Tolebrutinib Can Reverse Multiple Sclerosis-Induced Cerebrospinal Fluid Proteomic Alterations

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Anna S. Blazier[1], Gregory Wirak[1], Pavithra Krishnaswami[1], Mikhail Levit[1], Syed Ali Raza[2], Dimitry Ofengeim[1], Timothy J. Turner[1], Steven Jacobson[2], Daniel S. Reich[2]

[1]Sanofi, Cambridge, MA, USA; [2]NINDS, Bethesda, MD, USA

INTRODUCTION

- Proteins measured in the cerebrospinal fluid (CSF) may serve as a window into the central nervous system (CNS) and may provide accessible prognostic and/or predictive biomarkers for treatment[1,2]
- Olink proteomics is a high-throughput, multiplex immunoassay technology that enables the simultaneous measurement of up to 3072 proteins[3]
- In this study, we employed Olink proteomics technology to characterise the CSF proteome of people with untreated MS and to evaluate alterations to the MS CSF proteome upon extended therapeutic intervention with tolebrutinib, a brain-penetrant Bruton's tyrosine kinase (BTK) inhibitor[4]
- This study provides insights into both disease pathophysiology and the correlation with therapeutic intervention

OBJECTIVE/AIM

- To characterise the proteomic landscape in the CSF of people with MS treated with tolebrutinib after transitioning from B-cell depleting therapy

METHODS

We examined the CSF proteome using the Cardiometabolic, Inflammation, Neurology and Oncology Olink Explore 384 panels (1463 analytes total)

- CSF samples were from 31 healthy volunteers and 71 treatment-naïve people with MS

We used the same Olink panels to characterise CSF proteomic changes in participants from the Phase 2 BRaKe MS clinical trial (NCT04742400) treated with tolebrutinib after transitioning from an anti-CD20 B-cell depleting therapy (ocrelizumab)

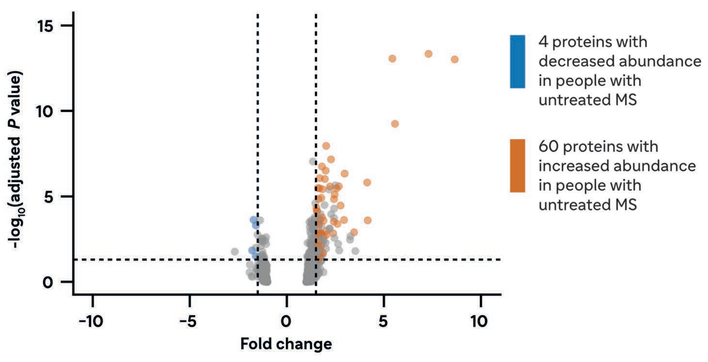
- Seven participants (age [mean ± standard deviation]: 48.2 ± 7.9 years; sex: 2 female) who had been treated with ocrelizumab for >6 months (median: 3.2 years; range: 1.8–4.0 years) were included
- Participants had no signs of acute focal inflammation based on magnetic resonance imaging
- CSF was collected before baseline[a], and at 12 and 48 weeks after transitioning to tolebrutinib 60 mg

[a]"Baseline" was within 6 months since the last ocrelizumab infusion.

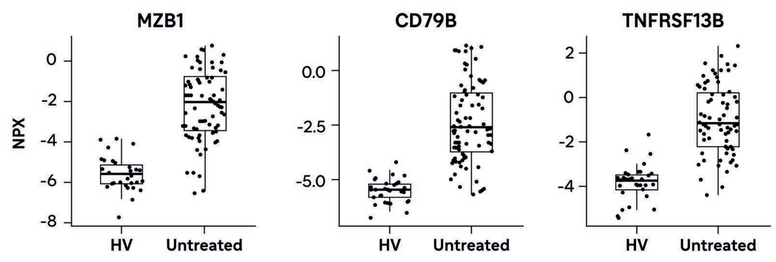
RESULTS

MS CSF proteome

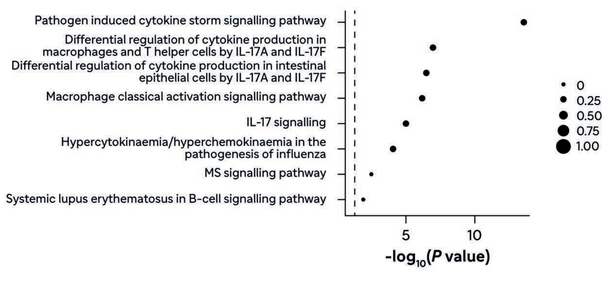
Differential abundance analysis[a]



Examples of proteins elevated in the CSF of people with untreated MS[b]



Pathway analysis of proteins differentially abundant in people with untreated MS

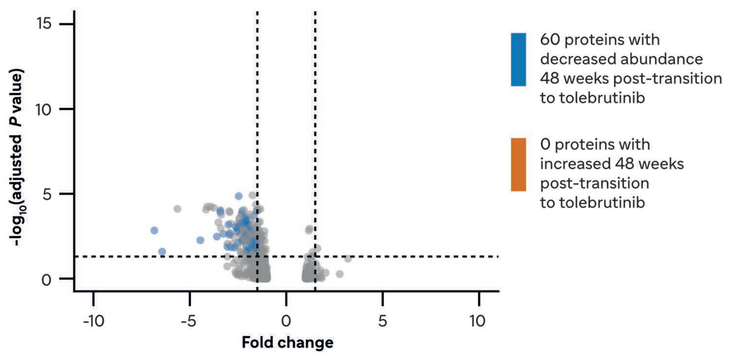


- Pathway analysis indicated increased markers of macrophage, B- and T-cell activation in MS CSF

