

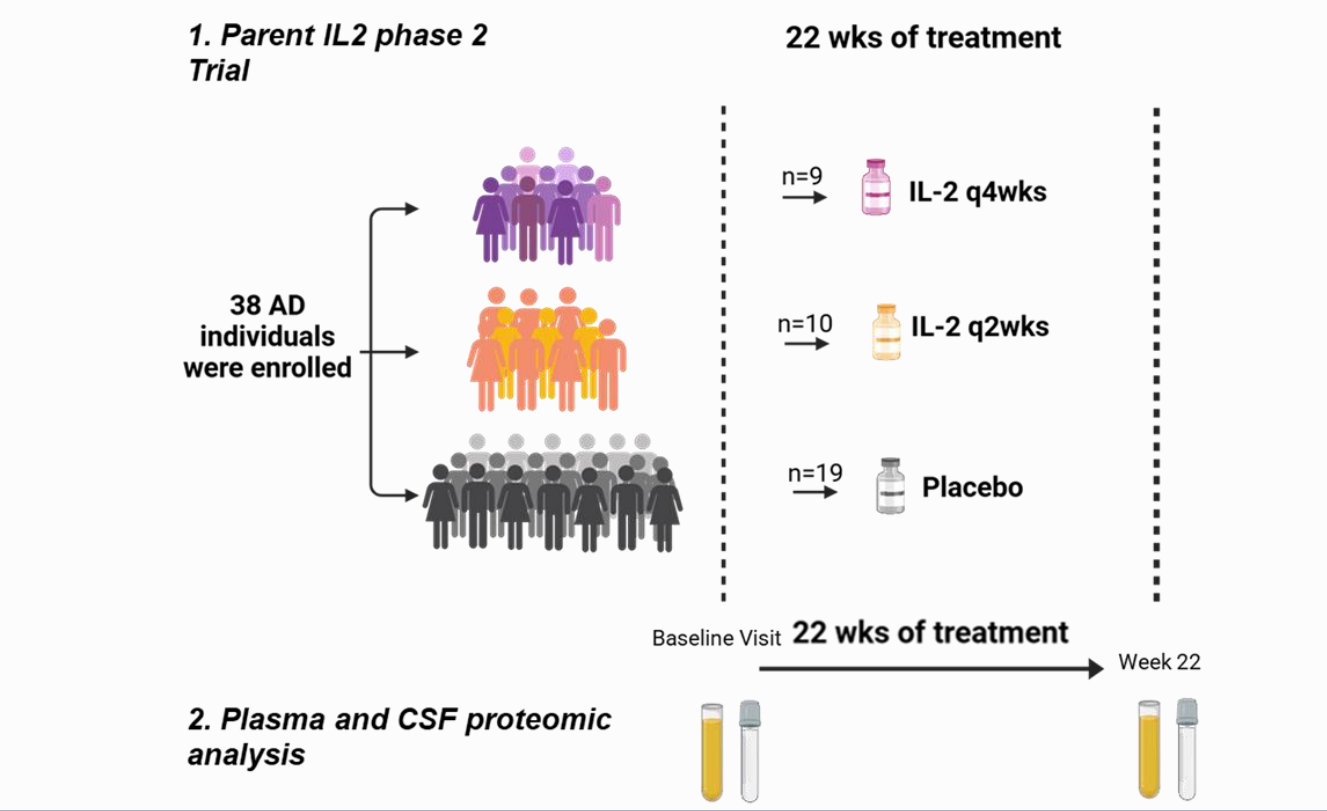
Frequency-dependent plasma and CSF proteomic signatures reveal distinct molecular responses to low-dose IL-2 treatment in Alzheimer’s disease

