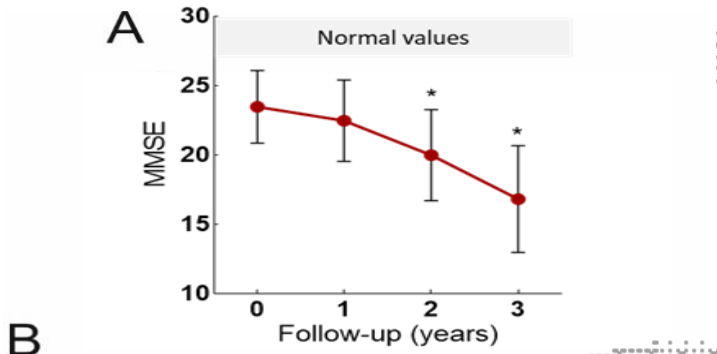
Age-matched

38 mild AD patients

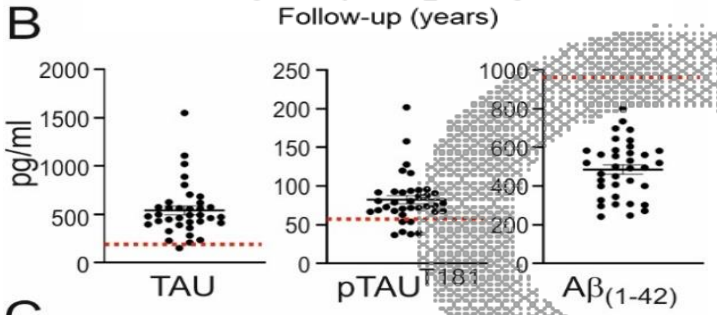
38 controls

Similar comorbidities

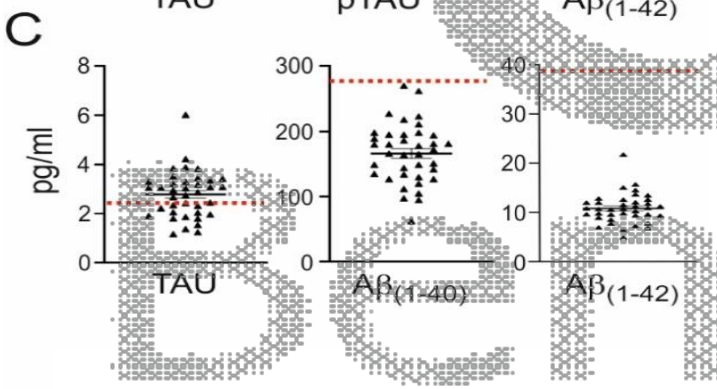
Mild AD diagnosis (NIA-AA criteria)



MMSE



CSF



Plasma

Gene expression study in blood

- **84 redox genes**: ROS producers, genes related to ROS metabolism and redox-responsive genes.
- **136 inflammation genes:** NFKB signaling and transcription of its target genes.

Housekeeping genes: HPRT1 and RPLPO (RefFinder analysis which integrates 4 computational programs (BestKeeper, NormFinder, Genorm and the comparative delta-Ct method).

Patients were not treated with AD-specific medication

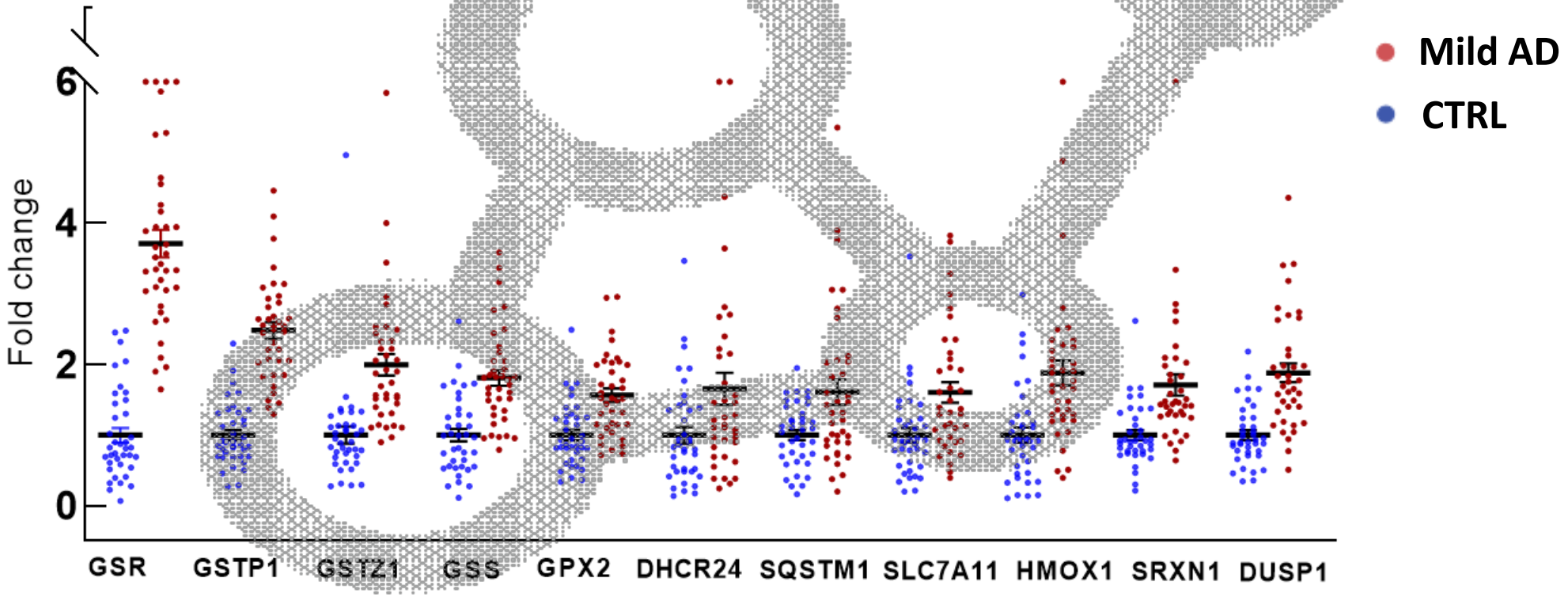
Over-expressed redox genes (FC > 2, p < 0.001) in the blood of patients *vs* controls

Genes encoding ROS producers	NCF1, DUOX1/2*
Genes encoding antioxidants	Glutathione metabolism: **GSR***, GPX3*, GSTP1 Thioredoxin metabolism: TXNRD2
Other genes involved in redox signaling	FOXM1*, HSPA1A*, SEPP1, CCS
Pathway activity signature	TRAPPC6A