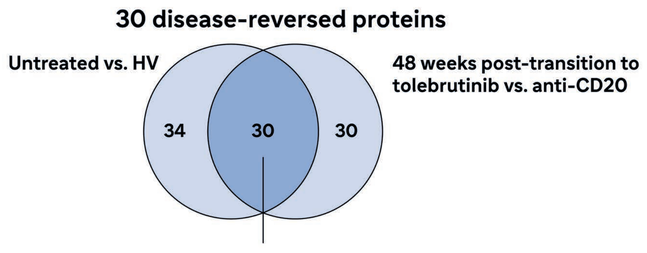
Data were analysed using a linear mixed effects regression model in R. Group, sex and age were treated as fixed effects. Patient was treated as random effect. Emmeans was employed *post hoc* for all pair-wise group comparisons. Markers significant for group and neither sex nor age were selected for downstream follow-up.
[a]Dashed lines reflect standard statistical thresholds for determining significantly differentially abundant proteins, with the horizontal line reflecting an adjusted *P* value of 0.05 and the two vertical lines reflecting fold change of 1.5 in both directions.
[b]These visualised proteins had the largest fold change between people with untreated MS and HV.
CD79B=cluster of differentiation 79B; CSF=cerebrospinal fluid; HV=healthy volunteer; IL=interleukin; MZB1=marginal zone B and B1 cell specific protein; NPX=Normalised Protein eXpression; TNFRSF13B=tumour necrosis factor receptor superfamily member 13B.

Changes in CSF proteome 48 weeks post-transitioning from anti-CD20 therapy to tolebrutinib

Differential abundance analysis[a]
48 weeks post-transition to tolebrutinib (n=6) vs. anti-CD20 baseline (n=7)



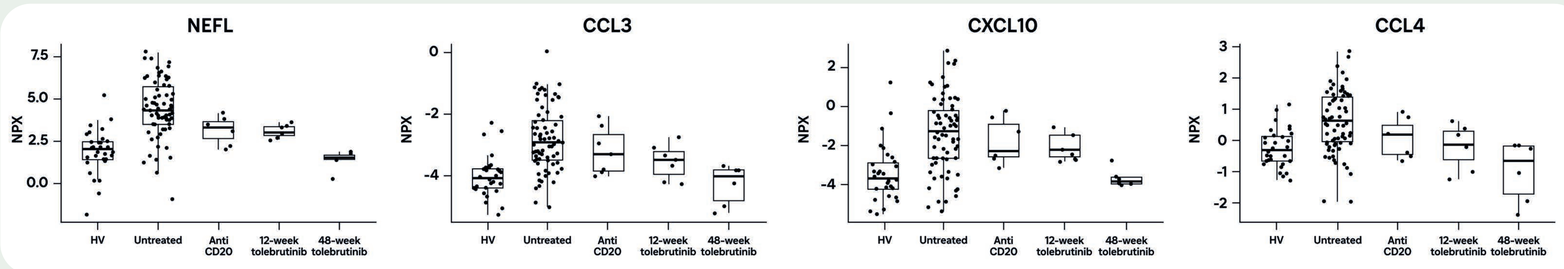
30 disease-reversed proteins



30 proteins with increased abundance in people with MS (relative to HV) were reversed in samples taken 48 weeks post-transitioning to tolebrutinib relative to their anti-CD20 baseline levels

[a]Dashed lines reflect standard statistical thresholds for determining significantly differentially abundant proteins, with the horizontal line reflecting an adjusted *P* value of 0.05 and the two vertical lines reflecting fold change of 1.5 in both directions.
HV=healthy volunteer.

Examples of disease-reversed proteins 48 weeks after transitioning from anti-CD20 therapy to tolebrutinib



CCL=chemokine (C-C motif) ligand; CXCL=chemokine (C-X-C motif) ligand; HV=healthy volunteer; NEFL=neurofilament light; NPX=Normalised Protein eXpression.

Comparing 48-week tolebrutinib vs. anti-CD20 proteomic CSF signature to *in vitro* and *in vivo* BTKi gene expression signatures

Green dots are the proteins that were differentially abundant in the 48-week tolebrutinib vs. anti-CD20 proteomic CSF comparison.
[a]iPSC-derived microglia, astrocytes and neurons.
BTKi=Bruton's tyrosine kinase inhibitor; CSF=cerebrospinal fluid; EAE=experimental autoimmune encephalomyelitis; IgG=immunoglobulin G; iPSC=induced pluripotent stem cell.

CONCLUSIONS

Olink analysis detected 64 proteins that had altered levels in the CSF of people with untreated MS

- Pathway analysis indicated increased markers of macrophage, B- and T-cell activation in MS CSF

The CSF proteome of people with MS was altered 48 weeks after transitioning to tolebrutinib from a B-cell depleting therapy, with 30 disease-associated proteins (47%) reverting towards levels observed in healthy volunteers

- Some overlap in the proteins that decreased in abundance after tolebrutinib treatment was observed between the participants with MS and IgG-stimulated *in vitro* microglial monoculture and neural tri-culture systems
- We are working actively to further understand the relative impacts of anti-CD20 therapies and BTK inhibition on these CSF proteomic changes using single-cell RNA-sequencing, flow cytometry, and proteomic analysis of matched serum samples

Our work contributes to an improved understanding of drug-induced protein alterations in the CSF of people with MS and proposes molecular biomarkers for evaluating therapeutic efficacy in the CNS

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Disclosures

Anna S. Blazier, Gregory Wirak, Pavithra Krishnaswami, Mikhail Levit, Dimitry Ofengeim and **Timothy J. Turner:** Employees of Sanofi (may hold shares and/or stock options in the company)
Syed Ali Raza and **Steven Jacobson:** Nothing to disclose
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