BACKGROUND

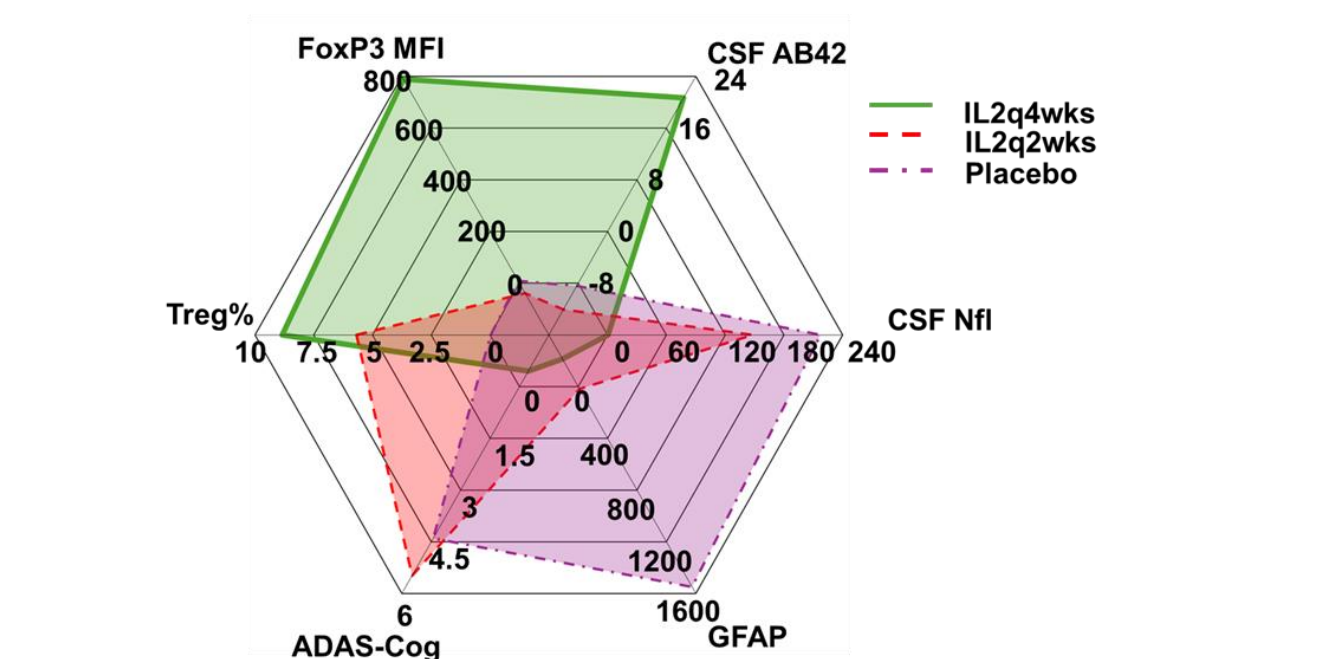
Low-dose interleukin-2 (IL-2) has emerged as a targeted immunomodulatory strategy designed to selectively expand regulatory T cells (Tregs) in Alzheimer’s disease (AD). However, the molecular mechanisms underlying IL-2-mediated effects in systemic and central nervous system (CNS) compartments, and the optimal dosing frequency to achieve benefit, remain unclear.

METHODS

This study was to analyze the proteomics of plasma and CSF from AD patients enrolled in a phase 2a, randomized, double-blinded, placebo-controlled trial as described earlier (Faridar et al, 2025). A total of 38 participants were aged 50-86 years with MMSE scores ranging from 12 to 26 were enrolled in the original trial. Enrolled AD patients were randomized into three arms: (i) five-day courses of low-dose IL-2 (10^6 IU/day) SC every 4 weeks (IL2q4wks); (ii) the same five-day courses of subcutaneous IL-2 (10^6 IU/day) administered every 2 weeks (IL2q2wks); and (iii) placebo for 22 weeks. All participants underwent lumbar puncture for CSF collection and blood draw for plasma collection at the screening visit and following completion of the treatment phase at week 22. We performed comprehensive plasma and cerebrospinal fluid (CSF) proteomic profiling (5,416 analytes; Olink Explore platform) on plasma and CSF samples. Differentially expressed protein analysis, protein-protein interaction networks, pathway and cell-type enrichment analyses were conducted to identify key molecular alterations and associated CNS cell populations.