*** Redox-responsive genes**

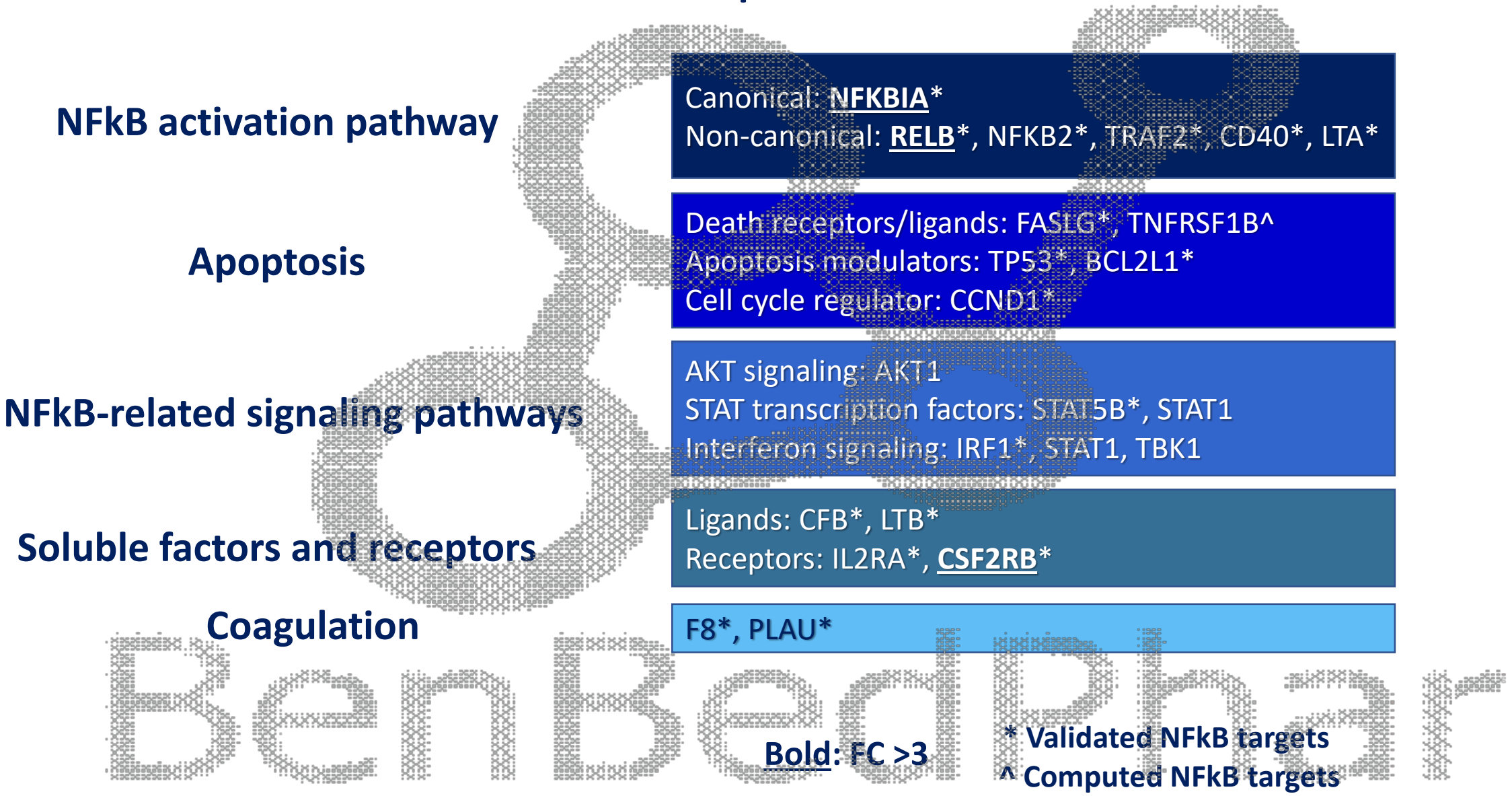
Bold: FC >3

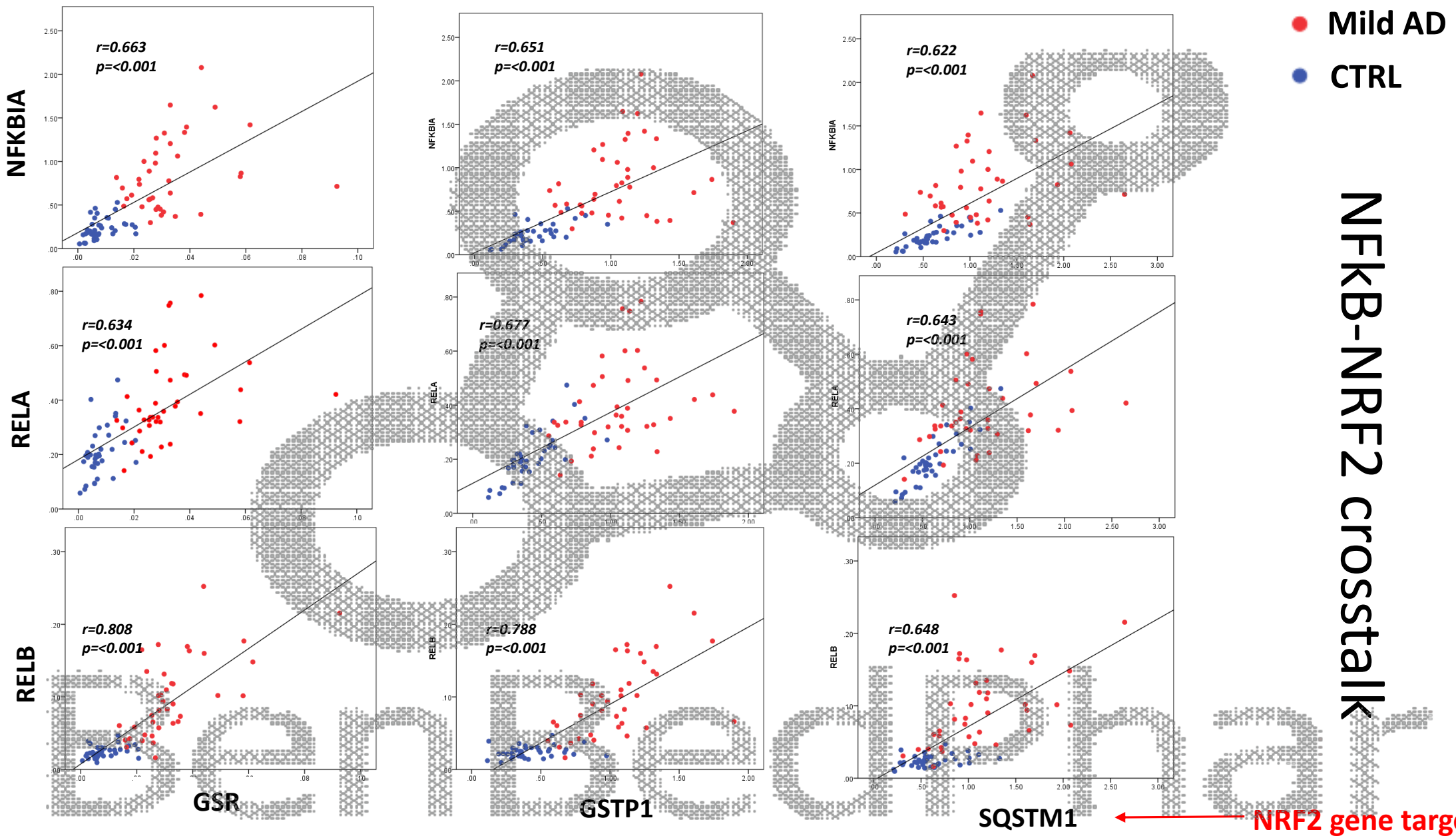
NRF2 signature in mild AD patients



NRF2 activation is apparently not able
to restore redox homeostasis in the blood of AD patients

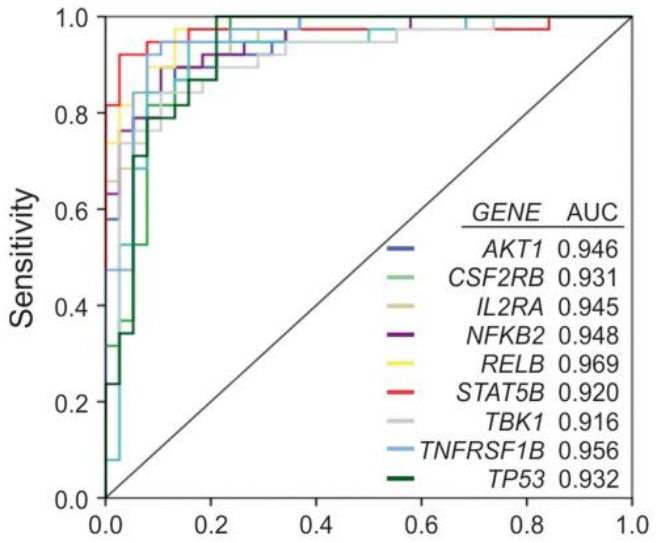
Over-expressed inflammation genes (FC > 2, p < 0.001) in the blood of AD patients *vs* controls



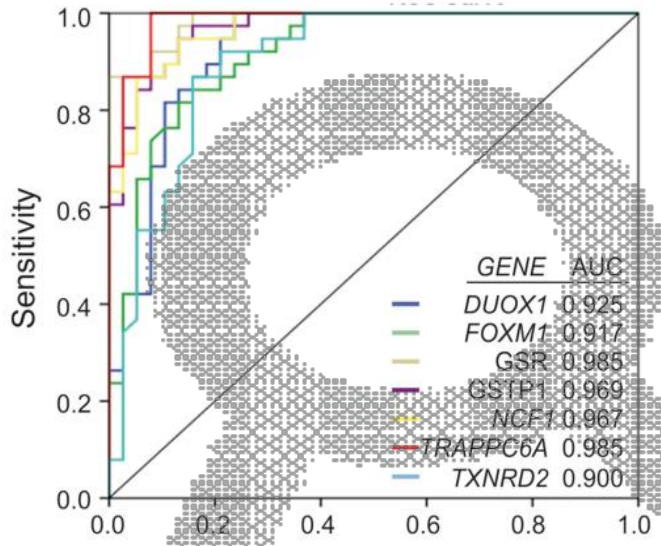

Mild AD
CTRL
NFKB-NRF2 crosstalk
NFKBIA
RELA
RELB
r=0.663
p=<0.001
r=0.651
p=<0.001
r=0.622
p=<0.001
r=0.634
p=<0.001
r=0.677
p=<0.001
r=0.643
p=<0.001
r=0.808
p=<0.001
r=0.788
p=<0.001
r=0.648
p=<0.001
GSR
GSTP1
SQSTM1
NRF2 gene targets

Receiver Operating Curve

Inflammation genes



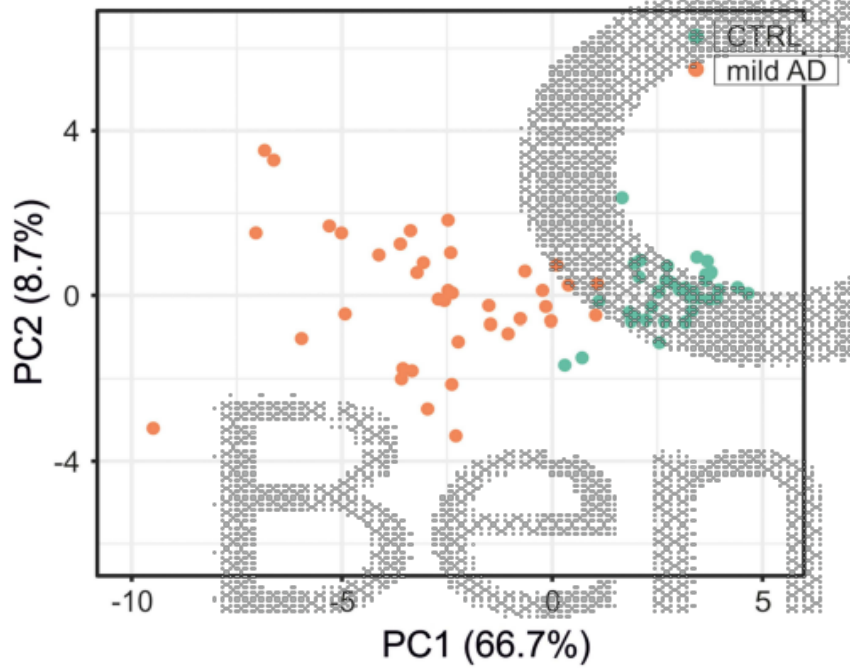
Redox genes



Genes (FC > 2, p < 0.001, AUC : 0.9) discriminating between mild AD and age-matched controls