ORIGINAL TRIAL FINDINGS

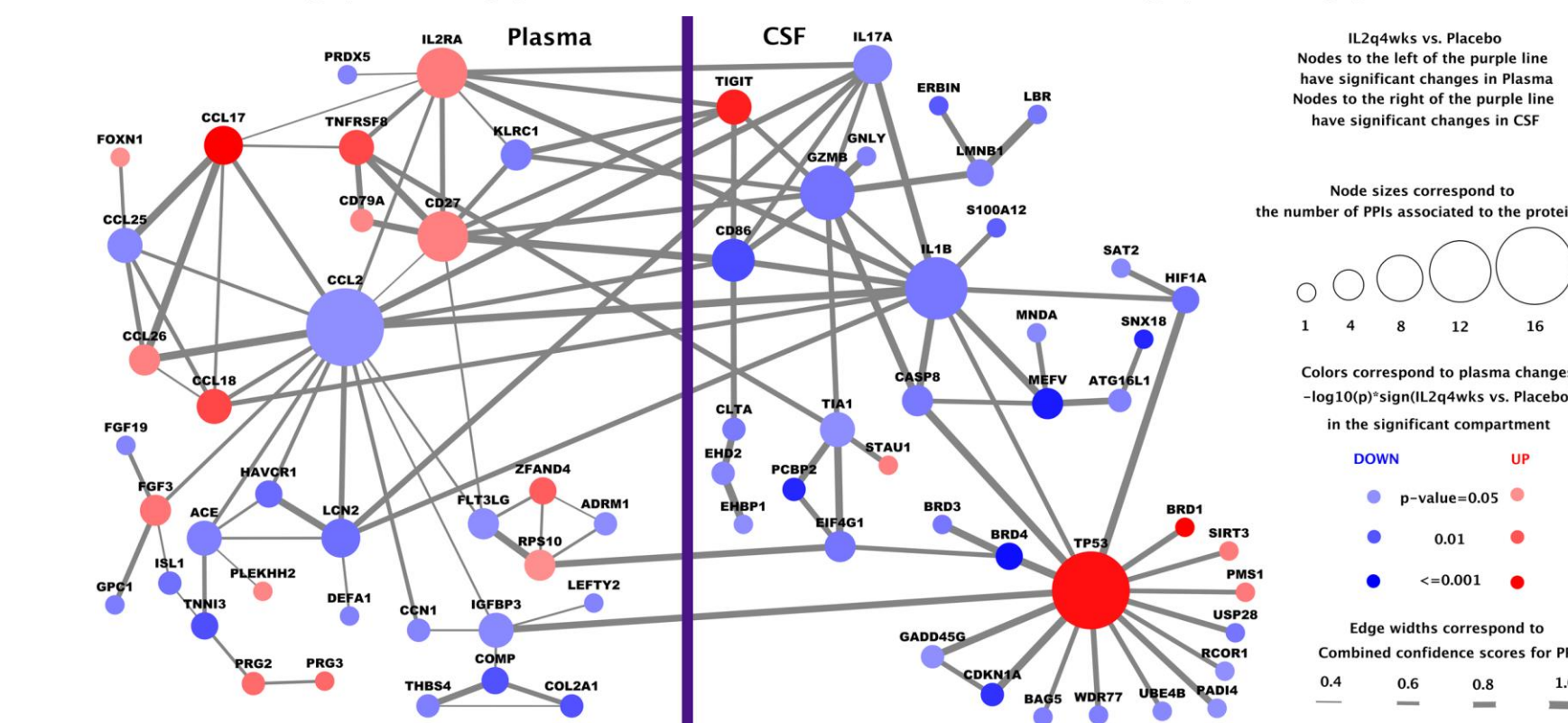
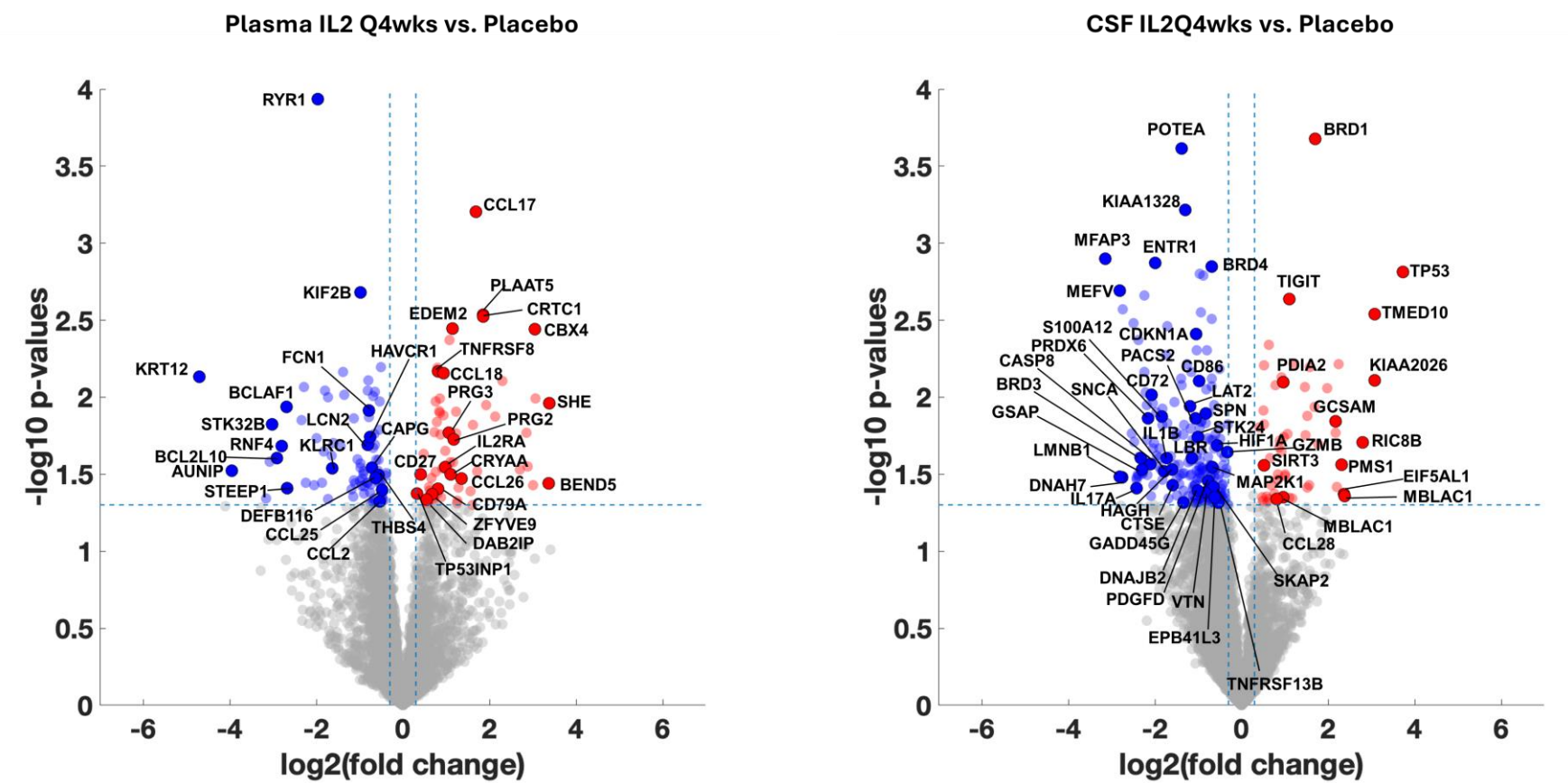


Arms	Treg%	FoxP3 MFI	CSF AB42	CSF NFL	CSF GFAP	ADAS-Cog
IL2q4wks	8.84±0.734**	788.7±106.6**	20.66±11.65*	0.48±89.92*	-214.1±139.91	-0.45±2.62*
IL2 q2wks	5.68±0.66**	-38.8±97.85	-12.06±10.46	148.07±85.21	17.41±1297.49	5.479±2.10

Placebo	-0.03±0.2	9.01±74.23	-8.42±7.72	217.34±65.39	1548.9±982.68	4.40±1.48
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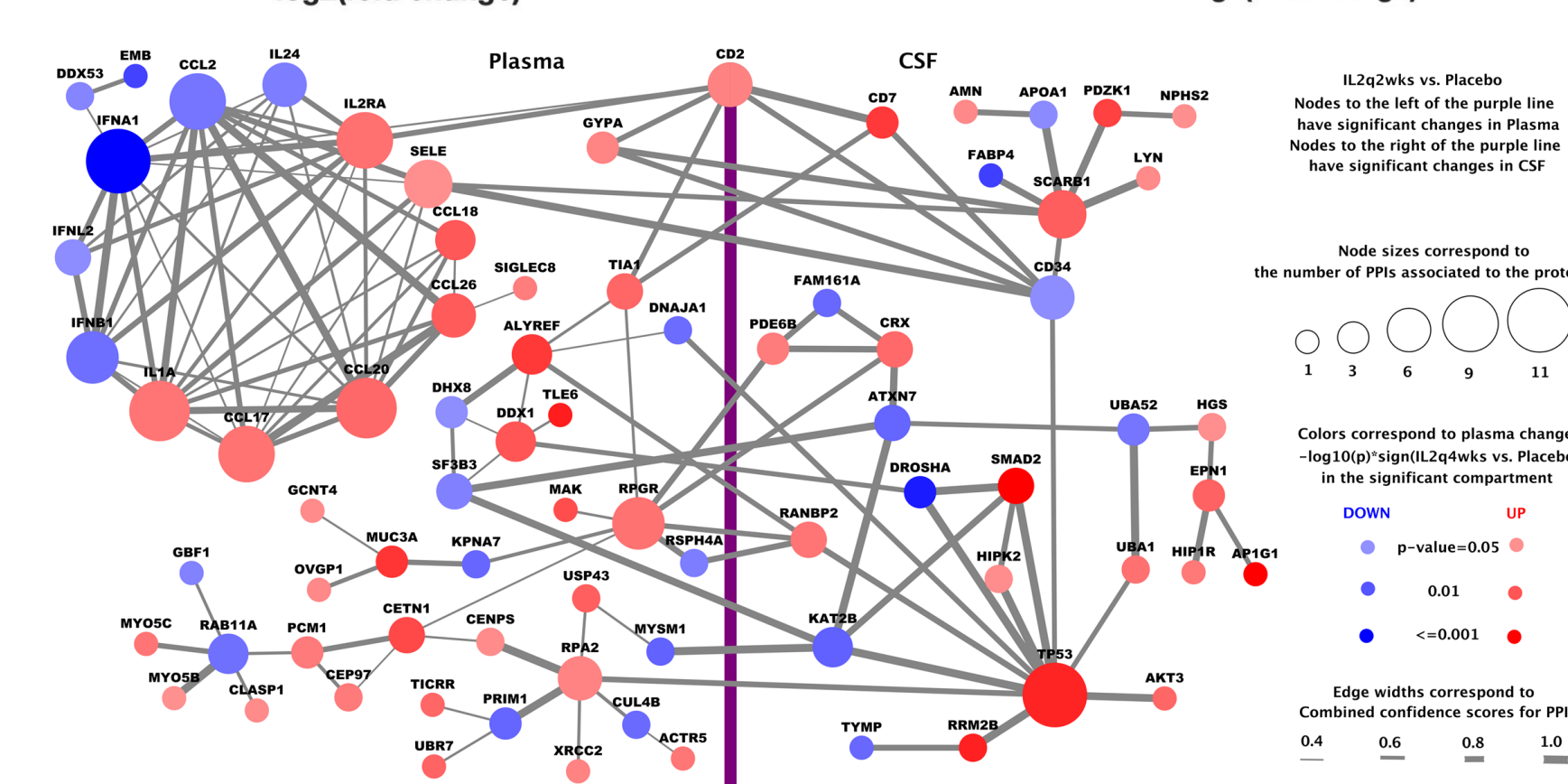