Redox genes

- ROS producers: **NCF1, DUOX1**
- **NRF2 targets: GSR, GSTP1**
- Other antioxidant mechanisms: TXNRD2
- DNA damage and mitosis: **FOXM1**
- Pathway signature: **TRAPPC6A**



Principal Component Analysis

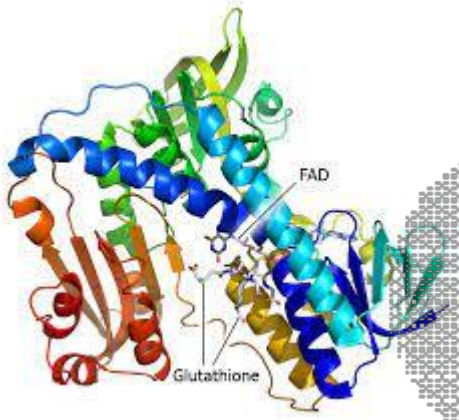
Inflammation genes

- Core NFkB genes: **RELB, NFKB2**
- Apoptosis-related genes: **TNFRSF1B, TP53**
- NFkB-related signaling pathways: **AKT1, STAT5B, TBK1**
- Immunity genes: **IL2RA, CSF2RB**

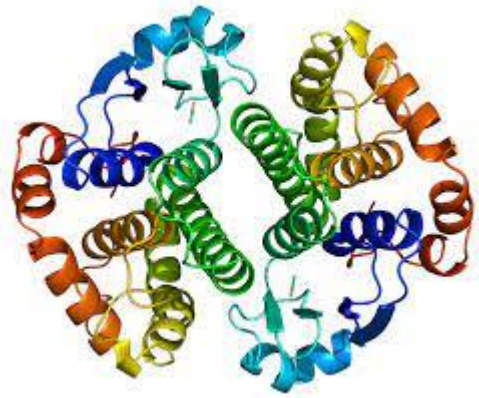
Genes that are relevant also in the AD brain (GSE122063)

Conclusion

Blood leukocytes are “primed” in the blood of mild AD patients.



GSR **GSTP1**



Candidate NRF2 biomarkers
in the blood of mild AD patients

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Journal of Inflammation Research

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ORIGINAL RESEARCH

Whole Blood Expression Pattern of Inflammation and Redox Genes in Mild Alzheimer's Disease

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Ana I Rojo[2–5]
José Jiménez-Villegas[2,3]
Estibaliz Capetillo-Zarate[5–7]
Carlos Matute[6,7]
Gerard Piñol-Ripoll[8]
Gina Manda[1]
Antonio Cuadrado[1–5]

Background: Although Alzheimer's disease (AD) is associated with alterations of the central nervous system, this disease has an echo in blood that might represent a valuable source of biomarkers for improved diagnosis, prognosis and for monitoring drug response.

Methods: We performed a targeted transcriptomics study on 38 mild Alzheimer's disease (AD) patients and 38 matched controls for evaluating the expression levels of 136 inflammation and 84 redox genes in whole blood. Patients were diagnosed as mild AD based on altered levels of total TAU, phospho-TAU and $Abeta_{(1-42)}$ in cerebrospinal fluid, and $Abeta_{(1-40)}$, $Abeta_{(1-42)}$ and total TAU levels in plasma. Whenever possible, blood and brain comparisons were made using public datasets.

Results: We found 48 inflammation and 34 redox genes differentially expressed in the blood of AD patients vs controls (FC >1.5, $p < 0.01$), out of which 22 pro-inflammatory and 12 redox genes exhibited FC >2 and $p < 0.001$. Receiver operating characteristic (ROC) analysis identified nine inflammation and seven redox genes that discriminated between AD patients and controls (area under the curve >0.9). Correlations of the dysregulated inflammation and redox transcripts indicated that *RELA* may regulate several redox genes including *DUOX1* and *GSR*. Based on the gene expression profile, we have found that the master regulators of inflammation and redox homeostasis, NFκB and NRF2, were significantly disturbed in the blood of AD patients, as well as several zinc finger and helix-loop-helix transcription factors.

Conclusion: The selected inflammation and redox genes might be useful biomarkers for monitoring anti-inflammatory therapy in mild AD.

Keywords: oxidative stress, neuroinflammation, gene expression, dementia, NRF2, NFkappaB

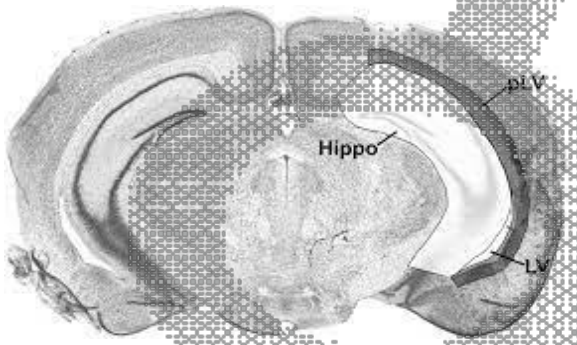
https://pubmed.ncbi.nlm.nih.gov/34848989/

The animal model

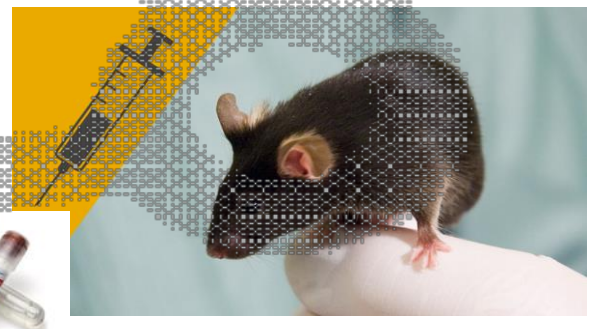
APP^{V717I} and TAU^{P301L} transgenic mice that recapitulate with aging a combined amyloid and TAU pathology.

Biologic samples

Hippocampus



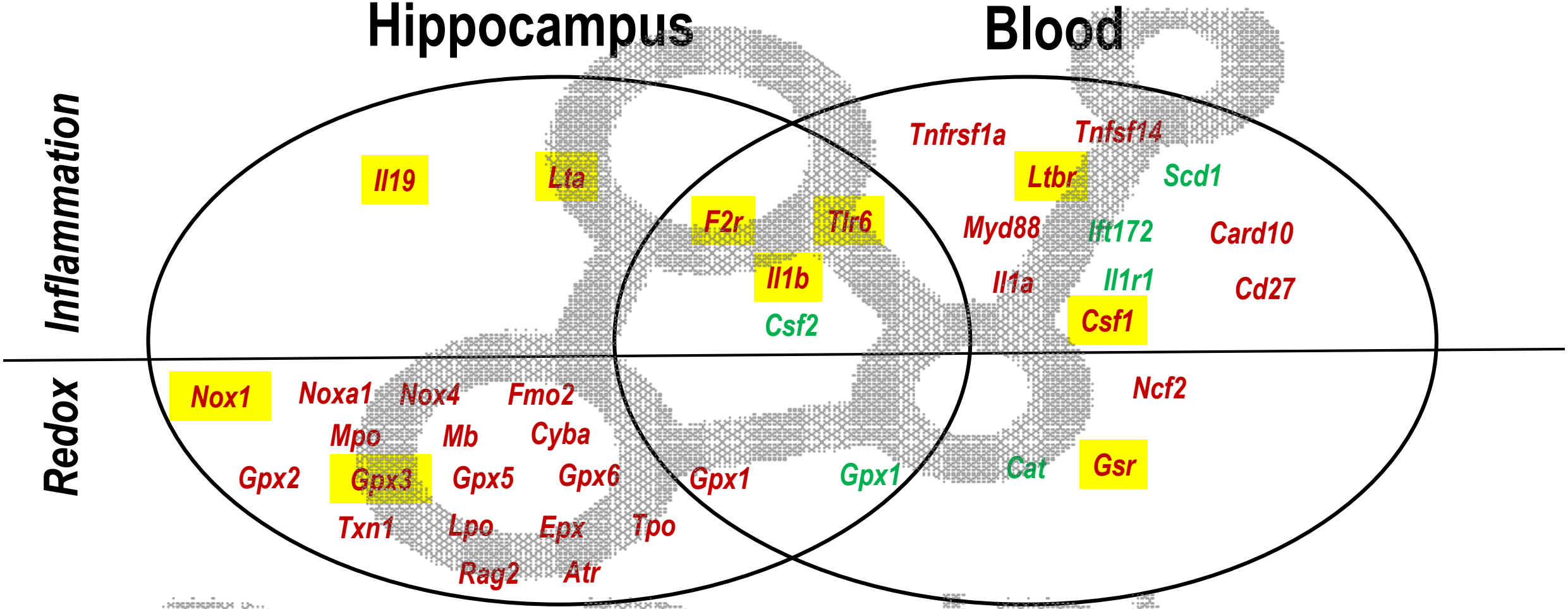
Blood



Investigations

Expression levels of 84 inflammation and 84 redox genes in AD mice (n=10) *vs* age-matched controls (n=8).

How much do these AD mice mimic the human disease in terms of NRF2, inflammation and redox biomarkers in the hippocampus and blood?”