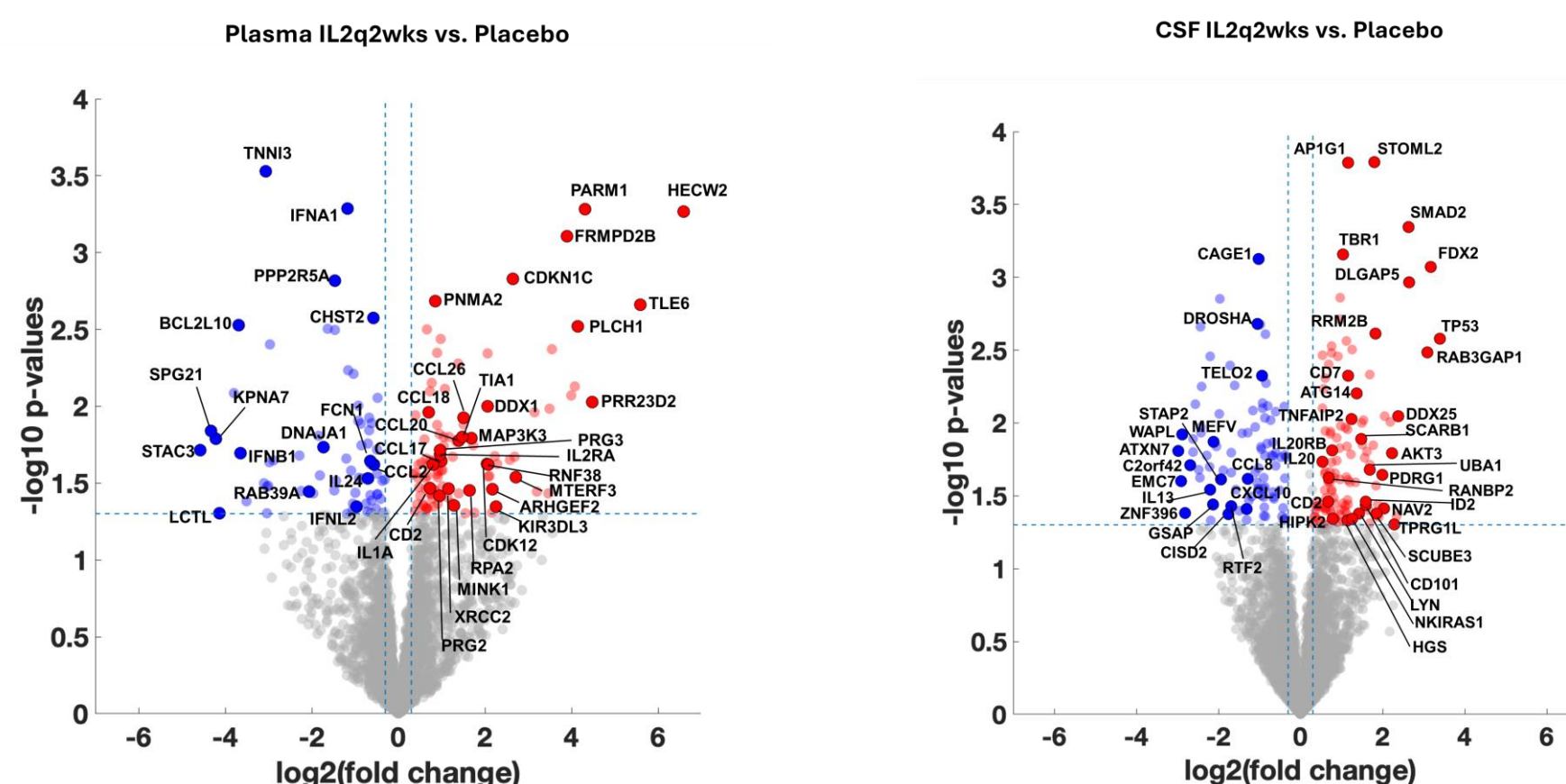
Participants in the IL2q4wks arm exhibited greater expansion of Tregs, higher FoxP3 mean fluorescence intensity (MFI), a significant increase in CSF A β ₄₂ concentrations, and trend toward stabilization of cognitive performance (ADAS-Cog scores) and CSF GFAP, NfL at week 22 compared with those receiving IL2q2wks dosing or placebo, relative to baseline

1. Differential plasma and CSF proteomic profiles following IL2q4wks treatment



- IL2q4wks administration suppressed plasma monocyte/lymphocyte activation chemokines and mediators, suggesting that IL2q4wks shifts the peripheral immune milieu from a pro-inflammatory, monocyte- and T-cell-activating/recruiting state toward a more regulatory, tolerance-promoting profile.
- CSF findings in the IL2q4wks arm indicate that this dosing schedule uniquely promotes a proteomic landscape characterized by suppression of the key inflammatory cytokine IL1B, other inflammasome-associated mediators (S100A12, MEFV), and additional pro-inflammatory/pro-apoptotic factors (GZMB, IL17A, CASP8, BAG5, CDKN1A, GADD45G, HIF1A, UBE4B, USP28).

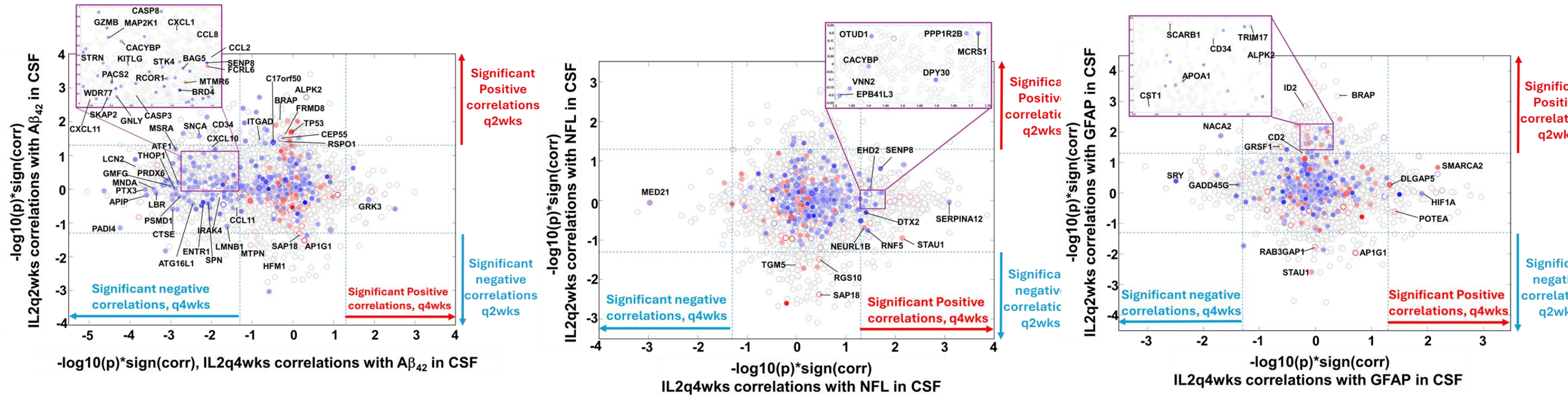
2. Differential plasma and CSF proteomic profiles following IL2q2wks treatment



- Immune mediators altered in the IL2q2wks plasma profile suggested a more mixed response, with suppression of pro-inflammatory CCL2 and IL24, increased expression of inflammation and leukocyte activation-associated molecules (ARHGEF2, CCL20, CD2, IL1A, SELE), and reduced expression of immunoregulatory or anti-inflammatory mediators (IFNB1, IFNL2/IL28A, NPY, NLRP14).
- The IL2q2wks schedule elicited a mixed CSF signature, characterized by insufficient suppression of inflammatory signaling and persistent cellular stress responses, paralleling their plasma proteomic findings.