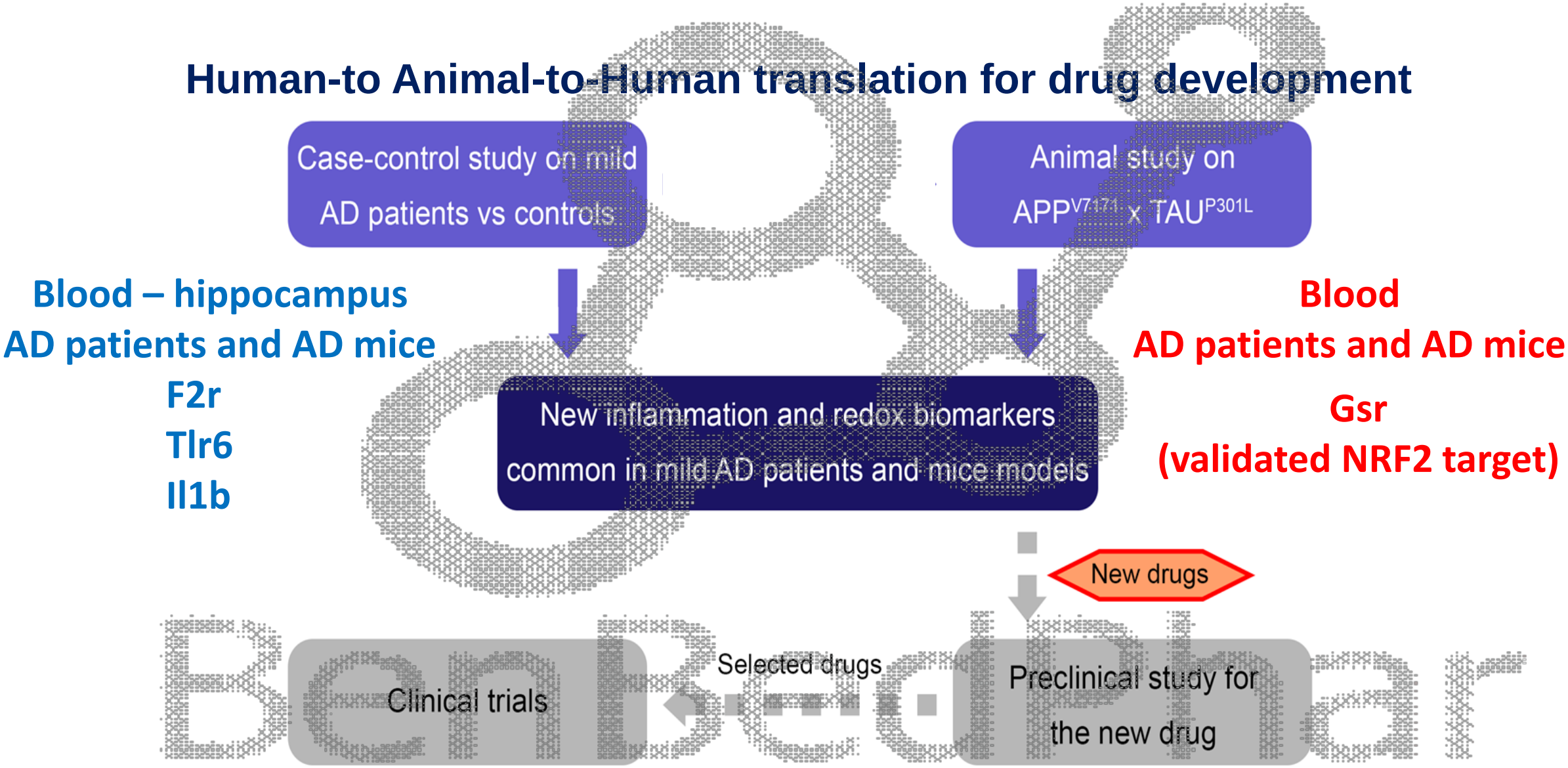

Hippocampus
Blood
Inflammation
Redox
Il19
Lta
F2r
Tlr6
Il1b
Csf2
Tnfrsf1a
Tnfsf14
Ltbr
Scd1
Myd88
Ift172
Card10
Il1a
Il1r1
Cd27
Csf1
Nox1
Noxa1
Nox4
Fmo2
Mpo
Mb
Cyba
Gpx2
Gpx3
Gpx5
Gpx6
Gpx1
Gpx1
Cat
Gsr
Ncf2
Txn1
Lpo
Epx
Tpo
Rag2
Atr
Red font – up-regulated genes, FC > 1.8, p < 0.05
Green font – down-regulated genes, FR < - 1.8, p <0.05
Genes with the same evolution trend in AD patients and mice

Validation of the GSR gene over-expression in the blood of AD mice

Gene	AD mice blood				Mild AD patients blood	
	21 AT vs. 12 WT		7 AT vs. 8 WT		38 MCI vs. 38 CTRL	
	48.5 ± 4.9 weeks		37.1 ± 0.8 weeks			
	FR	*p-value*	*FR*	*p-value*	*FR*	*p-value*
Gsr	2.08	<0.001	1.70	0.001	3.93	<0.001
Osgin1	2.72	<0.001	1.69	0.001	-	

Conclusion

Human-to Animal-to-Human translation for drug development



International Journal of
Molecular Sciences

MDPI

Article

Altered Blood and Brain Expression of Inflammation and Redox Genes in Alzheimer's Disease, Common to $APP^{V717I} \times TAU^{P301L}$ Mice and Patients

Catalina Anca Cucos [1,†], Elena Milanesi [1,†], Maria Dobre [1], Ioana Andreea-Musat [2], Gina Manda [1,*] and Antonio Cuadrado [1,3,4,5,*]

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Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), 28029 Madrid, Spain
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† These authors contributed equally to this work.

Abstract: Despite extensive research, the pathophysiology of Alzheimer's disease (AD) is still not fully understood, and currently there are no effective treatments. Therefore, there is an unmet need for reliable biomarkers and animal models of AD to develop innovative therapeutic strategies addressing early pathologic events such as neuroinflammation and redox disturbances. The study aimed to identify inflammatory and redox dysregulations in the context of AD-specific neuronal cell death and DNA damage, using the $APP^{V717I} \times TAU^{P301L}$ (AT) mouse model of AD. The expression of 84 inflammatory and 84 redox genes in the hippocampus and peripheral blood of double transgenic AT mice was evaluated against age-matched controls. A distinctive gene expression profile in the hippocampus and in the blood of AT mice was identified, addressing DNA damage, apoptosis and thrombosis, complemented by inflammatory factors and receptors along with ROS producers and antioxidants. Gene expression dysregulations that are common to AT mice and AD patients guided the most relevant blood candidate biomarkers. The identified inflammation and redox genes, common to AD patients and AT mice, might be valuable candidate biomarkers for preclinical drug development that could be readily translated to clinical trials.

Keywords: Alzheimer's disease; gene expression; inflammation; redox alterations; hippocampus; blood

Check for updates

Citation: Cucos, C.A.; Milanesi, E.; Dobre, M.; Musat, I.A.; Manda, G.; Cuadrado, A. Altered Blood and Brain Expression of Inflammation and Redox Genes in Alzheimer's Disease, Common to $APP^{V717I} \times TAU^{P301L}$ Mice and Patients. *Int. J. Mol. Sci.* **2022**, *23*, 5799. https://doi.org/10.3390/ijms23105799

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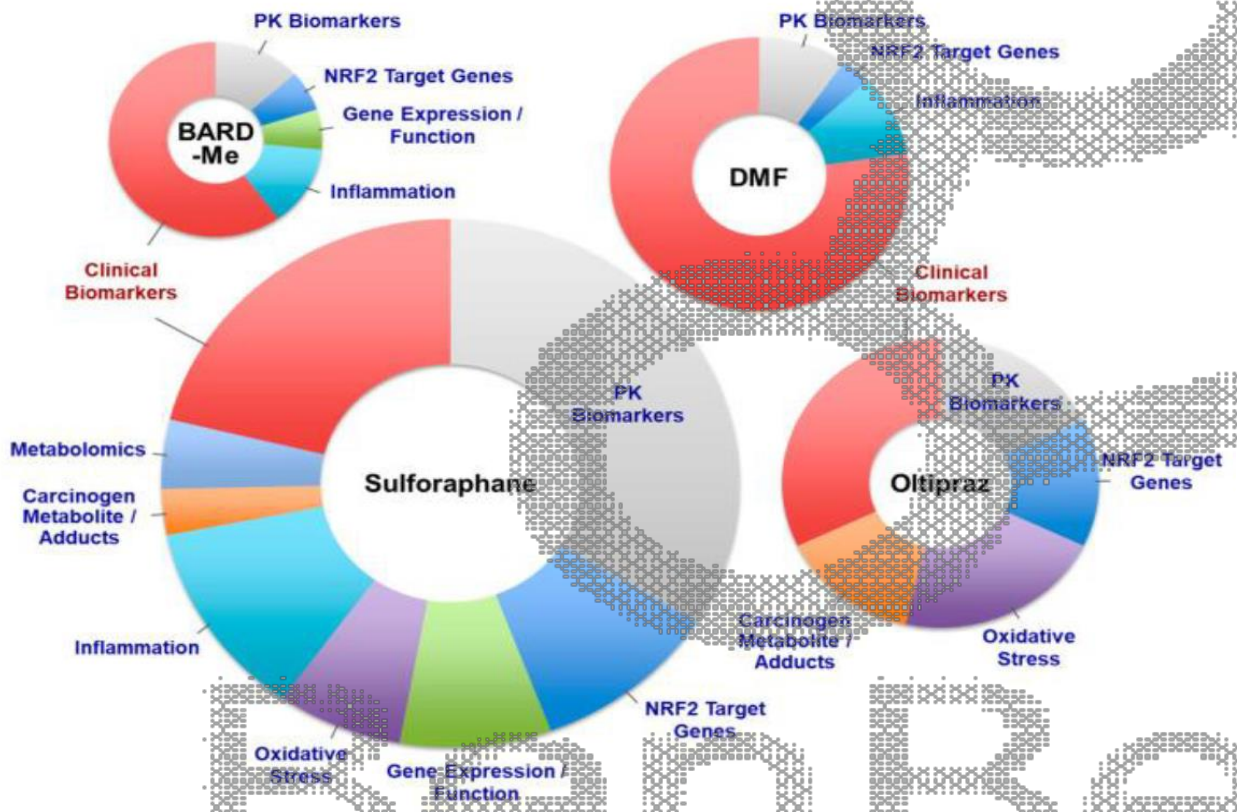
Published: 23 May 2022

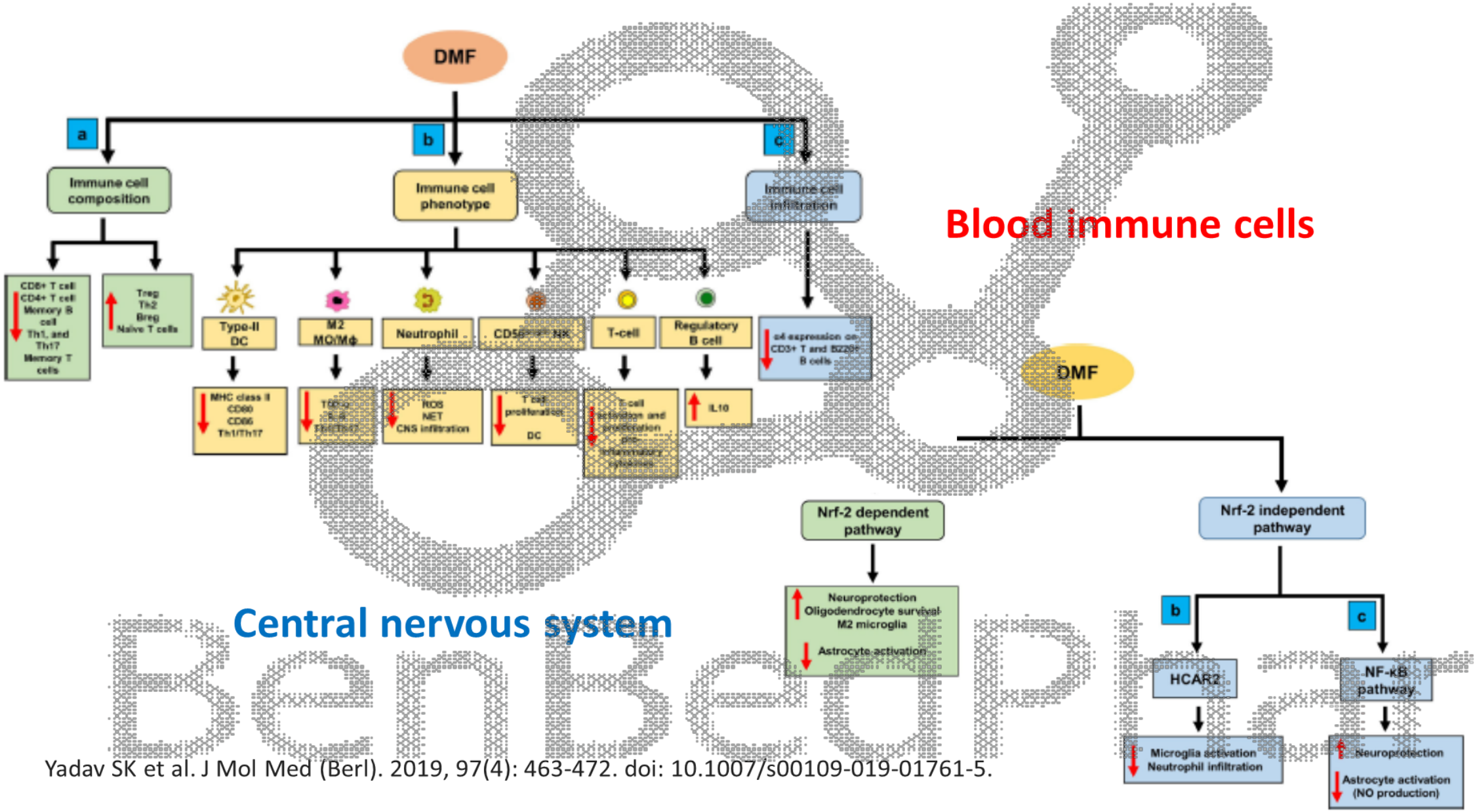
IV. Clinical trials on NRF2 activators



Predicting a clinical end-points and treatment effects in various diseases

BenBedPhar

The NRF2 pathway as biomarker for dimethyl fumarate treatment in relapsing – remitting multiple sclerosis



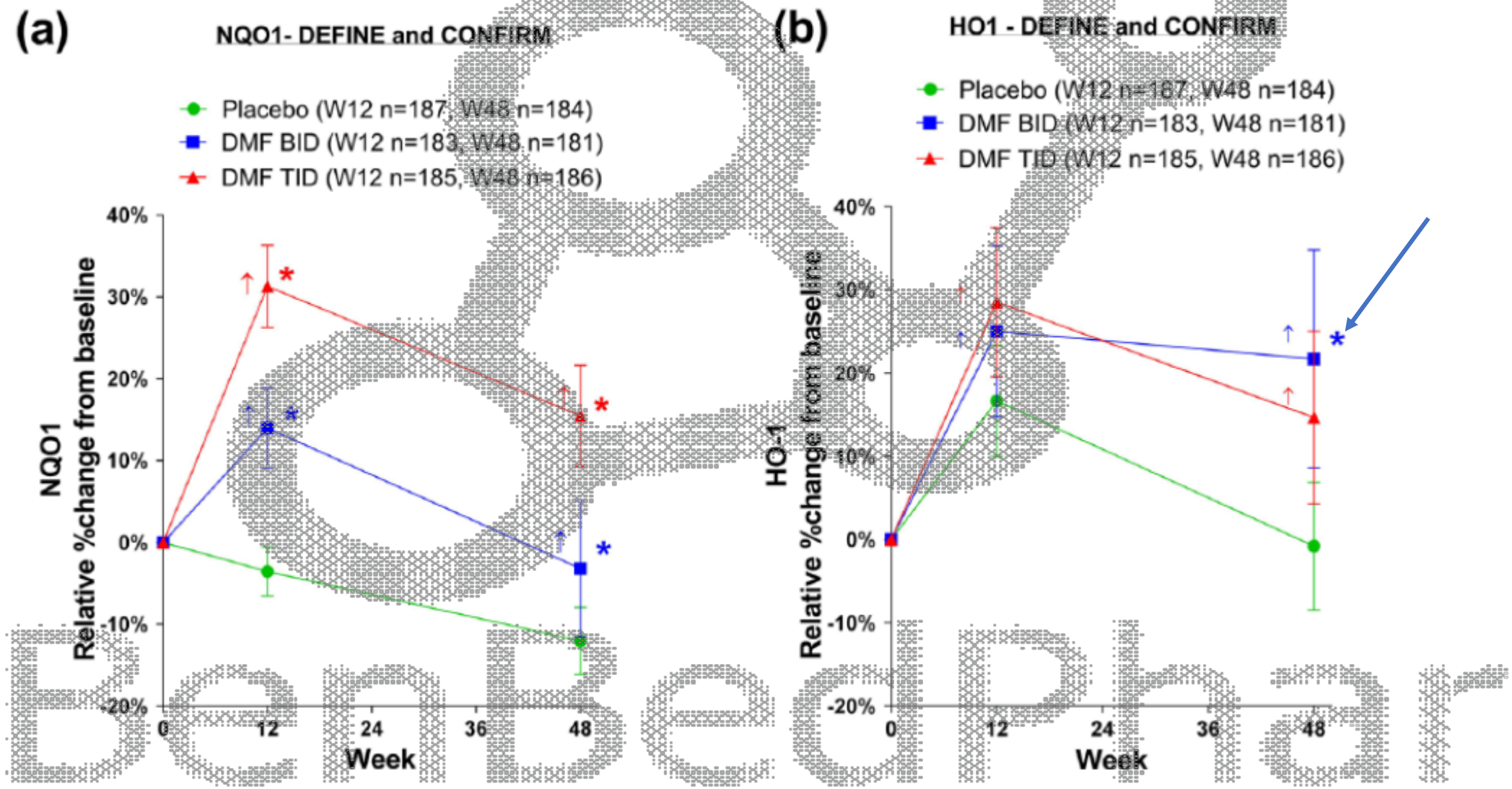
Yadav SK et al. J Mol Med (Berl). 2019, 97(4): 463-472. doi: 10.1007/s00109-019-01761-5.