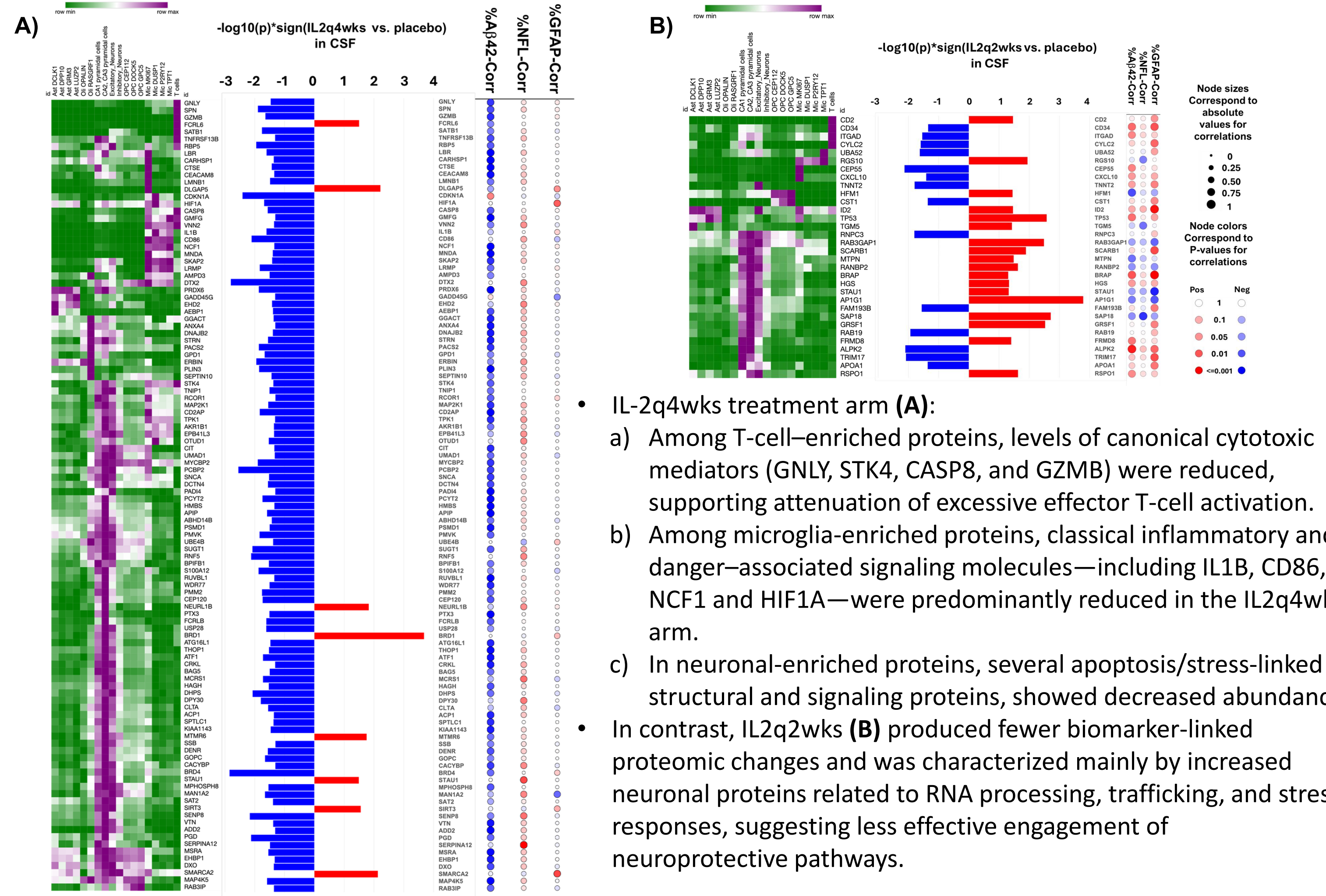
RESULTS

3. CSF proteomic correlation with AD core biomarker changes under IL-2 treatment

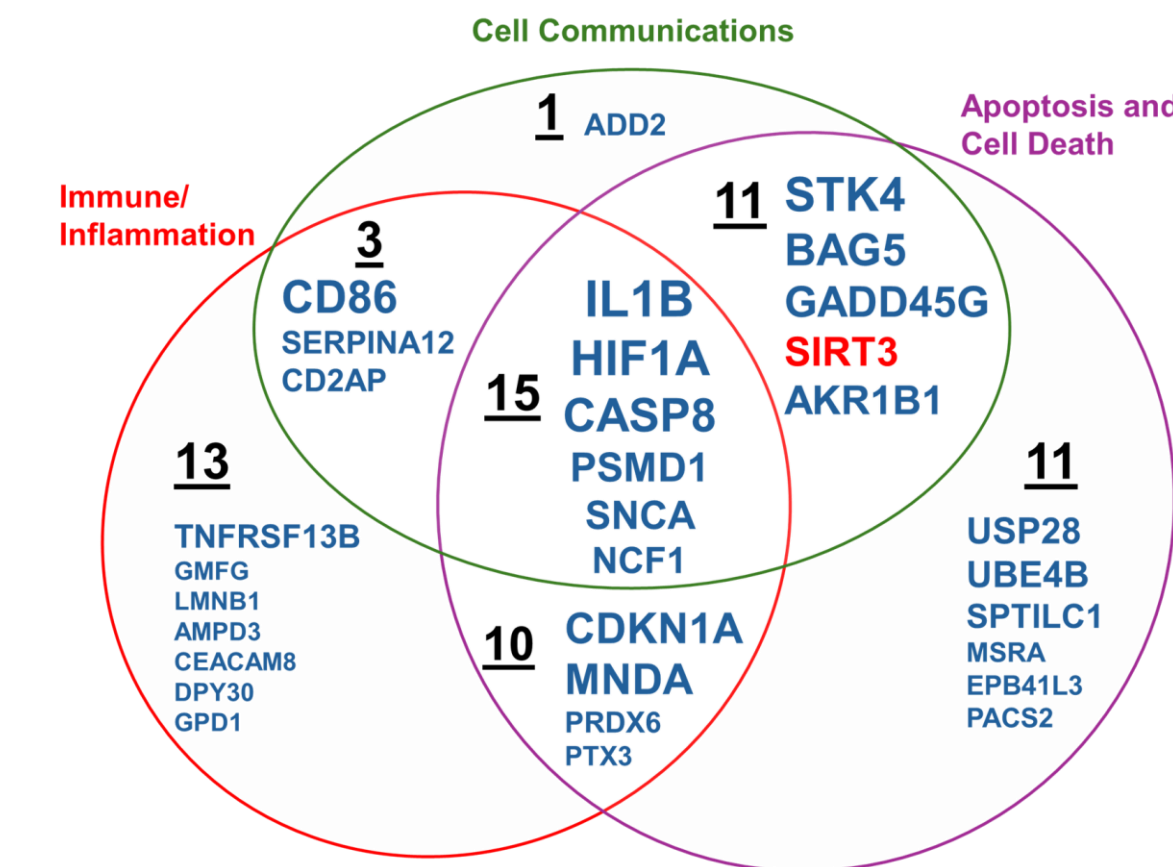


- IL2q4wks-associated suppression of specific CSF analytes inversely correlated with increases in CSF A β ₄₂, suggesting that IL2q4wks-induced proteomic recalibration aligns with improvements in amyloid pathology. In contrast, in the IL2q2wks arm, only four CSF DA proteins were negatively correlated with changes in CSF A β ₄₂.
- For NFL, IL2q4wks showed more positive correlations than negative correlations, whereas IL2q2wks exhibited fewer positives and more negatives.
- For GFAP, IL2q2wks displayed broader positive coupling with CSF analytes relative to IL2q4wks.

4. Cell-type enrichment of CSF proteomic alterations following IL2 treatment



5. Determining hub CSF proteomic signatures following IL2 treatment



- Pathway enrichment analysis revealed a coordinated downregulation of pathways associated with IL2q4wks regimen, which clustered into major biological categories of immune/inflammatory signaling, cell communication, and apoptosis/cell death.
- These categories were not independent but converged on a shared set of central hub proteins—IL1B, HIF1A, CASP8, PSMD1, SNCA, NCF1, S100A12, SPN, VTN, GZMB—suggesting that IL2q4wks induces suppression of an inflammation, stress and cell death responses.

CONCLUSIONS

In conclusion, our findings demonstrate that IL-2 dosing frequency critically remodel systemic and CNS immune-proteomic states in AD, with the IL2q4wks regimen uniquely suppressing inflammatory chemokines and leukocyte-associated inflammatory mediators in the plasma as well as inflammation, stress and apoptosis responses in CSF that aligns with favorable AD core biomarker changes and clinical trends. This exploratory integrated multi-compartment proteomic framework presented here provides a rationale and mechanistic foundation for the next phase IIb clinical trial.

ACKNOWLEDGEMENT

This research was supported by the Alzheimer’s Association Part-The-Cloud Award (PTCG-21-1818034) and National Institute of Health R01 grant (R01AG077685). The authors gratefully acknowledge the patients and their families for their participation in this clinical trial.