Phase 3 CONFIRM and DEFINE studies on DMF-treated MS patients *vs* placebo

Characteristic	Placebo (*N*=200)	DMF BID (*N*=200)	DMF TID (*N*=200)
Age (years)	38.7±9.2[b]	38.9±9.3	39.4±8.7
Female sex, no. (%)	135 (68)[c]	137 (69)	157 (79)
Weight (kg)	76.7±18.2	76.2±20.3	74.2±18.5
Race,[d] no. (%)			
White	175 (88)	178 (89)	175 (88)
Other	25 (13)	22 (11)	25 (13)
Time since diagnosis (years)	5.3±5.8	5.3±5.5	5.1±5.4
Subjects who took any prior MS medication,[e] no. (%)	103 (52)	97 (49)	96 (48)
Relapses in previous 12 months, no.	1.3±0.8	1.4±0.7	1.4±0.6
EDSS score at baseline,[f] no. (%)			
0.0	12 (6)	14 (7)	11 (6)
1.0 or 1.5	52 (26)	59 (30)	50 (25)
2.0 or 2.5	47 (24)	59 (30)	69 (35)
3.0 or 3.5	57 (29)	37 (19)	38 (19)
4.0 or 4.5	28 (14)	25 (13)	26 (13)
5.0	4 (2)	5 (3)	6 (3)
Mean score on EDSS	2.4±1.2	2.3±1.2	2.3±1.2

Phase 3 CONFIRM and DEFINE studies on DMF-treated MS patients *vs* placebo

qRT-PCR on blood samples collected in PAXgene tubes; B2M - reference gene



Gopal S et al. Mult Scler. 2017, 23(14): 1875-1883. doi: 10.1177/1352458517690617.

Additional proof on NRF2 activation in DMF-treated MS patients

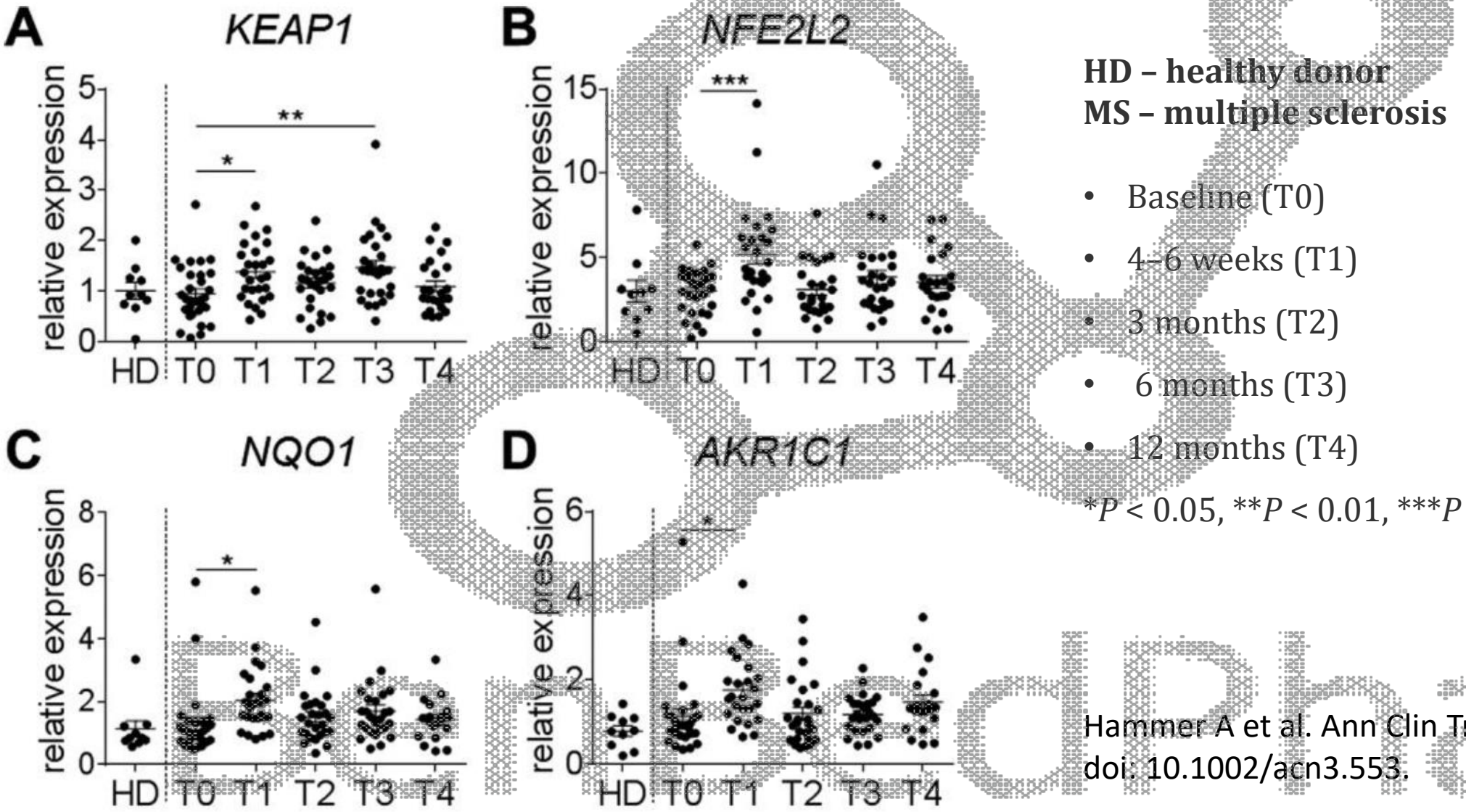
Cohort characteristic	Patient cohort (n=43)
Age, mean (SD)	36.3 (11.7)
Female, n (%)	31 (72.1)
Newly diagnosed within previous year, n (%)	19 (44.2 %)
Patients with prior treatment, n (%)	17 (39.5 %)
- Interferon β, n (%)	11 (25.6 %)
- Glatiramer acetate, n (%)	6 (13.9 %)
Patients with relapses in prior year, n (%)	30 (69.8 %)
EDSS score, median (IQR)	1.5 (1.0)

12 months therapy with Tecfidera®

- 22 of 28 (78.6%) patients showed no new MRI lesions
- 19/28 (67.9%) patients had no relapses
- EDSS (Expanded Disability Status Scale) remained stable in 16 out of 28 (57.1%) patients
- 9 of 28 (32.1%) patients gained No Evidence of Disease Activity (NEDA) status

Hammer A et al. Ann Clin Transl Neurol 2018, 5(6):668-676. doi: 10.1002/acn3.553.

DMF induces transiently an early transcriptional activity of NRF2 in PBMCs from HD (n=9–11) and DMF-treated MS patients (n=25–30)



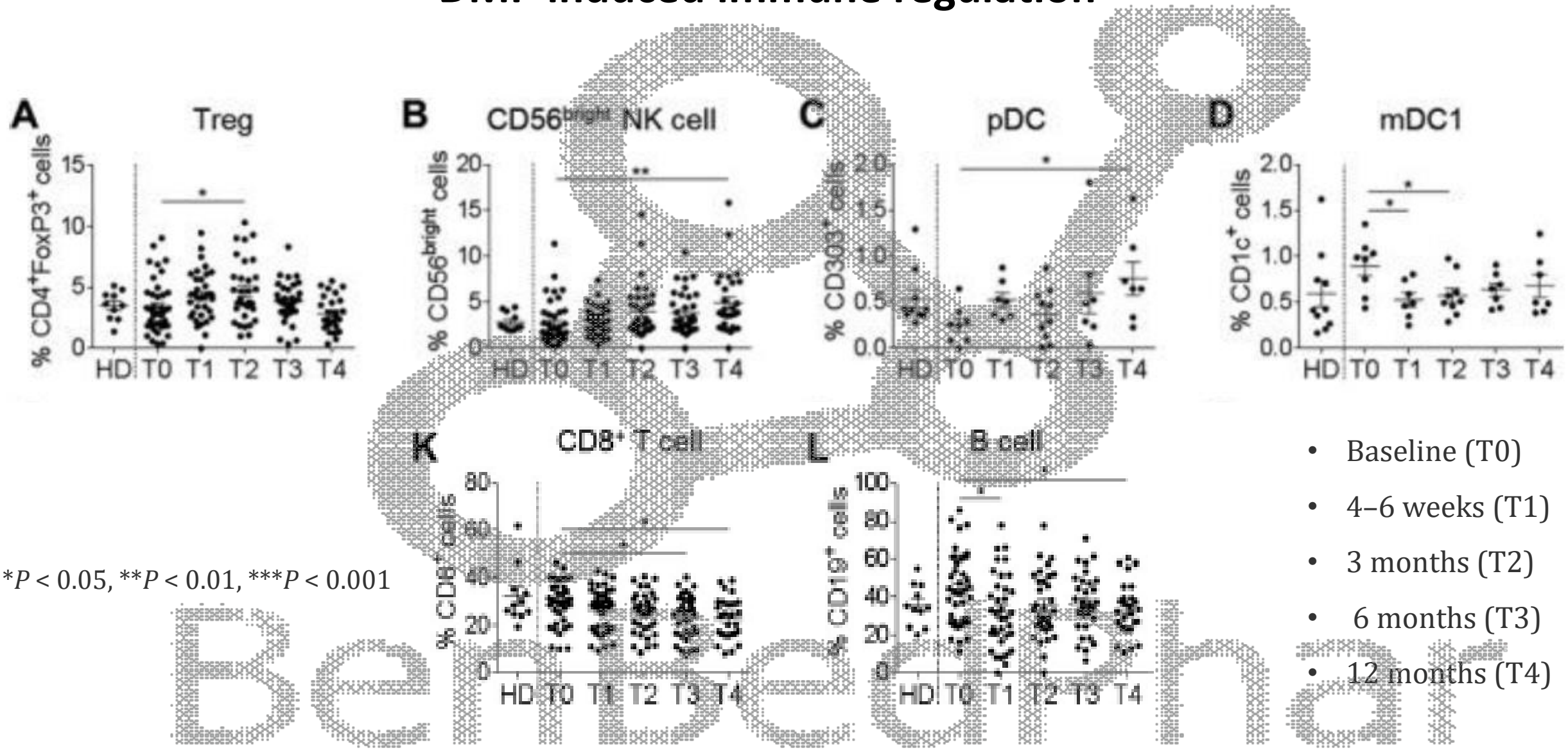
HD – healthy donor
MS – multiple sclerosis

- Baseline (T0)
- 4–6 weeks (T1)
- 3 months (T2)
- 6 months (T3)
- 12 months (T4)

$*P < 0.05$, $**P < 0.01$, $***P < 0.001$

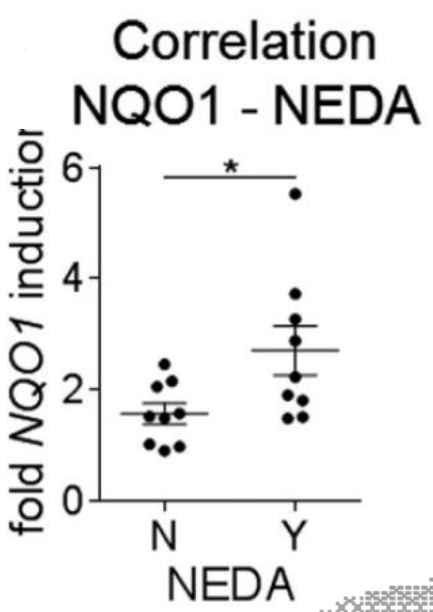
Hammer A et al. Ann Clin Transl Neurol.
doi: 10.1002/acn3.553.

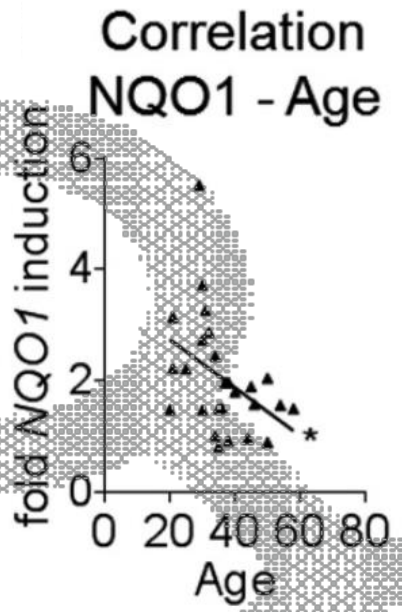
DMF-induced immune regulation

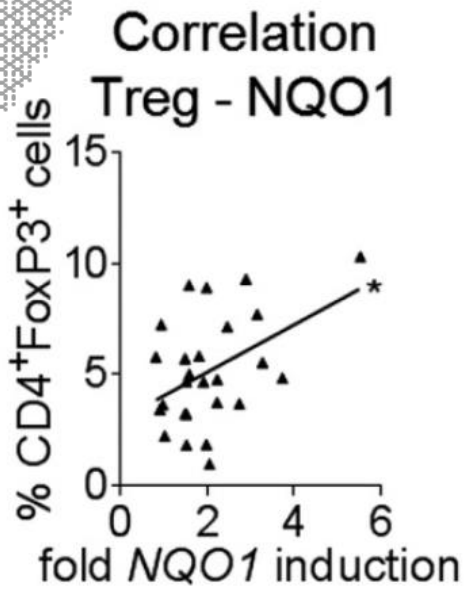


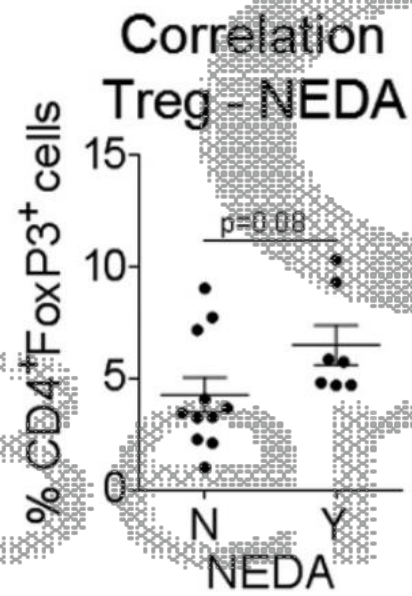
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

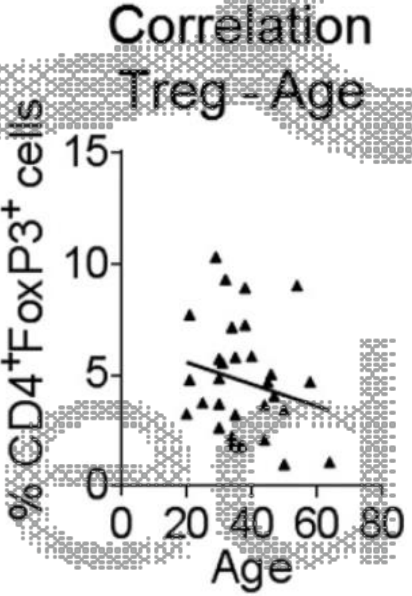
- Baseline (T0)
- 4–6 weeks (T1)
- 3 months (T2)
- 6 months (T3)
- 12 months (T4)









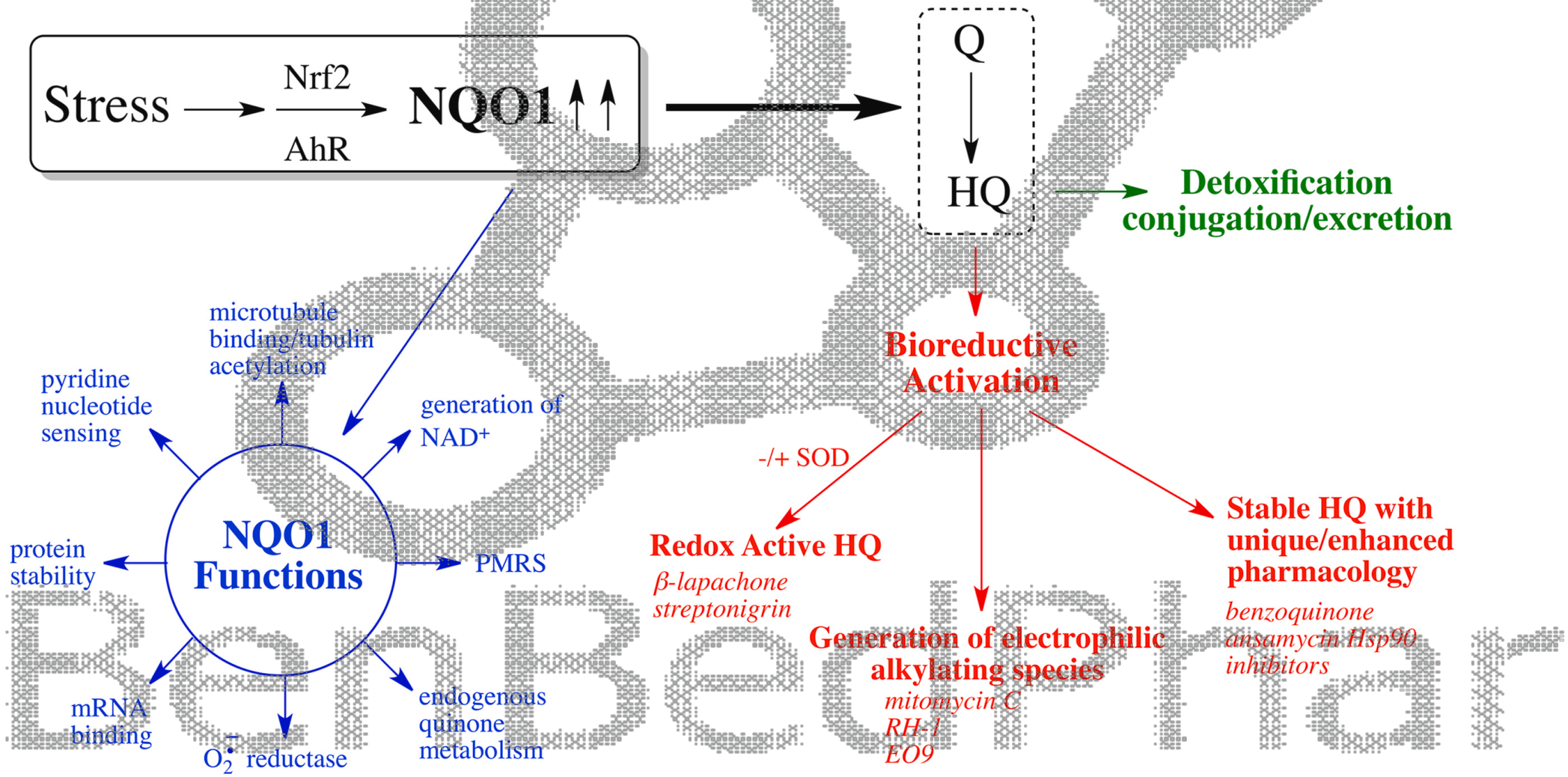


NEDA
No Evidence of Disease Activity

Ross D & Siegel D

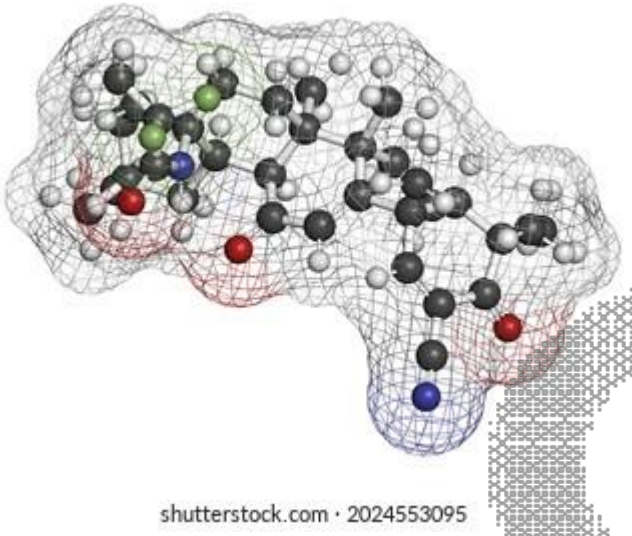
The diverse functionality of NQO1 and its roles in redox control.

Redox Biol. 2021 May;41:101950. doi: 10.1016/j.redox.2021.101950.



Highlight

A new player in the NRF2 pharmaceutics



Omaveloxolone (Skyclarys, Reata's Pharmaceutical's),

a semisynthetic oleanane triterpenoid that potently activates NRF2

was approved by FDA in February 2023

as the first treatment of Friedreich's ataxia,

a rare autosomal recessive degenerative disorder

characterized by an abnormal form of the frataxin gene and protein

that leads to important mitochondrial dysfunction, oxidative stress and

defective antioxidant defence due to the suppression of NRF2 signaling.

↓

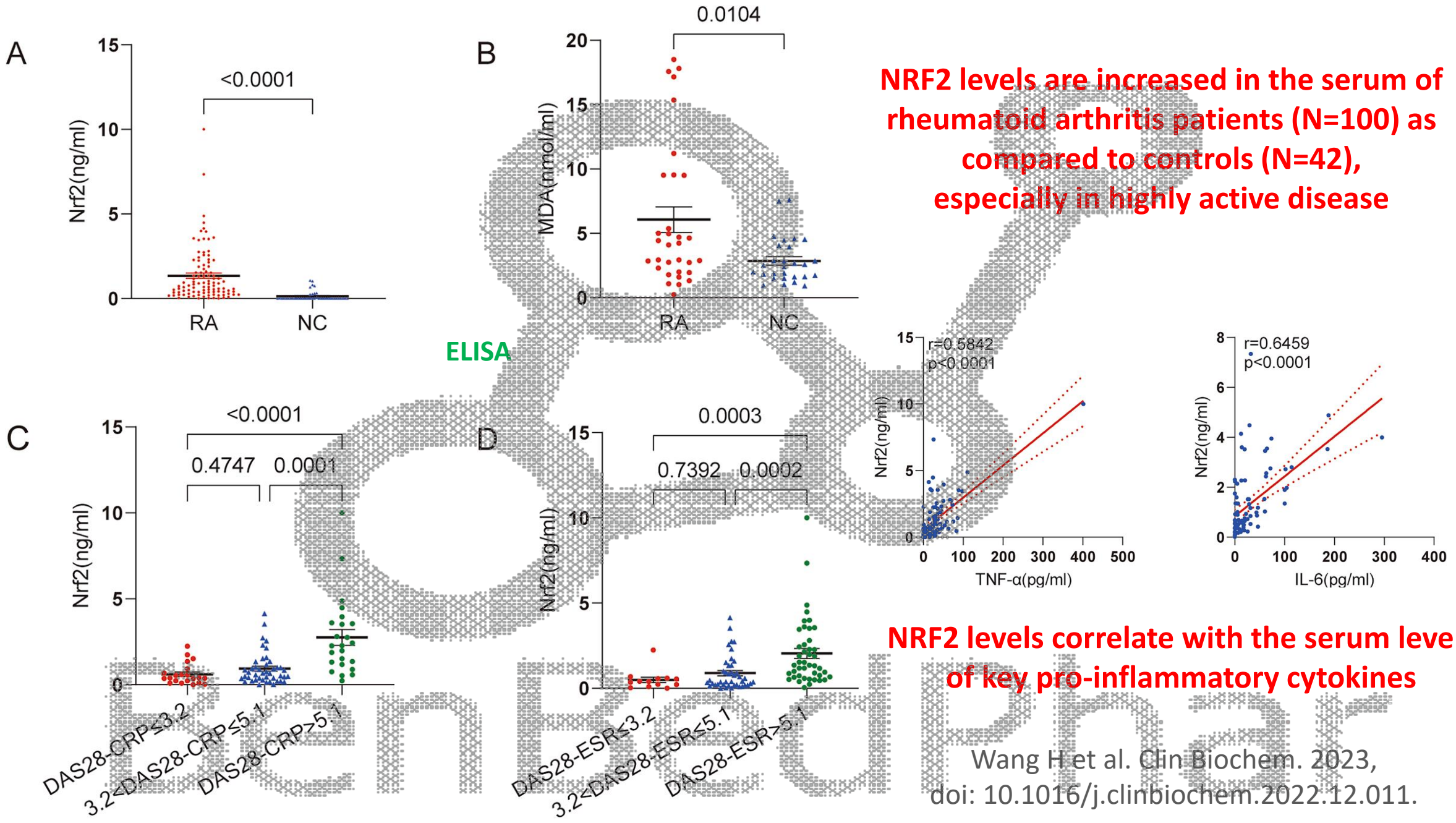
KEAP1 - Cys 151

↓

NRF2 activation

V. NRF2 levels in serum or plasma

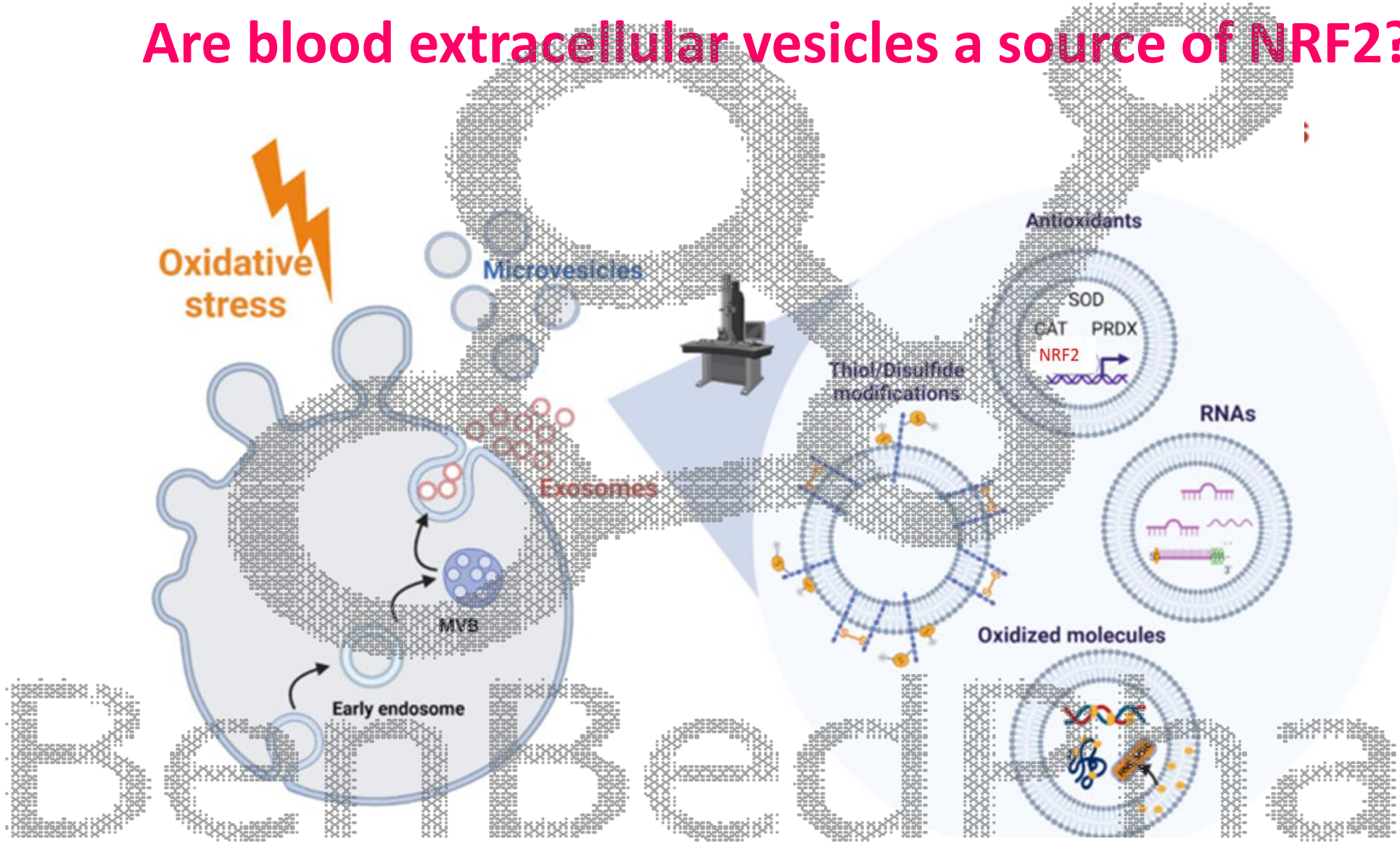
BenBedPhar



Wang H et al. Clin Biochem. 2023,
doi: 10.1016/j.clinbiochem.2022.12.011.

Where does serum/plasma NRF2 come from?

Are blood extracellular vesicles a source of NRF2?



Saeed-Zidane M et al. PLoS ONE, doi: 10.1371/journal.pone.0187569.

Zhang W et al. Oxid Med Cell Longev. 2022, 2022: 3553-3617. doi: 10.1155/2022/3553617.

Kahroba H et al. Biochimie. 2020, 171-172:103-109. doi: 10.1016/j.biochi.2020.02.011.

The story continues on June 29, 2023 with my talk on

Cancer and Radiotherapy

in the context of NRF2